DEPARTMENT OF

MEDICAL BIOCHEMISTRY

AND MICROBIOLOGY

ANNUAL REPORT

2008

INTRODUCTION

The past year has seen many positive developments at the Department of Medical Biochemistry and Microbiology (Institutionen för medicinsk biokemi och mikrobiologi, or IMBIM). Two research groups have joined the department, one led by Professor Birgitta Heyman and another by Associate Professor Anna-Karin Olsson. Professor Heyman's group accounts for much of the research in experimental immunology at the Medical Faculty in Uppsala. The group is a valuable addition to IMBIM, engendering the second 'I' in the 'IMBIM' acronym with quality and content. The research focus of Associate Professor Olsson and co-workers lies where the fields of immunology, vascular and tumor biology intercept, consolidating a prominent area of research at the Department. Furthermore, as part of a University initiative entitled 'Quality and Renewal 07', the Department expects to increase its scientific personnel with new professors and assistant professors. Recruitments to these new positions have already commenced and are expected to be finalized by the end of 2008 and early in 2009.

A major relocation of research laboratories was completed during 2008. The total laboratory space at the Department decreased, several groups moved to new corridors and most groups have reduced their space. This relocation has meant that groups with similar research interests have come closer thereby increasing possibilities for creative interactions. In addition, and importantly, the relocations decreased IMBIM's costs for rent; it can be hoped that a desire to decrease costs for rent will permeate all levels of the University.

During the year, Professor Emeritus Torvard C. Laurent received the prestigious large gold medal from the Royal Swedish Academy of Engineering Sciences for his seminal research on hyaluronan. IMBIM extends its sincere congratulations to Professor Laurent for this well-deserved mark of recognition. Research carried out at IMBIM during 2008 has been of the highest caliber, with the publication of more than 70 original scientific papers, many in high impact journals, around 20 reviews or commentaries and presentations at congresses and symposia. Five graduate students received their doctorates and many scientists at IMBIM successfully applied for external grants.

Details of the research activities at IMBIM are summarized in the following pages of this annual report. Here are just a few of the highlights:

- The white horse, an icon for dignity, has had a huge impact on human culture across the world. Dr Gerli Rosengren-Pielberg and her colleagues identified the mutation causing this spectacular trait. They showed that white horses carry a 4.5 kb duplication in intron 6 of syntaxin17. The mutation is a cis-acting regulatory mutation that up-regulates the expression of both syntaxin17 and the neighboring gene, NR4A3. The finding is of considerable medical significance as the mutation in humans predisposes the carrier to melanoma development (Rosengren-Pielberg *et al.,* Nature Genetics).

- Many domestic chickens have yellow skin. This is due to a recessive allele that allows the deposition of colorful carotenoids in the skin. Jonas Eriksson and his colleagues

demonstrated that this is caused by a regulatory mutation that inhibits the expression of the gene encoding the enzyme, β-carotene dioxygenase 2 (BCDO2), which cleaves colorful carotenoids into colorless apocarotenoids. This is the first identification of a gene controlling carotenoid-based pigmentation (Eriksson *et al.*, PLoS Genetics).

- Heparan sulfate proteoglycans influence embryonic development and adult physiology through interactions with protein ligands. The interactions depend on heparan sulfate structure. Recent results from Professor Lena Kjellén's group support a model in which the enzymes of heparan sulfate biosynthesis form a complex, a "GAGosome", where the relative levels of the enzymes determine the outcome of biosynthesis (Presto *et al.*, 2008 PNAS).

- Associate Professor Per Jemth and co-workers conducted studies on fundamental aspects of protein chemistry, such as the landscape of the protein folding reaction and the role of intra-domain allostery in protein-ligand interactions. They discovered a specific polypeptide sequence exhibiting great entropic freedom at the early stages of the folding reaction but much more restricted by native topology later on in the reaction. The study reassessed the function of an evolutionarily conserved amino acid network in a protein domain, and found that it did not contribute to ligand binding, as previously thought (Chi *et al.*, 2008, PNAS).

- DNA repair systems involved in repairing common spontaneous mutations caused by oxidized and deaminated bases strongly influence the GC content of bacterial genomes. Peter Lind and Professor Dan Andersson showed that mutations caused by the oxidized base, 8-oxo-G, are very common and if not repaired by the repair system (MutYM) these specific mutations result in rapid reduction in GC content. Intracellular parasitic and endosymbiotic bacteria typically have low GC content and also lack the MutYM repair system. This study suggests that it is likely that in such bacteria, unrepaired 8-oxo-G mutations generate a mutational bias towards reduced GC content (Lind and Andersson, 2008, PNAS).

IMBIM has invested significantly in undergraduate teaching and contributes to five of the study programs given by the Science Area of Medicine and Pharmacy at Uppsala University. Starting in 2006, the MD program is now being radically changed so that each course is centered on a specific medical topic. To stimulate student interest, medical cases are discussed in small groups of about eight students. The department, therefore, no longer offers coherent courses in the core subjects of IMBIM. Rather, our teachers are active over several semesters and strive to ensure that the core curriculum of the various subjects is filled when the diversified courses are integrated. The major part of the teaching carried out by IMBIM staff takes place during the first three semesters of the academic year. Therefore most of the time-consuming planning in conjunction with the change of the study program has now been accomplished.

The Bologna process is a strategy aimed at harmonizing study programs throughout Europe. This reform has meant that many four-year university programs in Sweden (*magister)* have

been or are being converted into programs consisting of bachelor and master components requiring three and two years of studies, respectively. This change has had a major effect on only one of the programs in which teachers from IMBIM are involved, that of Biomedicine. The modifications are now gradually being implemented. In addition to teaching during the bachelor component of this program, IMBIM is also responsible for a master program in Infection Biology, which is presently in the planning stage.

About one third of the faculty funding to IMBIM income is derived from undergraduate teaching. Funding is based on the number of students who have passed the exams. This year, fewer students than planned passed their first year exams in the Biomedicine and the Dispensing Pharmacy programs, partly due to fewer enrolled students but also due to a higher failure rate. The reasons for this are complex and several. One possibility is that the high school students attracted to these programs are less well prepared for university studies. Another possibility is that the courses have failed to maintain the motivation of the students. In an effort to improve motivation, the syllabus of the course in Molecular Biology and Gene Technology in the Dispensing Pharmacy program has now been revised to better reflect future professional needs.

Several researchers from IMBIM have ongoing collaborations with industry, including local enterprises. Dr. Cecilia Annerén, a senior scientist at GE Healthcare in Uppsala, has placed her academic research, funded by the Swedish Science Council and the Juvenile Diabetes Foundation (USA), at IMBIM. We welcome the possibilities for collaboration with GE Healthcare that may arise from this initiative. In addition, several other research groups at IMBIM maintain established collaborations with industrial partners. The Department also participates in various programs with visiting high school students. Researchers at IMBIM have also contributed several papers in popular science journals during 2008.

Semi-autonomous groups directed by principal investigators carry out research at the Department, many of which have been successful in their quest for external grants during 2008. Research and graduate (research) education are largely funded by external grants. The Department is completely dependent on the success of our scientists in their search for external grants to maintain a sizeable and internationally recognized body of research graduate education. Furthermore, the distribution of competitive government funds to the universities will become increasingly dependent on external grants and citations of published work. It is therefore of central importance for IMBIM to provide the conditions and an environment that is conducive to the work carried out by the research groups in order to maintain their motivation.

The situation for the small to medium sized groups requires particular scrutiny. These groups form the core of any research department and, thanks to their number, they are responsible for most of the publications that generate citations and attract grants. However, an increasing number of initiatives, legislation and rulings from central agencies at the university and governmental bodies impose a threat to the possibility for individual scientists to carry out quality research at a reasonable cost of effort. This is also a potential problem for teaching in undergraduate courses. To maintain the academic tradition of teachers as active researchers and

thereby contribute to the content of courses with the most up to date information, we need to find ways to guarantee that small and medium sized groups continue to produce internationally competitive research. It remains a major task for IMBIM to increase its competitiveness by allowing the system of competitive grants to work as effectively as possible. To achieve this, IMBIM must keep overheads down and reduce unnecessary administrative burdens. Furthermore, it is important to generate structures where several small to medium-sized groups co-operate.

In common with most other preclinical departments in Sweden, graduate education at IMBIM is largely funded by external grants. Together with increasing salary costs, this is likely to result in a reduction of graduate students. Due to the recent growth of IMBIM, the relative number of graduate students has indeed decreased over the last ten years. The number of graduate students from non-EU states that have financial support from their country of origin has, however, increased in recent years. Currently around 15% of the graduate students belong to this latter category. This development is welcomed and enriches IMBIM in many ways.

It is a pleasure to report many positive developments at IMBIM in 2008. There is, however, a need for caution and a readiness to meet potential future problems, including financial problems. An important task in the immediate future is to recapture some of the atmosphere and interactions of a coherent scientific department that were lost during the re-organization of the faculty during the 1990s. This could possibly be achieved by creating scientific areas with a higher degree of autonomy.

Kristofer Rubin, chairman

Uppsala, January, 2009

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SCIENTIFIC REPORTS

FUNCTIONAL GENOMICS

FUNCTIONAL GENOMICS IN DOMESTIC ANIMALS

Leif Andersson

The overall objective of the group is to use genetics and genomics to advance our understanding of the genetic mechanisms underlying phenotypic variation. We are using domestic animals models because domestication and animal breeding have caused major changes in many phenotypic traits. This gives a unique opportunity to unravel the genes underlying phenotypic variation. The research includes genetic studies of both monogenic traits, like inherited disorders and coat colour, and multifactorial traits, like muscle development, fat deposition, autoimmune disorders and general disease resistance. The major research projects involve genetic analysis of divergent intercrosses in chicken and genome-wide association analysis in the dog but the ongoing research also includes genetic studies in horses and pigs. The ultimate goal of the research is to identify the genes and mutations affecting a certain trait and thereafter study the mechanism and biological significance of the detected mutations.

Members of the group during 2008

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Publications from 2004 to 2008

1. Clop,A., Amills,M., Noguera,J.L.; Fernández,A., Capote,J., Misericòrdia Ramón,M., Kelly,L., Kijas, J.M.H., Andersson,L. and Sànchez,A. 2004. Estimating the frequency of

Asian *cytochrome B* haplotypes in European standard and local pig breeds. Genetics Selection Evolution 36:97-104.
2. Mahlapuu,M., Johansson,C., Lindgren,K., Hjälm,G., Barnes,B., Krook,A., Zierath,J., Andersson,L., and Marklund,S. (2004) Expression profiles representing the γ-subunit isoforms of AMP-activated protein kinase suggest a major role for γ3 in white skeletal muscle fibers of mammals. Am. J. Physiol. Endocrinol. Metab. 286:E194-E200.
3. Schütz,K., Kerje,S., Jacobsson,L., Carlborg,Ö., Forkman,B., Andersson,L., and Jensen,P. 2004. Major growth QTLs in fowl are related to fearful behavior – possible genetic links between fear responses and production traits in a red junglefowl x White Leghorn intercross. Behavior Genetics 34: 121-130.
4. Barnes,B.R., Marklund,S. Steiler,T.L., Walter,M., Hjälm,G., Amarger,V., Mahlapuu,M., Leng,Y., Johansson,C., Galuska,D., Lindgren,K., Åbrink,M., Stapleton,D., Zierath,J.R., and Andersson,L. 2004. The AMPK-γ3 isoform has a key role for carbohydrate and lipid metabolism in glycolytic skeletal muscle. Journal of Biological Chemistry 279: 38441-38447.
5. Moller,M., Berg,F., Riquet,J., Pomp,D., Archibald,A., Anderson,S., Feve,K., Zhang,Y., Rothschild,M., Milan,D., Andersson,L., and Tuggle,C. 2004. High-resolution comparative mapping across pig chromosome 4 (SSC4), emphasising the *FAT1* region. Mammalian Genome 15: 717-731.
6. Kerje,S., Sharma,P., Gunnarsson,U., Kim,H., Bagchi,S., Fredriksson,R. Schütz,K., Jensen,P., von Heijne,G., Okimoto,R., and Andersson,L. 2004. The *Dominant white, Dun* and *Smoky* color variants in chicken are associated with insertion/deletion polymorphisms in the *PMEL17* gene. Genetics 168:1507-1518.
7. Jacobsson,L, Park,H.-B., Wahlberg,P., Jiang,S. Siegel,P.B., and Andersson,L. 2004. Assignment of 14 microsatellite markers to the chicken linkage map. Poultry Science 83: 1825-1831.
8. Fitzsimmons,C.J., Savolainen,P., Amini,B., Hjälm,G., Lundeberg,J., and Andersson,L. 2004. Detection of sequence polymorphisms in red junglefowl and White Leghorn ESTs. Animal Genetics 35: 391-396.
9. Keeling,L., Andersson,L., Schütz,K.E., Kerje,S., Fredriksson,R., Carlborg,Ö., Cornwallis,C.K., Pizzari,T., and Jensen,P. 2004. Feather-pecking and victim pigmentation. Nature 431: 645-646.
10. Braunschweig,M.H., Van Laere,A.-S., Buys,N., Andersson,L., and Andersson,G. 2004. *IGF2* antisense transcript expression in porcine postnatal muscle is affected by a quantitative trait nucleotide in intron 3. Genomics 84:1021-1029.
11. International Chicken Polymorphism Map Consortium (L.Andersson is one of the corresponding authors). 2004. A genetic variation map for chicken with 2.8 million single-nucleotide polymorphisms. Nature 432:717-722.
12. International Chicken Genome Sequence Consortium. 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 432:695-716.
13. Jungerius,B.J., Van Laere,A.-S., te Pas,M.F.W., van Oost,B.A., Andersson,L.; and Groenen,M.A.M. 2004. The *IGF2*-intron3-G3072A substitution explains a major imprinted QTL effect on backfat thickness in a Meishan X European white pig intercross. Genetical Research 84:95-101.
14. Barnes,B.R., Glund,S., Long,Y.C., Hjälm,G., Andersson,L., and Zierath,J.R. 2005. 5'-AMP-activated protein kinase regulates skeletal muscle glycogen content and ergogenics. FASEB J. 19:773-779.

15. Larson,G., Dobney,K., Albarella,U., Fang,M., Matisoo-Smith,E., Robins,J., Lowden,S., Finlayson,H., Brand,T., Rowley-Conwy,P., Andersson,L. and Cooper,A. 2005. Worldwide phylogeography of wild boar reveals multiple centres of pig domestication. Science 307:1618-1621.
16. Cassini,P., Montironi,A., Botti,S., Hori,T., Okhawa,H., Stella,A., Andersson,L., Giuffra,E. 2005. Genetic analysis of anal atresia in pigs: evidence for segregation at two main loci. Mammalian Genome 16:164-170.
17. Johansson,A., Pielberg,G., Andersson,L., and Edfors-Lilja,I. 2005. Polymorphism at the porcine Dominant white/KIT locus influence coat colour and peripheral blood cell measures. Animal Genetics 36:288-296.
18. Wattrang,E., Almqvist,M., Johansson,A., Fossum,C., Wallgren,P., Pielberg,G., Andersson,L., and Edfors-Lilja, I. 2005. Confirmation of QTL on porcine chromosomes 1 and 8 influencing leukocyte numbers, haematological parameters and leukocyte function. Animal Genetics 36:337-345.
19. Savolainen,P., Fitzsimmons,C., Arvestad,L., Andersson,L., and Lundeberg,J. 2005. ESTs from brain and testis of White Leghorn and red junglefowl: annotation, bioinformatic classification of unknown transcripts and analysis of expression levels. Cytogenetics Genome Research 111:79-87.
20. Pielberg,G., Mikko,S., Sandberg,K., and Andersson,L. 2005. Comparative linkage mapping of the *Grey* coat colour gene in horses. Animal Genetics 36:390-395.
21. Jensen,P., Keeling,L., Schutz,K., Andersson,L., Mormede,P., Brändström,H., Forkman,B., Kerje,S., Fredriksson,R., Ohlsson,C., Larsson,S., Mallmin,H., and Kindmark,A. 2005. Feather pecking in chickens is genetically related to behavioural and developmental traits. Physiology & Behavior 86:52-60.
22. Essen-Gustavsson,B., Jensen-Waern,M., Jonasson,R., and Andersson,L. 2005. Effect of exercise on proglycogen and macroglycogen content in skeletal muscles of pigs with the Rendement Napole mutation. Am J Vet Res. 66:1197-1201.
23. Jacobsson,L., Park,H.-B., Wahlberg,P., Fredriksson,R., Perez-Enciso,M., Siegel,P.B., and Andersson,L. 2005. Many QTLs with minor additive effects are associated with a large difference in growth between two selection lines in chickens. Genetical Research 86:115-125.
24. Barnes,B.R., Long,Y.C., Steiler,T.L., Leng,Y., Galuska,D., Wojtaszewski,J., Andersson,L., and Zierath,J.R. 2005. Changes in exercise-induced gene expression in AMPK gamma-3 null and gamma-3 R225Q transgenic mice. Diabetes 54:3484-3489.
25. Long,Y.C., Barnes,B.R., Mahlapuu,M., Steiler,T.L., Martinsson,S., Leng,Y., Wallberg-Henriksson,H., Andersson,L., and Zierath,J.R. 2005. Role of AMP-activated protein kinase in the coordinated expression of genes controlling glucose and lipid metabolism in mouse white skeletal muscle. Diabetologia 48:2354-2364.
26. Drogemuller,C., Giese,A., Martins-Wess,F., Wiedemann,S., Andersson,L., Brenig,B., Fries,R., and Leeb,T. 2005. The mutation causing the black-and-tan pigmentation phenotype of Mangalitza pigs maps to the porcine *ASIP* locus but does not affect its coding sequence. Mamm Genome. 2006 17:58-66.
27. Park,H.-B., Jacobsson,L., Wahlberg,P., Siegel,P.B., and Andersson,L. 2006. QTL analysis of body composition and metabolic traits in an intercross between chicken lines divergently selected for growth. Physiological Genomics 25:216-223.
28. Carlborg,Ö., Jacobsson,L., Åhgren,P., Siegel,P.B., and Andersson,L. 2006. Epistasis and the release of genetic variation during long-term selection. Nature Genetics 38:418-420.
29. Nilsson,E.C., Long,Y.C., Martinsson,S., Glund,S., Garcia-Roves,P., Svensson,L.T., Andersson,L., Zierath,J.R., and Mahlapuu M. 2006. Opposite transcriptional regulation in

skeletal muscle of AMPK gamma 3 R225Q transgenic versus knock-out mice. J. Biol. Chem. 281:7244-7252.
30. Berg,F., Stern,S., Andersson,K, Andersson,L., and Moller M. 2006. Refined localization of the FAT1 quantitative trait locus on pig chromosome 4 by marker-assisted backcrossing. BMC Genet. 7:17.
31. Karlskov-Mortensen,P., Bruun,C.S., Braunschweig,M.H., Sawera,M., Markljung,E., Enfalt,A.C., Hedebro-Velander,I., Josell,A., Lindahl,G., Lundstrom,K., von Seth,G., Jorgensen,C.B., Andersson,L. and Fredholm,M. 2006. Genome-wide identification of quantitative trait loci in a cross between Hampshire and Landrace I: carcass traits. Animal Genetics 37:156-162.
32. Fang,M. and Andersson,L. 2006. Mitochondrial diversity in European and Chinese pigs is consistent with population expansions that occurred prior to domestication. Proc Biol Sci. 273:1803-1810.
33. Treebak,J.T., Glund,S., Deshmukh,A., Klein,D.K., Long,Y.C., Jensen,T.E., Jorgensen,S.B., Viollet,B., Andersson,L., Neumann,D., Wallimann,T., Richter,E.A., Chibalin,A.V., Zierath,J.R. and Wojtaszewski,J.F. 2006. AMPK-mediated AS160 phosphorylation in skeletal muscle is dependent on AMPK catalytic and regulatory subunits. Diabetes 55:2051-2058.
34. Bruun,C.S., Jørgensen,C.B, Nielsen,V.H, Andersson,L. and Fredholm,M. 2006. Evaluation of the porcine Melanocortin-4 receptor gene (*MC4R*) as a positional candidate for a fatness QTL in a cross between Landrace and Hampshire. Animal Genetics 37:359-362.
35. Fang,M., Berg,F., Ducos,A. and Andersson,L. 2006. Mitochondrial haplotypes of European wild boars with 2n=36 are closely related to those of European domestic pigs with 2n=38. Animal Genetics 37:459-464.
36. Berg,F., Gustafson,U. and Andersson,L. 2006. The uncoupling protein 1 gene (*UCP1*) is disrupted in the pig lineage -a genetic explanation for the poor thermoregulation in piglets PLoS Genetics 2:e129.
37. Brunberg,E., Andersson,L., Cothran,G., Sandberg,K., Mikko,S. and Lindgren,G. 2006. A missense mutation in PMEL17 is associated with the Silver coat color in the horse. BMC Genetics 7:46.
38. Wright,D., Kerje,S., Lundström,K., Babol,J., Schutz,K., Jensen,P. and Andersson,L. 2006. Quantitative trait loci analysis of egg and meat production traits in a red junglefowl x White Leghorn cross. Anim. Genet. 37:529-534.
39. Natt,D., Kerje,S., Andersson,L. and Jensen,P. 2007 Plumage Color and Feather Pecking-Behavioral Differences Associated with PMEL17 Genotypes in Chicken (*Gallus gallus*).Behav. Genet. 37:399-407.
40. Gunnarsson,U., Hellström,A.R., Tixier-Boichard,M., Minvielle,F., Bed'hom,B., Ito,S., Jensen,P., Rattink,A., Vereijken,A., and Andersson,L. 2007. Mutations in *SLC45A2* cause plumage color variation in chicken and Japanese quail. Genetics 175:867-877.
41. Rubin,C.J., Brandstrom,H., Wright,D., Kerje,S., Gunnarsson,U., Schutz,K., Fredriksson,R., Jensen,P., Andersson,L., Ohlsson,C., Mallmin,H., Larsson,S. and Kindmark A. 2007. Quantitative Trait Loci for Bone Mineral Density and Bone Strength in an Intercross between Domestic and Wild Type Chicken. J. Bone Miner. Res. 22:375-384.
42. Wahlberg,P., Strömstedt,L., Tordoir,X., Foglio,M., Heath,S., Lechner,D., Hellström,A.R, Tixier-Boichard,M., Lathrop,M., Gut,I.G. and Andersson,L. 2007. A high-resolution linkage map for the Z chromosome in chicken reveals hot spots for recombination. Cytogenetics and Genome Research 117:22-29.

43. Rubin,C.J., Lindberg,J., Fitzsimmons,C., Savolainen,P., Jensen,P., Lundeberg,J., Andersson,L. and Kindmark A. 2007. Differential gene expression in femoral bone from red junglefowl and domestic chicken, differing for bone phenotypic traits. BMC Genomics 8:208.
44. Karlsson,E.K., Baranowska,I., Wade,C.M., Hillbertz,N.H.C., Zody,M.C., Andersson,N., Biagi,T.M., Patterson,N., Rosengren Pielberg,G., Kulbokas, E.J.III, Comstock,K.E., Keller,E.T., Mesirov,J.P., von Euler,H., Kämpe,O., Hedhammar,A., Lander,E.S., Andersson,G., Andersson,L. and Lindblad-Toh,K. 2007. Efficient mapping of Mendelian traits in dogs through genome-wide association analysis. Nature Genetics 39:1321-1328.
45. Hillbertz,N.H.C., Isaksson,M., Karlsson,E.K., Hellmén,E., Rosengren Pielberg,G., Savolainen,P., Wade,C.M., von Euler,H., Gustafson,U., Hedhammar,A., Nilsson,M., Lindblad-Toh,K., Andersson,L. and Andersson,G. 2007. A duplication of *FGF3*, *FGF4*, *FGF9* and *ORAOV1* causes the hair ridge and predisposes to dermoid sinus in Ridgeback dogs. Nature Genetics 39:1318-1320.
46. Wright,D., Kerje,S., Brändström,H., Schütz,K., Kindmark,A., Andersson,L., Jensen,P. and Pizzari,T. 2008. The genetic architecture of a female sexual ornament. Evolution 62:86-98.
47. Eriksson,J., Larson,G., Gunnarsson,U., Bed'hom,B., Tixier-Boichard,M., Strömstedt,L., Wright,D., Jungerius,A., Vereijken,A., Randi,E., Jensen,P. and Andersson,L. 2008. Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken. PLoS Genetics 4: e1000010.
48. Markljung,E., Braunschweig,M.H., Karlskov-Mortensen,P, Bruun,C.S., Sawera,M., Cho,I.C., Hedebro-Velander,I, Josell,A, Lundstrom,K, von Seth,G, Jorgensen,C.B., Fredholm,M,, and Andersson,L. 2008. Genome-wide identification of quantitative trait loci in a cross between Hampshire and Landrace II: meat quality traits. BMC Genetics 9:22.
49. Rosengren Pielberg,G., Golovko,A., Sundström,E., Curik,I., Lennartsson,J., Seltenhammer,M.H., Druml,T., Binns,M., Fitzsimmons,C., Lindgren,G., Sandberg,K., Baumung,R., Vetterlein,M., Strömberg,S., Grabherr,M., Wade,C., Lindblad-Toh,K., Pontén,F., Heldin,C.-H., Sölkner,J. and Andersson,L. 2008. A cis-acting regulatory mutation causes premature hair greying and susceptibility to melanoma in the horse. Nature Genetics 40: 1004 - 1009.
50. Fang,M., Larson,G., Soares Ribeiro,H., Li,N., and Andersson,L. 2008. Contrasting mode of evolution at a coat color locus in wild and domestic pigs. PLoS Genetics in press.

Reviews from 2004 to 2008

1. Georges,M. and Andersson,L. 2004. Positional identification of structural and regulatory quantitative trait nucleotides in livestock. In: The Genome of Homo Sapiens (eds. Stillman,B. and Stewart,D. Cold Spring Harbor Symposia on Quantitative Biology, Vol. LXVIII, pp 179-187, Cold Spring Harbor Laboratory.
2. Andersson,L. and Georges,M. 2004. Domestic animal genomics: deciphering the genetics of complex traits. Nature Review Genetics 5:202-212.
3. Andersson,L. 2005. Farm Animals. In: Encyclopedia of Genetics, Genomics, Proteomics and Bioinformatics (eds. Jorde,L.B., Little,P.F.R., Dunn,M.J. and Subramaniam,S.) John Wiley & Sons Ltd. Chichester, Volume3, pp. 1171-1183.
4. Jensen,P. and Andersson,L. 2005. Genomics meets ethology: a new route to understanding domestication, behaviour and sustainability in animal breeding. Ambio 34:320-324.

5. Ka,S., Fitzsimmons,C., Jacobsson,L., Savolainen,P., Lundeberg,J., Siegel,P.B., Andersson,L., and Hallböök,F. 2005. Expression analysis of growth and energy regulation-associated genes in two divergent chicken strains. Ann N Y Acad Sci. 1040:357-359.
6. Wick,G., Andersson,L., Hala,K., Gershwin,M.E., Selmi,C.F., Erf,G.F., Lamont,S.J., and Sgonc,R. 2006. Avian models with spontaneous autoimmune diseases. Advances in Immunology 92:71-117.
7. Siegel,P.B., Dodgson,J.B. and Andersson,L. 2006. Progress from chicken genetics to the chicken genome. Poultry Science 85:2050-2060.
8. Pielberg,G. and Andersson,L. 2006. Gene copy number detection in animal studies. Methods Mol. Biol. 373:147-156.
9. Andersson,L. 2007. The molecular basis for phenotypic changes during pig domestication. In: Pigs and humans: 10,000 years of interaction(eds. Albarella,U., Dobney,K., Ervynck,A., and Rowley-Conwy,P. Oxford University Press, Oxford, pp. 42-54.
10. Andersson,L. 2008. Domestic animals - a treasure trove for comparative genomics. In: Comparative Genomics: Basic and Applied Research (ed. Brown,J.R.) Taylor and Francis Group, Boca Raton, Fl., pp. 341-362.
11. Andersson,L. 2008. Genome-wide association analysis in domestic animals – a powerful approach for genetic dissection of trait loci. Genetica in press.
12. Sundström,E. and Andersson,L. 2008. Den vita hästens gåta löst. Forskning och Framsteg 8:40-43.

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GENETIC ANALYSIS OF DIVERGENT INTERCROSSES IN THE CHICKEN

Jonas Eriksson, Ulrika Gunnarsson, Anders Hellström, Freyja Imsland, Greger Larson, Ranran Liu, Jennifer Meadows, Per Wahlberg, Dominic Wright, Leif Andersson

We have in collaboration with Prof. Per Jensen (Linköping) and Prof. Paul Siegel (Blacksburg, USA) developed two unique resource pedigrees for genetic dissection of multifactorial traits. One pedigree was generated by crossing a single Red junglefowl male with females from a line of White Leghorn selected for egg production. The populations differ markedly in growth, fertility (number of eggs and size of eggs), behaviour and body composition (fat vs. protein content). The other intercross involves two lines (High and Low) that have been divergently selected for growth for more than 40 generations. The selection response has been remarkable and the 8-week weight shows approximately a 9-fold difference between lines, about 1.8 kg for the H line in contrast to about 0.2 kg for the L line. The two lines show a dramatic difference in appetite (hyperphagia vs. anorexia) and the body

composition has been altered with the H line developing obesity. The HxL intercross is a unique resource for understanding the genetic regulation of growth, appetite and obesity. Both pedigrees comprise almost 1,000 animals from three generations. This experimental design gives an excellent power in the genetic analysis. A broad collection of phenotypic data and DNA samples have been obtained from all animals. Our strategy is to combine genetic marker and gene expression analysis for gene discovery. The analysis of the Red Junglefowl intercross has localized more than 40 quantititave trait loci (QTL) controlling growth, body composition, fertility, egg weight, behaviour and bone density. Using these resources we have identified the genes causing dominant white colour, dominant black colour, silver plumage colour, yellow skin and Pea-comb.

The project is carried out with collaborators at Uppsala university, Linköping university, Karolinska Institute, INRA (France) and Virginia Polytechnic Institute (Blacksburg, USA).

GENETIC ANALYSIS OF THREE CHICKEN MODELS FOR AUTOIMMUNE DISORDERS IN HUMANS

Susanne Kerje, Navya Laxman, Dominic Wright, Leif Andersson

We have intiated cross-breeding experiments and genome scans for three lines of chickens representing novel models for three autoimmune disorders in humans, Hashimoto's thyroiditis, systemic sclerosis and vitiligo. The Obese strain (OS) chickens develop a spontaneous autoimmune thyroiditis closely resembling Hashimoto's thyroiditis in human. The strain was established in the 1960's and has been widely used as an animal model to reveal various aspects of the disease. The University of California at Davis line 200 (UCD200) chickens develop an inherited syndrome with features very similar to human systemic sclerosis including fibrotic destruction of the skin and internal organs. Finally, the Smyth line (SL) represents an animal model for vitiligo in which 70-90% of the birds express a post-hatch autoimmune destruction of melanocytes leading to feather de-pigmentation at 6–14 weeks of age. Interestingly the incidence of vitiligo is dramatically increased (from ~15% to ~85%) after immunization with a Herpes virus vaccine. Virus infections are generally believed to trigger autoimmune disorders in humans. The intercross pedigrees will be used for genome scans with the ultimate goal of identifying genes underlying these autoimmune disorders. The identification of disease-related genes will lead to a better understanding of pathogenesis, as well as of general mechanisms underlying autoimmune diseases, thus facilitating the development of better diagnostic, prognostic and therapeutic tools. The work is carried out in collaboration with Drs. Olov Ekwall and Olle Kämpe at Department of Medical Sciences and Dr. Örjan Carlborg at SLU.

A REGULATORY MUTATION IN *IGF2* AFFECTING MUSCLE GROWTH, FAT DEPOSITION AND THE SIZE OF THE HEART IN PIGS

Ellen Markljung, Lin Jiang, Göran Hjälm, Kerstin Lindblad-Toh, Leif Andersson

We have previously reported that a paternally expressed Quantitative Trait Locus (QTL) segregating in our wild boar intercross maps in the vicinity of the paternally expressed *IGF2* locus encoding insulin-like growth factor 2. The allele inherited from the domestic pig

increases muscle mass by 3-4%, reduces backfat thickness (subcutaneous fat) and increases the size of the heart but it has no effect on birth weight. We also demonstrated that this QTL is caused by a single point mutation in the middle of an intron. The mutation occurs at an evolutionary conserved CpG island and we have experimental evidence showing that it affects transcriptional regulation of *IGF2* in a tissue-specific fashion. We observed a three-fold increase of *IGF2* transcription in postnatal skeletal muscle but not in prenatal skeletal muscle or in postnatal liver. Thus, the mutation apparently fine-tunes the expression of *IGF2*. We have now identified the repessor interacting with the mutated site. We are using RNAi, transfection experiments and immunohistochemistry to study the functional significance of the new factor. The project is carried out in collaboration with Dr. Göran Andersson, SLU

POSITIONAL IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF THE MUTATION CAUSING GREYING WITH AGE IN HORSES

Gerli Pielberg, Anna Golovko, Elisabeth Sundström, Leif Andersson

Grey is a dominant coat colour mutation that is common in horses and found in a variety of breeds including Arabian horses, Lippizzaner horses, Thoroughbreds, Swedish Warmblood and Icelandic horses. A grey horse is born coloured (bay, black or chestnut) but for each year it becomes gradually greyer and eventually all hair become completely white. A remarkable feature of this coat colour variant is that there is a very high incidence of melanomas in old grey horses. It has been estimated that ~80% of grey horses older than 15 years have melanomas whereas this is a very rare condition in horses with other colours. Thus, the positional cloning of the *Grey* gene provides a unique opportunity to generate new basic knowledge about tumour development of melanocytes. We have now identified that the causal mutation is a 4.5 kb duplication located in intron 6 of *Syntaxin17*. We also demonstrated that this is cis-acting regulatory mutation that upregulates both *Syntaxin17* and the neighbouring gene *NR4A3* encoding an orphan nuclar receptor. We are currently exploring the mechanism leading to premature greying and melanoma development using transfection experiments and we are planning transgenic experiments.

STUDIES OF EPIGENTICS USING FISSION YEAST AS A MODEL SYSTEM

Pernilla Bjerling

A lot of what is known today about epigenetics stems from studies in fission yeast, *Schizosaccharomyces pombe*. Epigenetics are changes in the genome function that does not occur in the DNA sequence and that can be inherited to daughter cells and sometimes to the offspring. Epigenetic changes in the genome is believed to be of major contribution in several diseases like cancer, diabetes type II and obesity. Still, very little is known about how to reverse disease-causing changes in the epigenome. One mechanism of epigenetics is the formation of different types of chromatin. The basic unit of chromatin is the nucleosome, consisting of a core of histone proteins that the DNA is wrapped around. The histones have different types of modifications that influence gene expression. Another, not so well studied mechanism of epigenetics concerns the influence of subnuclear localisation of the chromatin/expression status of a locus/gene. By using fission yeast as a model system we can learn more about what determines the epigenome and how switches between different types of chromatin can occur. We mainly do our localisation studies by live analysis using real-time fluorescence microscopy. A region of interest in the genome is labelled with Green Fluorescent Protein (GFP). GFP is fused to the *E. coli* Lac Repressor protein (Lac-R) and expressed ectopically in the cell where tandem repeats of the *lacO* sequence has been inserted into a specific locus of the chromosome via homologous recombination. In addition, in these cells, the nuclear membrane (NM) and the Spindle Pole Body (SPB) are labelled with other fluorochromes.

Members of the group during 2008

Jenny Alfredsson-Timmins, PhD student
Pernilla Bjerling, assistant professor
Carolina Kristell, PhD student

Project students during 2008

Mia Billquist, undergraduate student
Johan Lindqvist, undergraduate student

Publications from 2004 to 2008

1. Bjerling P., Ekwall K., Egel R., and Thon G. (2004) A novel type of silencing factor, Clr2, is necessary for transcriptional silencing at various chromosomal locations in the fission yeast *Schizosaccharomyces pombe* NAR 32: 4421-4428
2. Walfridsson J., Bjerling P., Thalen M., Yoo E. J., Park S. D., and Ekwall K. (2005) CHD remodelling factor stimulates CENP-A loading to centromeres NAR 33: 2868-2879.
3. Alfredsson-Timmins J., Henningson F., and Bjerling P. (2007) The Clr4 methyltransferase determines the subnuclear localization of the mating-type region in fission yeast. JCS 120:1935-1943

4. Alfredsson-Timmins J., Kristell C., Henningson F., Lyckman S. and Bjerling P. (2009) Reorganization of chromatin is an early response to nitrogen starvation in *Schizosaccharomyces pombe*. Chromosoma 118: 99-112

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ORGANISATION OF THE MATING-TYPE CHROMATIN

Jenny Alfredsson-Timmins

In *S. pombe* there are three main regions where a special form of transcriptionally repressed chromatin, named heterochromatin, is formed; the pericentromeric region, the subtelomeric region and in the mating-type region. The molecular characteristics of heterochromatin is conserved between fission yeast and human with low acetylation levels of the histones and methylation of lysine 9 at histone H3 (H3AcK9) by histone methyltransferase Clr4. The H3AcK9 modification creates a binding site for chromodomain proteins, HP1 in human, and Swi6, Chp1 and Chp2 in *S. pombe*. The three centromeres localise at the nuclear membrane by attaching to the spindle pole body (SPB), while the telomeres are found in two to four clusters at the NM at the opposite side of the nucleus where the nucleolus is found. In addition, in a wild-type strain the mating-type region is positioned in proximity of the SPB together with the centromeres. This localisation is dependent on Clr4 and partially dependent on *cis*-acting boundary elements (Alfredsson-Timmins et al. 2007). Since we found that Clr4 is essential for correct localisation we are currently investigating which of the chromodomain proteins are needed for correct localisation. Our preliminary data suggests that boundary elements together with chromodomain proteins in balanced dosage and composition cooperate in organising the mating-type chromatin.

LOCALISATION OF GENES INDUCED DURING NITROGEN STARVATION

Jenny Alfredsson-Timmins and Carolina Kristell

The aim of this project is to investigate in detail the induction of highly regulated genes. A previous study identified two clusters of genes upregulated after one hour of nitrogen starvation using DNA-microarray technology. We have labelled these loci using the *lacO*/LacR-GFP strategy. During normal growth conditions the gene clusters localize to the nuclear periphery at the opposite side of the nucleus as compared to the spindle pole body (SPB). This constrained localization is dependent on the histone deacetylase Clr3, known to transcriptionally repress genes in these clusters. Already 20 minutes after nitrogen depletion drastic changes in subnuclear localization of the two loci are observed, away from the NM towards the nuclear interior. At least for one of the clusters the movement is clearly transcription dependent (Alfredsson-Timmins et al. 2008). By expression profiling we have identified 118 genes that are upregulated after 20 minutes of nitrogen starvation. Furthermore, we have done genome-wide Chromatin Immunoprecipitation (ChIP) CHIP assays to detect changes in nucleosome occupancy and histone acetylation levels using tiling microarrays with 20 bp resolution from Affymetrix. We are currently in the process of identifying the

transcription factors and chromatin remodelling enzymes responsible for the gene activation by a candidate approach. When these factors are identified we will return to our GFP labelled strains and investigate which factors are needed for the nitrogen depletion induced change in localisation of the genes.

FUNCTION OF SILENCING FACTOR CLR2

Carolina Kristell

Cryptic loci regulator 2 (Clr2) was identified in a screen for mutants alleviating transcriptional silencing in the mating-type region. Clr2 is a general silencing factor in *S. pombe*. In addition, a strain lacking Clr2 has elevated levels of histone acetylation in the mating-type region (Bjerling et a. 2004). Recently Clr2 was purified as a part of the SHREC complex together with Clr1, a zinc-finger protein with uncharacterised molecular functions, Clr3 a histone deacetylase and Mit1, a nucleosome-remodelling factor. The binding sites throughout the genome of SHREC were determined by ChIP CHIP analysis. However, the molecular function of Clr2 is unknown. We are currently doing a deletion analysis study of the Clr2 protein in order to understand its function in heterochromatin formation.

CANINE DISEASE GENE MAPPING AND FUNCTIONAL GENOMICS

Kerstin Lindblad-Toh

The unique breeding history of the domestic dog offers an unparalleled opportunity to explore the genetic basis of both disease and other traits. Since domestication ~30,000 years ago, humans have selectively bred dogs for specific attributes, a process magnified by the strict breed standards in recent centuries. This history has created a genome structure that makes disease gene mapping particularly easy in dogs. We have developed a powerful strategy, with initial genome wide mapping in a single breed and subsequent fine-mapping in multiple breeds, as well as generated and validated a canine SNP array for genome-wide association. To more easily identify actual mutations – many of which will be regulatory in nature – in both canine and human patients we are working on strategies to effectively sequence targeted regions of a genome using next generation sequencing technology. Once a disease gene and mutation has been identified we will further study its function and examine the corresponding gene in human patients. In 2008, we published the genes for canine ectodermal dysplasia in Chinese-crested dogs and cone-rod dystrophy in Dachshounds. In addition, genome-wide association scans from several cancers, autoimmune diseases and neurological traits are promising, and we expect to find the mutations in several of these and also examine human patients in the next year.

Members of the group during 2008

Kerstin Lindblad-Toh, guest professor “
Mia Olsson, graduate student
Michael C Zody, graduate student “
Snaevar Sigurdsson, post doc (started in February)
Katarina Tengvall, graduate student (started in March)
Katarina Truve, graduate student *
Patricio Rivera, graduate student *
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International exchange during 2008

Kerstin Lindblad-Toh (Broad Institute)
Michael C Zody (Broad Institute)
Katarina Tengvall (visit to the Broad Institute)
Mia Olsson (visit to the Broad Institute)
Patricio Rivera (visit to the Broad Institute)
Elinor Karlsson, (visit from Broad Institute)
Noriko Tonomura (visit from Broad Institute)
Marco Galaverni (visit from Italy)

Publications from 2004 to 2008

1. Jaillon, O., Aury, J.M., Brunet, F., Petit, J.L., Stange-Thomann, N., Mauceli, E., Bouneau, L., Fischer, C., Ozouf-Costaz, C., Bernot, A., Nicaud, S., Jaffe, D., Fisher, S., Lutfalla, G., Dossat, C., Segurens, B., Dasilva, C., Salanoubat, M., Levy, M., Boudet, N., Castellano, S., Anthouard, V., Jubin, C., Castelli, V., Katinka, M., Vacherie, B., Biemont, C., Skalli, Z., Cattolico, L., Poulain, J., De Berardinis, V., Cruaud, C., Duprat, S., Brottier, P., Coutanceau, J.P., Gouzy, J., Parra, G., Lardier, G., Chapple, C., McKernan, K.J., McEwan, P., Bosak, S., Kellis, M., Volff, J.N., Guigo, R., Zody, M.C., Mesirov, J., Lindblad-Toh, K., Birren, B., Nusbaum, C., Kahn, D., Robinson-Rechavi, M., Laudet, V., Schachter, V., Quetier, F., Saurin, W., Scarpelli, C., Wincker, P., Lander, E.S., Weissenbach, J., Roest Crollius, H. (2004) Genome duplication in the teleost fish Tetraodon nigroviridis reveals the early vertebrate proto-karyotype. Nature 431(7011):946-57.
2. International Human Genome Sequencing Consortium. (2004) Finishing the euchromatic sequence of the human genome. Nature 431:931-45.
3. Lindblad-Toh, K (2004) Genome sequencing: three's company. Nature 428:475-6.
4. Bernstein, BE, Kamal, M, Lindblad-Toh, K, Bekiranov, S, Kampa, D, Huebert, DJ, McMahon, S, Kulbokas, EJ, Gingeras, TR, Schreiber, SL, and Lander, ES. (2005) High-resolution maps of human chromatin and comparison with mouse. Cell 120:169-181.
5. Xie, Xiaohui, Lu, Jun, Kulbokas, EJ, Golub, TR, Lindblad-Toh, K, Lander, ES, and Kellis, M, and. (2005) Systematic identification of regulatory elements in the human genome by comparison of multiple mammalian species. Nature. 434:338-45
6. Margulies, EH, Vinson, J, NISC Comparative Sequencing Program, Miller, W, Jaffe, JL Chang, DB, Lindblad-Toh, K, Green, ED, Lander, ES, Mullikin, JC, and Clamp, M. (2005) An Initial Strategy for the Systematic Identification of Functional Elements in the Human Genome by Low-Redundancy Comparative Sequencing. PNAS 102: 4795-4800
7. Chimpanzee genome sequencing consortium. Initial comparison of the chimpanzee and human genomes. (2005) Nature 437:69-87.
8. Jaime F. Modiano, Matthew Breen, Robert C. Burnett, Heidi G. Parker, Seidu Inusah, Rachael Thomas, Paul R. Avery, Kerstin Lindblad-Toh, Elaine A. Ostrander, Gary C. Cutter, Anne C. Avery2 (2005) Distinct B and T Cell Lymphoproliferative Disease Prevalence among Dog Breeds Indicates Heritable Risk (2005) Cancer Res. 65:5654-61
9. Hitte C, Jennifer Madeoy2, Ewen F. Kirkness3, Catherine Priat1, Travis D. Lorentzen2, Fabrice Senger1, Dan Thomas4, Thomas Derrien1, Christina Ramirez2, Carol Scott4, Gwenaelle Evanno1, Barbara Pullar2, Edouard Cadieu1, Vinay Oza2, Kristelle Lougant1, David B. Jaffe5, Sandrine Tacher1 , Stéphane Dréano1, Nadia Berkova1, Catherine Andre1, Panagiotis Deloukas4, Claire Fraser3, Kerstin Lindblad-Toh5, Elaine A. Ostrander2,6, and Francis Galibert1 Facilitating genome navigation: survey sequencing and dense radiation-hybrid gene mapping. (2005) Nat Rev Genet. 6:643-8
10. Rachael Thomas, Allyson Scott, Cordelia F. Langford, Susan P. Fosmire, Cristan M. Jubala, Travis D. Lorentzen, Christophe Hitte, Elinor K. Karlsson, Ewen Kirkness, Elaine A. Ostrander, Francis Galibert, Kerstin Lindblad-Toh, Jaime F. Modiano and Matthew Breen Construction of a 2Mb resolution BAC-microarray for CGH analysis of canine tumors. Running title: A 2Mb canine CGH microarray (2005) Genome Research 15:1831-7.

11. Quignon P, Giraud M, Rimbault M, Lavigne P, Tacher S, Morin E, Retout E, Valin AS, Lindblad-Toh K, Nicolas J, Galibert F. Related Articles, Links Free in PMC The dog and rat olfactory receptor repertoires. (2005) Genome Biol. 6:R83
12. Lindblad-Toh, Kerstin, et al. Genome Sequence, Comparative Analysis and haplotype structure of the domestic dog. (2005) Nature 438:803-819
13. Pedersen JS, Bejerano G, Siepel A, Rosenbloom K, Lindblad-Toh K, Lander ES, Kent J, Miller W, Haussler D. Related Articles, Links Free in PMC Identification and Classification of Conserved RNA Secondary Structures in the Human Genome. (2006) PLoS Comput Biol. 2:e33
14. Opossum Genome Sequencing Consortium Lindblad-Toh, K (senior authors) (2007) Genome of the marsupial Monodelphis domestica reveals lineage-specific innovation in coding and non-coding sequences. Nature. 447(7141): 167-77.
15. Baker ML, Indiviglio S, Nyberg AM, Rosenberg GH, Lindblad-Toh K, Miller RD, Papenfuss AT (2007) Analysis of a set of Australian northern brown bandicoot expressed sequence tags with comparison to the genome sequence of the South American grey short tailed opossum. BMC Genomics 8:50
16. Duke SE, Samollow PB, Mauceli E, Lindblad-Toh K, Breen M. (2007) Integrated cytogenetic BAC map of the genome of the gray, short-tailed opossum, Monodelphis domestica. J Vet Intern Med. 21(1): 199-203
17. Parra ZE, Baker ML, Schwarz RS, Deakin JE, Lindblad-Toh K, Miller RD. (2007) A unique T cell receptor discovered in marsupials. A unique T cell receptor discovered in marsupials. Proc Natl Acad Sci USA 104(23): 9776-81
18. ENCODE Project Consortium (2007) Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature. 447(7141): 799-816
19. Margulies EH, Cooper GM, Asimenos G, Thomas DJ, Dewey CN, Siepel A, Birney E, Keefe D, Schwartz AS, Hou M, Taylor J, Nikolaev S, Montoya-Burgos JI, Loytynoja A, Whelan S, Pardi F, Massingham T, Brown JB, Bickel P, Holmes I, Mullikin JC, Ureta-Vidal A, Paten B, Stone EA, Rosenbloom KR, Kent WJ, Bouffard GG, Guan X, Hansen NF, Idol JR, Maduro VV, Maskeri B, McDowell JC, Park M, Thomas PJ, Young AC, Blakesley RW, Muzny DM, Sodergren E, Wheeler DA, Worley KC, Jiang H, Weinstock GM, Gibbs RA, Graves T, Fulton R, Mardis ER, Wilson RK, Clamp M, Cuff J, Gnerre S, Jaffe DB, Chang JL, Lindblad-Toh K, Lander ES, Hinrichs A, Trumbower H, Clawson H, Zweig A, Kuhn RM, Barber G, Harte R, Karolchik D, Field MA, Moore RA, Matthewson CA, Schein JE, Marra MA, Antonarakis SE, Batzoglou S, Goldman N, Hardison R, Haussler D, Miller W, Pachter L, Green ED, Sidow A. (2007) Analyses of deep mammalian sequence alignments and constraint predictions for 1% of the human genome. Genome Res. 6:760-74
20. Astrof S, Kirby A, Lindblad-Toh K, Daly M, Hynes RO (2007) Heart development in fibronectin-null mice is governed by a genetic modifier on chromosome 4. Mech Dev 124:551-8.
21. Xie X, Mikkelsen TS, Gnirke A, Kellis M, Lindblad-Toh K, and Lander ES. (2007) A genome-wide catalog of conserved regulatory motifs in the human genome reveals thousands of insulator sequences Proc Natl Acad Sci U S A. 104:7145-50
22. Karlsson EK Baranowska I Wade C Hillbertz NHC, Zody MC, Andersson N, Biagi B, Patterson N, Kulbokas EJIII, Comstock K, Keller E, von Euler H, Kämpe O, Hedhammar A, S. Lander ES, Andersson G, Andersson L & Lindblad-Toh (2007) Genome-wide association mapping in dogs- a powerful approach for gene discovery Nature Genetics 39:1321-8.

23. Hillbertz, N.H., Isaksson M. Karlsson E, Hellmen E, Rosenberg Pielberg G., Savolainen P, Wade C., von Euler H., Gustafsson U. Hedhammar Å, Milsson M, Lindblad-Toh K., Andersson, L. Andersson, G. (2007) A duplication of FGF3, FGF4, FGF9 and ORAOV1 causes dorsal hair ridge and predisposes to dermoid sinus in ridgeback dogs. Nature Genetics 39:1318-20.
24. Drosophila Genome Sequencing Consortium (2007) Evolution of genes and genomes on the Drosophila phylogeny. Nature. 450:203-18.
25. Miller W, Rosenbloom K, Hardison RC, Hou M, Taylor J, Raney B, Burhans R, King DC, Baertsch R, Blankenberg D, Kosakovsky Pond SL, Nekrutenko A, Giardine B, Tyekucheva S, Diekhans M, Pringle TH, Murphy WJ, Lesk A, Weinstock GM, Lindblad-Toh K, Gibbs RA, Lander ES, Siepel A, Haussler D, Kent WJ. (2007) 28-Way Vertebrate Alignment and Conservation Track in the UCSC Genome Browser. Genome Res 17(12):1797-808
26. Pontius JU, Mullikin JC, Smith DR; Agencourt Sequencing Team, Lindblad-Toh K, Gnerre S, Clamp M, Chang J, Stephens R, Neelam B, Volfovsky N, Schäffer AA, Agarwala R, Narfström K, Murphy WJ, Giger U, Roca AL, Antunes A, Menotti-Raymond M, Yuhki N, Pecon-Slattery J, Johnson WE, Bourque G, Tesler G; NISC Comparative Sequencing Program, O'Brien SJ. (2007) Initial sequence and comparative analysis of the cat genome. Genome Res. 17:1675-89.
27. Menotti-Raymond M, David VA, Pflueger SM, Lindblad-Toh K, Wade CM, O'Brien SJ, Johnson WE. (2008) Patterns of molecular genetic variation among cat breeds. Genomics. Genomics. 91:1-11
28. Clamp M, Fry B, Kamal M, Xie X, Cuff J, Lin MF, Kellis M, Lindblad-Toh K, Lander ES. (2007) Distinguishing protein-coding and noncoding genes in the human genome. Proc Natl Acad Sci U S A. 104:19428-33
29. Xie X, Mikkelsen TS, Gnirke A, Kellis M, Lindblad-Toh K, and Lander ES. (2007) A genome-wide catalog of conserved regulatory motifs in the human genome reveals thousands of insulator sequences Proc Natl Acad Sci U S A 104:7145-50.
30. van den Berg L, Vos-Loohuis M, Schilder MB, van Oost BA, Hazewinkel HA, Wade CM, Karlsson EK, Lindblad-Toh K, Liinamo AE, Leegwater PA. (2008) Evaluation of the Serotonergic Genes htr1A, htr1B, htr2A, and slc6A4 in Aggressive Behavior of Golden Retriever Dogs. Behav Genet. 38:55-66
31. Wiik AC, Wade C, Biagi T, Ropstad E-O, Bjerkås E, Lindblad-Toh K^2, and Lingaas F. (2008) A mutation for recessive cone-rod dystrophy in standard wire haired dachshund. Genome Research 18: 1415 – 1421.
32. Drogemüller C, Karlsson EK, Hytönen HK, Perloski M, Dolf G, Sainio K, Lohi H, Lindblad-Toh K & Leeb T. (2008) Identification of the canine hairless mutation reveals an essential role for *FOXI3* in ectodermal development Science 321:1462.
33. Rosengren Pielberg G, Golovko A, Sundström E, Curik I, Lennartsson J, Seltenhammer MH, Druml T, Binns M, Fitzsimmons C, Lindgren G, Sandberg K, Baumung R, Vetterlein M, Strömberg S, Grabherr M, Wade C, Lindblad-Toh K, Pontén F, Heldin C-H, Sölkner J & Andersson L (2008) A cis-acting regulatory mutation causes premature hair greying and susceptibility to melanoma in the horse Nat Genet 40:1004-9
34. Miller W, Drautz D, Ratan A, Pusey B, Lesk A, Tomsho L, Packard M, Gilbert T, Sher A, Raney B, Lindblad-Toh K, Lander E, Knight J, Irzyk GP, Perbost CS, Fredrikson KM, Harkins TT, Sheridan S, Pringle T & Schuster SC. (2008) Sequencing the nuclar genome of the extinct wolly mammoth. Nature 456: 456(7220):387-90
35. Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, Sougnez C, Greulich H, Muzny DM, Morgan MB, Fulton L, Fulton RS, Zhang Q, Wendl MC,

Lawrence MS, Larson DE, Chen K, Dooling DJ, Sabo A, Hawes AC, Shen H, Jhangiani SN, Lewis LR, Hall O, Zhu Y, Mathew T, Ren Y, Yao J, Scherer SE, Clerc K, Metcalf GA, Ng B, Milosavljevic A, Gonzalez-Garay ML, Osborne JR, Meyer R, Shi X, Tang Y, Koboldt DC, Lin L, Abbott R, Miner TL, Pohl C, Fewell G, Haipek C, Schmidt H, Dunford-Shore BH, Kraja A, Crosby SD, Sawyer CS, Vickery T, Sander S, Robinson J, Winckler W, Baldwin J, Chirieac LR, Dutt A, Fennell T, Hanna M, Johnson BE, Onofrio RC, Thomas RK, Tonon G, Weir BA, Zhao X, Ziaugra L, Zody MC, Giordano T, Orringer MB, Roth JA, Spitz MR, Wistuba II, Ozenberger B, Good PJ, Chang AC, Beer DG, Watson MA, Ladanyi M, Broderick S, Yoshizawa A, Travis WD, Pao W, Province MA, Weinstock GM, Varmus HE, Gabriel SB, Lander ES, Gibbs RA, Meyerson M, Wilson RK. Somatic mutations affect key pathways in lung adenocarcinoma. *Nature*. 2008 Oct 23;455(7216):1069-75.

36. McCarroll SA, Huett A, Kuballa P, Chilewski SD, Landry A, Goyette P, Zody MC, Hall JL, Brant SR, Cho JH, Duerr RH, Silverberg MS, Taylor KD, Rioux JD, Altshuler D, Daly MJ, Xavier RJ. Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. *Nat Genet*. 2008 Sep;40:1107-12.
37. Zody MC, Jiang Z, Fung HC, Antonacci F, Hillier LW, Cardone MF, Graves TA, Kidd JM, Cheng Z, Abouelleil A, Chen L, Wallis J, Glasscock J, Wilson RK, Reily AD, Duckworth J, Ventura M, Hardy J, Warren WC, Eichler EE. Evolutionary toggling of the MAPT 17q21.31 inversion region. *Nat Genet*. 2008 Sep;40:1076-83.
38. Dutt A, Salvesen HB, Chen TH, Ramos AH, Onofrio RC, Hatton C, Nicoletti R, Winckler W, Grewal R, Hanna M, Wyhs N, Ziaugra L, Richter DJ, Trovik J, Engelsen IB, Stefansson IM, Fennell T, Cibulskis K, Zody MC, Akslen LA, Gabriel S, Wong KK, Sellers WR, Meyerson M, Greulich H. Drug-sensitive FGFR2 mutations in endometrial carcinoma. *Proc Natl Acad Sci U S A*. 2008 Jun 24;105(25):8713-7.
39. Thomas R, Duke SE, Karlsson EK, Evans A, Ellis P, Lindblad-Toh K, Langford CF, Breen M. (2008) A genome assembly-integrated dog 1 Mb BAC microarray: a cytogenetic resource for canine cancer studies and comparative genomic analysis. Cytogenet Genome Res. 122:110-21

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GENETIC MAPPING TO FIND GENES RESPONSIBLE FOR AUTOIMMUNE DISEASE IN DOGS

Mia Olsson

Autoimmunity occurs when the immune system reacts against the individual's own tissue often resulting in damage and eventual dysfunction of target organs. Autoimmune diseases are often caused by a combination of multiple genes and environmental factors including

viruses or parasites. Many disorders are lethal if left untreated and most treatment options today involve replacement therapy and/or immuno-suppression. Many autoimmune disorders in humans also occur frequently in dogs, for instance Familial Fever syndromes, diabetes mellitus, Addison disease and hyperthyroidism. This project aims to identify genetic risk factors for several autoimmune diseases in the dog. Exocrine pancreas insufficiency (EPI) is a progressive loss of the pancreatic acinar cells that normally produce enzymes needed for the digestion of food. The disorder also occurs in humans but as a secondary condition to other underlying diseases such as cystic fibrosis and Shwamann-Diamond syndrome. In dogs EPI appears with a significantly increased prevalence in German shepherd, Eurasians and Rough coated Collie. Genome-wide association mapping is being undertaken in one breed and will be followed by fine mapping in all three breed. Shar-Pei Fever a disease characterized by unexplained fever episodes, swollen hocks and arthritis has been postulated as a good model for human Familial Periodic Fever syndromes. Genome-wide association mapping in ~40 cases and ~40 controls from the Shar-Pei breed has identified two candidate loci containing only a few genes. Fine-mapping and mutation detection is ongoing.

IDENTIFYING GENES PREDISPOSING TO IMMUNE-MEDIATED DISEASES IN DOGS

Katarina Tengvall

Autoimmunity occurs when the central immunological tolerance is broken and the immune system fails to recognise its own tissue as self. Addison's disease (hypoadrenocorticism) is an organ-specific autoimmune disease and is generally caused by an immune-mediated destruction of the adrenal cortex tissue leading to glucocorticosteroid (e.g. cortisol) and mineralcorticoid (e.g. aldosterone) deficiencies. The diagnosis is defined by an ACTH stimulation test where artificial ACTH is injected and the cortisol levels in the sera are measured before and after stimulation. This disease affects both humans and dogs and is caused by many genes and most likely influenced by environmental factors. In dogs, Standard Poodles, Bearded collies and Portuguese Waterdogs are predisposed for getting the disease. We aim to identify the genetic risk factors in these breeds. Whole genome association mapping has been conducted in 50 cases and 50 controls of Standard Poodles. Several loci have shown association and fine-mapping is ongoing. Another disease, Canine Atopic Dermatitis is defined as an allergic skin disease. The characteristic clinical features are most commonly associated with IgE antibodies directed towards environmental allergens. Numerous environmental allergens such as dust and storage mite antigens, house dust, grass, tree and weed pollens, mould spores have shown to be involved. Typical signs for a dog with atopic dermatitis are pruritus of the face, ears, paws, extremities, and/or ventrum. High-risk breeds include for example Bullterriers, West Highland white terriers, Boxers and German Shepherds. We aim to perform a Whole genome association analysis on 100 cases and 100 controls of German Shepherds.

INVESTIGATION OF MECHANISMS OF MUTATION AND SELECTION BY COMPARATIVE SEQUENCING

Michael C. Zody

Genomes evolve by a combination of two forces: mutation and selection. The availability of both genomic reference sequence of multiple species and variation data within species allows us to study fundamental aspects of both of these forces. This thesis project aims to study a number of diverse cases including: 1) The relationship between duplication and rearrangement in the primate lineage, by developing a history of structural changes on human chromosomes 15 and 17 and inferring a model of their relationship to duplication. This model can be extended to examine currently variable regions (copy number variants and polymorphic inversions) within human populations. 2) Identification of regions in the human genome under recent positive selection by comparison of human diversity data to sequence information from the chimpanzee genome. If possible, we will assign function to these regions. 3) Search the chicken genome for signatures of recent selection in domesticated lines by comparison of variation and copy number to the genome of a red jungle fowl. If possible, we will associate regions under selection with specific phenotypes that are divergent between domesticated and wild chickens or between different domesticated populations.

IDENTIFYING OSTEOSARCOMA GENES IN DOGS AND HUMANS

Snaevar Sigurdsson

Osteosarcoma (OSA) is the most common form of bone cancer in humans, and the sixth most common type of cancer in children. Most of the cases involve the long bone of the leg and usually affect teenagers. The cause of OSA is unknown, and an understanding of the underlying genetics would enable better treatment.

In dogs the OSA characteristics such as progression, histology and tumor expression patterns are similar to human patients. Large dog breeds are at a much higher risk for this disease, suggesting that inherited risk factors are involved. In a genome wide association study we have recently identified several regions of the dog genome associated with an increased risk for OSA in Rottweilers and Greyhounds, and fine-mapping data from ten other breeds support these findings. The aim of this study is to identify the actual genes and mutations causing the increased risk for OSA in dogs and test if the same genes confer risk to OSA in humans.

To identify candidate OSA mutations we will develop a sequence capture technology to enable selection of megabase sized candidate regions, before sequencing of these on the next generation sequencing instruments.

The identified genes will be screened for common and rare variants in human patients. Candidate mutations will be tested using molecular biological methods to determine their function. This work could lead to the development of genetic tests for OSA and perhaps more importantly provide novel drug targets to improve treatments.

FUNCTIONAL GENOMICS IN MODEL ORGANISMS

Hans Ronne

We are using two small model organisms, the budding yeast *Saccharomyces cerevisiae* and the moss *Physcomitrella patens,* to study basic cellular processes in eukaryotes. We have three main projects. The first project deals with the control of gene expression in eukaryotes. Within this project, we are studying the yeast zinc finger protein Gis1 and the jmjC protein domain, which was recently found to be a histone demethylase. Our second project uses functional genomics in yeast to study the uptake and mechanisms of action of anticancer drugs. Our third project uses the new model plant *Physcomitrella* (muddermossa) to study how the primary carbon and energy metabolism is regulated in plants.

Members of the group during 2008

Mattias Carlsson, graduate student
Marie Gustavsson, graduate student
Monika Johansson, postdoc
Jenni Hammargren, postdoc (from May)
Guo-Zhen Hu, research engineer
Eva Murén, research engineer
Anders Nilsson, graduate student
Niklas Nordberg, graduate student
Hans Ronne, professor
Susanna Tronnersjö, postdoc (to February)
Mikael Ulfstedt, graduate student

Project workers during 2008

Kerstin Naeslund

Publications from 2004 to 2008

1. Thelander, M., Olsson, T., and Ronne, H. (2004) Snf1-related protein kinase 1 is needed for growth in a normal day-night light cycle. *EMBO J.* 23, 1900–1910
2. Hallberg, M., Polozkov, G., Hu, G.-Z., Beve, J., Gustafsson, C., Ronne, H. and Björklund, S. (2004) Phosphorylation of the yeast Mediator subunit Med2 by the Srb10 cyclin-dependent kinase. *Proc. Natl. Acad. Sci. USA* 101, 3370-3375
3. Bourbon, H.-M., ... , Ronne, H., ..., Kornberg, R.D. [45 authors] (2004) A unified nomenclature for protein subunits of Mediator complexes linking transcriptional regulators to RNA polymerase II. *Mol. Cell* 14, 553-557
4. Thelander, M., Olsson, T. and Ronne, H. (2005) Effect of the energy supply on filamentous growth and development in *Physcomitrella patens*. *J. Exp. Bot.* 56, 653-662
5. Beve, J., Hu, G.-Z., Myers, L. C., Balciunas, D., Werngren, O., Hultenby, K., Wibom, R., Ronne, H. and Gustafsson, C. (2005) The structural and functional role of Med5 in yeast Mediator tail module. *J. Biol. Chem.* 280, 41366-41372

6. Hallberg, M., Hu, G.-Z., Tronnersjö, S., Balciunas, D., Shaikhibrahim, Z., Björklund, S. and Ronne, H. (2006) Functional and physical interactions within the middle domain of yeast Medaitor. *Mol. Genet. Genomics* 276, 197-210
7. Wadskog, I., Forsmark, A., Rossi, G., Konopka, C., Öyen, M., Goksör, M., Ronne, H., Brennwald, P. and Adler, L. (2006) The yeast tumour suppressor homologue Sro7p is required for targeting of the sodium pumping ATPase to the cell surface. *Mol. Biol. Cell* 17, 4988-5003.
8. Tronnersjö, S., Hanefalk, C., Balciunas, D., Hu, G.-Z., Nordberg, N., Murén, E. and Ronne, H. (2007) The jmjN and jmjC domains of the yeast zinc finger protein Gis1 interact with 19 proteins involved in transcription, sumoylation and DNA repair. *Mol. Genet. Genomics* 277, 57-70.
9. Thelander, M., Nilsson, A., Olsson, T., Johansson, M., Girod, P.-A., Schaefer, D., Zrÿd,J.-P. and Ronne, H. (2007) The moss genes *PpSKI1* and *PpSKI2* encode nuclear SnRK1 interacting proteins with homologues in vascular plants. *Plant Mol. Biol.* 64, 559-573.
10. Gustavsson, M. and Ronne, H. (2008) Evidence that tRNA modifying enzymes are important in vivo targets for 5-Fluorouracil in yeast. *RNA* 14, 666-674.
11. Ståhl, U., Stålberg, K., Stymne, S. and Ronne, H. (2008) A family of eukaryotic lysophospholipid acyltransferases with broad specificity. *FEBS Lett.* 582, 305-309.
12. Stålberg, K., Neal, A., Ronne, H. and Ståhl, U. (2008) Identification of a novel glycerophosphocholine acyltransferase activity and a new pathway for phosphatidylcholine biosynthesis in *Saccharomyces cerevisiae*. *J. Lipid Res.* 49, 1794-1806.
13. Gustavsson, M., Barmark, G., Larsson, J., Murén, E. and Ronne, H. (2008) Functional genomics of monensin sensitivity in yeast: implications for post-Golgi transport and vacuolar H^+-ATPase function. *Mol. Genet. Genomics* 280, 233-248.
14. Thelander, M., Nilsson, A. and Ronne, H. (2008) Carbon and energy allocation. In The Moss Physcomitrella patens, Eds. Perroud P.-F., Knight, C. and Cove, D., Blackwell Publishing, Annual Plant Reviews Series (in press)
15. Orzechowski Westholm, J., Xu, F., Ronne, H. and Komorowski, J. (2008) Genome-scale study of the importance of binding site context for transcription factor binding and gene regulation. *BMC Bioinformatics* 9, 484.
16. Orzechowski Westholm, J., Nordberg, N., Murén, E., Ameur, A., Komorowski, J. and Ronne, H. (2008) Combinatorial control of gene expression by the three yeast repressors Mig1, Mig2 and Mig3. BMC Genomics 9, 601.

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CONTROL OF GENE EXPRESSION IN YEAST

Jenni Hammargren, Niklas Nordberg, Susanna Tronnersjö

We are studying the jmjC domain, a highly conserved protein domain which is found in many transcription factors from plant, animals and fungi (Balciunas and Ronne, 2000, TiBS 25, 274-276). We have previously used a 2-hybrid screen to identify several yeast proteins that bind to this domain. They include the SUMO ligase Nfi1 and Nis1, a protein that interacts with Nfi1. We are now using a combination of genetic, molecular genetic and biochemical methods to study the jmjC-interacting proteins and how they contribute to gene regulation by Gis1, a yeast zinc finger protein that contains a jmjC domain. In a collaboration with Jakub Orzechowski Westholm and Jan Komorowski at the Linnaeus Centre for Bioinformatics, we are also using microarray experiments to study the role of the three repressors Mig1, Mig2 and Mig3 in glucose repression, and the role of Gis1 and the related yeast protein Rph1 in the control of gene expression during the transition from logarithmic growth to stationary phase.

FUNCTIONAL GENOMICS IN YEAST

Mattias Carlsson, Marie Gustavsson, Guo-Zhen Hu

We are studying basic cellular processes that are common to all eukaryotes, using the budding yeast *Saccharomyces cerevisiae* as experimental system. The fact that knockout mutants for all yeast genes are available makes it an ideal model system in functional genomics. We are using yeast functional genomics to study the uptake and mechanisms of action of anticancer drugs. Our work is focused on 5-fluorouracil (5-FU), one of the oldest and most widely used anticancer drugs. We have screened a yeast knockout mutant collection for mutants that show increased or decreased sensitivity to 5-FU, and identified 138 5-FU sensitive mutants. Several of the most sensitive mutants are deleted for genes encoding tRNA modification enzymes. These modifications are important for tRNA stability, and our findings therefore suggest that destabilisation of tRNA may contribute to the *in vivo* effects of 5-FU. We are currently investigating the effects of 5-FU on tRNA metabolism and stability using a combination of genetic and biochemical methods. In a separate line of work, we have cloned several genes that confer resistance to 5-FU when overexpressed in yeast. They include the *CPA1* and *CPA2* genes encoding the arginine-specific carbamoyl phosphate synthase. Carbamoyl phosphate synthesis (by a different isozyme) is also the first step in pyrimidine biosynthesis, which suggests that the mechanism of 5-FU resistance in this case is an upregulated uracil synthesis.

THE NEW MODEL PLANT *PHYSCOMITRELLA*

Monika Johansson, Eva Murén, Anders Nilsson, Mikael Ulfstedt

Recent advances in molecular genetics have made the small moss *Physcomitrella* patens (muddermossa) an important plant model organism. First, it is the only land plant in which gene knockouts can be made as easily as in yeast. Second, *Physcomitrella* is haploid, which

means that the effect of knockouts and other recessive mutations are visible without further crosses. Third, *Physcomitrella* is easy to handle since it can be grown on agar plates. Fourth, the *Physcomitrella* genome has recently been sequenced.

Our work focuses on the carbon and energy metabolism. In particular, we are studying the mechanisms that control the balance between anabolic and catabolic processes, and therefore decide if storage compounds are accumulated. A key regulator is the Snf1 kinase, also known as the AMP-activated kinase (AMPK), which has been called the energy gauge of the eukaryotic cell. We have cloned and characterized the two genes encoding this kinase in *Physcomitrella*. Another enzyme that we are studying is hexokinase. Apart from its role in the primary carbon metabolism, hexokinase has been implicated in sugar sensing and signaling. *Physcomitrella* has eleven hexokinase genes. We are characterizing these genes using knockouts to study their functions and fusions to the green fluorescent protein (GFP) to study their intracellular localizations. In a separate project, we are studying plasmid replication in *Physcomitrella*, with the aim of developing a shuttle vector that can be used for overexpression genetics. Finally, we are in the process of resequencing the *Physcomitrella* genome from an ecoptype that differs significantly from the already sequenced ecotype.

GLYCOBIOLOGY

HEPARAN SULFATE - STRUCTURE, BIOSYNTHESIS AND BIOLOGICAL FUNCTIONS

Lena Kjellén, Jin-ping Li, Ulf Lindahl, Dorothe Spillmann

Heparan sulfate (HS, abbreviation used in subsequent abstracts) polysaccharide chains occur covalently bound to proteins (proteoglycans) on surfaces of most cells and in the extracellular matrix. The polysaccharide is composed of alternating units of hexuronic acid [D-glucuronic acid (GlcA) or L-iduronic acid (IdoA)] and glucosamine (GlcN), that are sulfated in various positions. Due to variable sequence and sulfation patterns, HS shows extreme structural variability that appears to be under strict biosynthetic control. Different HS species bind in a specific and selective fashion to proteins and thus affect a multitude of biological phenomena, such as embryonic patterning, growth factor action, inflammatory reactions, tumor metastasis, blood coagulation, microbial adhesion etc. The various HS-oriented groups, described in more detail below, pursue two major lines of research.

Biosynthesis of HS. The process is initiated by the assembly of a $(GlcA\text{-}GlcNAc)_n$ polymer, that subsequently undergoes *N*-deacetylation/*N*-sulfation, C5-epimerization of GlcA to IdoA units, and finally *O*-sulfation in various positions. Several of the enzymes catalyzing these reactions have been cloned in the group. Their structures, substrate specificities, mode of action and concerted regulation are studied. Induced derangements of the biosynthetic process are studied *in vitro* and *in vivo*.

Functions of HS. HS domains corresponding to the minimal saccharide sequences required for interaction with selected proteins (e.g. growth factors/cytokines or their receptors, matrix proteins, cell adhesion molecules, enzymes/inhibitors) are isolated and sequenced using novel techniques. The results are related to mechanisms of HS function (for instance, in growth factor activation) and may provide the basis for generating new drugs.

FUNCTIONAL STUDIES OF HEPARAN SULFATE AND ITS BIOSYNTHESIS

Lena Kjellén

The aim of our research is to understand how specific sulfation patterns are generated during biosynthesis of heparan sulfate (HS) and the functional significance of these patterns. HS is important during embryonic development but also during adult life in situations where growth factors and cytokines influence cells to migrate or proliferate. Cancer is one such condition. Our focus has been on the biosynthesis enzyme glucosaminyl N-deacetylase/N-sulfotransferase, NDST, which has a key role in HS design during biosynthesis in the Golgi compartment. NDST removes acetyl groups from glucosamine residues and replaces them with sulfate groups. These N-sulfate groups are important for further modifications including O-sulfation in various positions and epimerization of glucuronic acid to iduronic acid. Four NDST isoforms, transcribed from four genes, have been identified. The different parts of the project are:

I. Identification of genes important for regulation of HS structure.
II. Posttranslational regulation of HS biosynthesis enzyme activity.
III. In vitro differentiation of embryonic stem cells with defective HS biosynthesis.
IV. HS biosynthesis in zebrafish.

Members of the group during 2008

Pernilla Carlsson, graduate student/postdoc
Anders Dagälv, graduate student
Inger Eriksson, research engineer
Katarina Holmborn, postdoc
Lena Kjellén, professor, group leader

Publications from 2004 to 2008

1. Karlsen, T.V., Iversen, V.V., Forsberg, E., Kjellén, L., Reed, R.K. & Gjerde, EA. (2004) ”Neurogenic inflammation in mice deficient in heparin synthesizing enzyme” *Am. J. Physiol. Heart Circ. Physiol*. 286, H884-H888
2. Holmborn, K.*, Ledin, J.*, Smeds, E., Eriksson, I.,Kusche-Gullberg, M. & Kjellén, L. (2004) "Heparan sulfate synthesized by mouse embryonic stem cells deficient in NDST1 and NDST2 is 6-O-sulfated but contains no N-sulfate groups" *J. Biol. Chem*. 279, 42355-42358 *equal contribution
3. Ledin, J., Staatz, W., Li, J-P, Götte, M., Selleck, S., Kjellén, L. & Spillmann, D. (2004) "Heparan sulfate structure in mice with genetically modified heparan sulfate production" *J. Biol. Chem*. 279, 42732-42741
4. Jakobsson, L., Kreuger, J., Holmborn, K., Lundin, L., Eriksson, I. Kjellén, L. & Claesson-Welsh, L. (2006) ”Heparan sulfate *in trans* potentiates VEGFR-mediated angiogenesis” *Dev. Cell* 10, 625-634

5. Kurup, S., A. Abramsson, J.P. Li, U. Lindahl, L. Kjellen, C. Betsholtz, H. Gerhardt, and D. Spillmann. (2006) “Heparan sulphate requirement in platelet derived growth factor B-mediated pericyte recruitment” *Biochem. Soc. Transact.* 34, 454-455
6. van den Born, J., Pisa, B., Bakker, M.A., Celie, J.W., Straatman, C., Thomas, S., Viberti, G.C., Kjellén, L. & Berden, J.H. (2006) “No change in glomerular heparan sulfate structure in early human and experimental diabetic nephropathy” *J. Biol. Chem*. 281, 29606-29613
7. Ledin, J., Ringvall, M., Thuveson, M., Eriksson, I., Wilén, M., Kusche-Gullberg, M., Forsberg, E. & Kjellén, L. (2006) “Enzymaticaly active N-deacetylase/N-sulfotransferase-2 is present in liver but does not contribute to heparan sulfate N-sulfation” *J. Biol. Chem.* 281, 35727-35734
8. Abramsson, A., Kurup, S., Busse, M., Yamada, S., Lindblom, P., Schallmeiner, E., Stenzel, D., Sauvaget, D., Ledin, J., Ringvall, M., Landegren, U., Kjellén, L., Bondjers, G., Li, J-P, Lindahl, U., Spillmann, D., Betsholtz, C. & Gerhardt, H. (2007) ”Defective N-sulfation of heparan sulfate proteoglycans limits PDGF-BB binding and pericyte recruitment in vascular development” *Genes Dev.* 21, 316-331
9. Busse, M., Feta, A., Presto, J., Wilén, M., Grønning, M., Kjellén, L. & Kusche-Gullberg, M. (2007) ”Contribution of EXT1, EXT2 and EXTL3 to heparan sulfate chain elongtion” *J. Biol. Chem*. 282, 32802-32810
10. Presto, J., Thuvesson, M., Carlsson, P., Busse, M., Wilén, M., Eriksson, I., Kusche-Gullberg, M. & Kjellén, L. (2008) ”Heparan sulfate biosynthesis enzymes EXT1 and EXT2 affect NDST1 expression and heparan sulfate sulfation” *Proc. Natl. Acad. Sci. USA* 105, 4751-4756
11. Carlsson, P., Presto, J., Spillmann, D., Lindahl, U. & Kjellén, L. (2008) ”Heparin/heparan sulfate biosynthesis: Processive formation of N-sulfated domains” *J. Biol. Chem*. 283, 20008-20014
12. Kawamura, H., Li, X., Goishi, K., van Meeteren, L.A., Jakobsson, L., Cébe-Suarez, S., Shimizu, A., Edholm, D., Ballmer-Hofer, K., Kjellén, L**.,** Klagsbrun, M. and Claesson-Welsh, L. (2008) “Neuropilin-1 in regulation of VEGF-induced activation of p38MAPK and endothelial cell organization” *Blood* 112, 3638-3649

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IDENTIFICATION OF GENES IMPORTANT FOR REGULATION OF HS STRUCTURE

Inger Eriksson, Lena Kjellén

A sensitive method using reverse-phase ion-pair HPLC for structural characterization of HS from small tissue samples and cell cultures is used in different model systems to correlate phenotype of organs and cells with HS structure. Current major projects include:

1. Studies on genes important for HS structural regulation in a breast cancer metastasis models (collaboration with Stefan Wiemann and Dorit Arlt, German Cancer Research Center, Heidelberg).
2. Characterization of HS isolated from different organs of brachymorphic mice carrying a mutation in PAPS synthase II, one of the two mammalian enzymes responsible for synthesis of PAPS, the sulfate donor in HS biosynthesis (collaboration with Nancy Schwartz, Chicago).

POSTTRANSLATIONAL REGULATION OF HS BIOSYNTHESIS ENZYME ACTIVITY

Pernilla Carlsson, Inger Eriksson

Our recent results support a GAGosome model where biosynthesis enzymes are assembled into modifying units and the composition of the unit determines the outcome of biosynthesis. We are also exploring the regulatory role of the sulfate donor PAPS. Our results indicate that PAPS binding transforms the NDST enzyme from randomly attacking its substrate to instead work in a processive manner. A splice variant of NDST1 is also being characterized with regard to tissue distribution and enzymatic properties.

IN VITRO DIFFERENTIATION OF EMBRYONIC STEM CELLS WITH DEFECTIVE HS BIOSYNTHESIS

Anders Dagälv, Katarina Holmborn

We have generated mice deficient in NDST1 and NDST2, respectively. While $NDST1^{-/-}$ mice die due to lung failure with a systemic alteration in HS structure and neuronal and skeletal defects, $NDST2^{-/-}$ mice are viable and fertile but contain reduced levels of connective tissue type mast cells lacking sulfated heparin. Double knockouts lacking both NDST1 and NDST2 have also been generated. Embryos with this genotype die early during development. ES-cells from blastocysts lacking one or both NDST isoforms have been established and various in vitro differentiation protocols are now being used to study the ability of the cells to form different cell types. Collaborators in this project include Johan Kreuger, Karin Nilsson, Maud Forsberg, Magnus Åbrink, Lena Claesson-Welsh and Filip Farnebo.

HS BIOSYNTHESIS IN ZEBRAFISH

Pernilla Carlsson

We are in collaboration with Johan Ledin characterizing zebra fish NDSTs with regard to expression and isoform function. Studies on the role of HS in axon guidance in zebrafish are also ongoing (collaboration with Chi-Bin Chien and Kuberan Balagurunathan, Salt Lake City) and chararacterization of HS structure in mutants with altered PAPS metabolism will be initiated.

HEPARAN SULFATE: IMPLICATIONS TO ANIMAL DEVELOPMENT AND PATHOPHYSIOLOGICAL PROCESSES

Jin-ping Li

The research of this group is mainly aimed at elucidating the functional properties of heparan sulfate (HS) during animal development and homeostasis as well as the roles that HS plays in amyloid-related and tumor pathological processes. These goals are approached through investigating the metabolism of HS with focus on two critical enzymes, glucuronyl C5-epimerase that is a key enzyme in HS biosynthesis, and heparanase that is involved in HS degradation. Various techniques, including biochemical, molecular and immunohistological tools, are applied, with focus on mouse models and cell lines that are genetically manipulated.

Members of the group during 2008

John Ancsin PhD, visiting scientist (since June)
Eva Gottfridsson, research engineer
Helena Grundberg, technician (until April)
Eva Hjertson, research engineer
Robert Kisilevsky, visiting Prof. emeritus (2 months, May, Oct)
Juan Jia, MD, graduate student
Jin-ping Li, MD, PhD, group leader
Ulf Lindahl, PhD, professor emeritus
Lars Lundin, Post-doc (part time)
Fredrik Noborn, graduate student
Elina Sandwall, graduate student

Publications from 2004 to 2008

1. Zcharia, E., Metzger, S., Chajek-Shaul, T., Aingorn, H., Elkin, M., Friedmann, Y., Weinstein, T., Li, J.-P., Lindahl, U. and Vlodavsky, I. (2004) Transgenic expression of mammalian heparanase uncovers physiological functions of heparan sulfate in tissue morphogenesis, vascularization and feeding behavior *FASEB. J*. 18, 252-263
2. Hagner-McWhirter, Å., Li, J.-P.*, Oscarson, S. and Lindahl, U. (2004) Irreversible glucuronyl C5-epimerization in the biosynthesis of heparan sulfate *J. Biol. Chem*. 279, 14631-14638
3. Ledin, J., Staatz, W., Li, J.-P., Gotte, M., Selleck, S., Kjellen, L. and Spillmann, D. (2004) Heparanase uptake is mediated by cell membrane heparan sulfate proteoglycans *J. Biol. Chem* 279, 42732 - 42741.
4. Yang, Z., Li, J.-P. and Guan, H.S. (2004) Preparation and characterization of oligomannuronates from alginate degraded by hydrogen peroxide, *Carbohydr. Polymers.* 58, 115-121
5. Li, J.-P.*, Escobar, M., Gong, F, Zhang, X., Zcharia, E., Metzger, S., Vlodavsky, I and Lindahl, U. (2005) In vivo fragmentation of heparan sulfate by heparanase overexpression renders mice resistant to amyloid protein A amyloidosis, *PNAS.* 102:18, 6473-6477

6. Lindahl, U., Li, J.-P., Kusche-Gullberg, M., Salmivirta, M., Alaranta, S., Veromaa, T., Emeis, J., Roberts, I., Taylor, C., Oreste, P., Zoppetti, G., Naggi, A., Torri, G. and Casu, B. (2005) Generation of "neoheparin" from *E. coli* K5 capsular polysaccharide, *J. Med. Chem*. 48:2; 349-352
7. Ma, R., Kanders, E., Sundh, U.B., Geng, M., Ek, P., Zetterqvist, Ö. and Li, J.-P.* (2005) Mutational study of human phosphohistidine phosphatase: Effect on enzymatic activity, *BBRC*, 337, 887-891
8. Nasser, N.J., Sarig, G., Brenner, G., Nevo, E., Goldshmidt, O., Zcharia, E., Li, J.-P. and Vlodavsky, I (2006), Heparanase neutralizes the anticoagulation properties of heparin and low-molecular-weight heparin, *J. Thromb. Haemost.* Mar;4(3):560-565
9. Maccarana, M., Olander, B., Malmström, J., Tiedemann, K., Lindahl, U. Li, J.-P. and Malmström, A. (2006) Biosynthesis of dermatan sulfate. Chondroitin glucuronate C5-epimerase is identical to SART2, *J. Biol. Chem*. 28;281(17):11560-11568
10. Feyerabend, T., Li, J.-P.* ,Lindahl, U. and Rodewald, H.R. (2006) Heparan sulfate C5-epimerase is essential for heparin biosynthesis in mast cells, *Nat. Chem. Biol.* 2(4):195-196
11. Kurup, S., Abramsson, A., Li, J.-P., Lindahl, U., Kjellen, L., Betsholtz, C., Gerhardt, H. and Spillmann, D. (2006). Heparan sulphate requirement in platelet-derived growth factor B-mediated pericyte recruitment, *Biochem Soc Trans.* 34(Pt 3):454-455
12. Kreuger, J., Spillmann, D., Li, J.-P. and Lindahl, U. (2006) Interactions between heparan sulfate and proteins: the concept of specificity, *J. Cell. Biol*. 174(3) 323- 327
13. Abramsson, A., Kurup, S., Busse, M., Yamada, S., Lindblom, P., Schallmeiner, E., Stenzel, D., Sauvaget, D., Ledin, J., Ringvall, M., Landegren, U., Kjellen, L., Bondjers, G., Li, J.-P., Lindahl, U., Spillmann, D., Betsholtz, C. and Gerhardt, H. (2007) Defective N-sulfation of heparan sulfate proteoglycans limits PDGF-BB binding and pericyte recruitment in vascular development, *Genes Dev*. 21(3):316-331
14. Kurup, S., Wijnhoven, TJ., Jenniskens, GJ., Kimata, K., Habuchi, H., Li, J.-P., Lindahl, U., van Kuppevelt ,TH. and Spillmann, D.. (2007) Characterization of anti-heparan sulfate phage display antibodies AO4B08 and HS4E4, *J. Biol. Chem*. 282(29) 21032-21042
15. Yang, Y., Macleod, V., Miao, HQ., Theus, A., Zhan, F., Shaughnessy, JD Jr., Sawyer, J., Li, J.-P., Zcharia, E., Vlodavsky, I. and Sanderson, RD. (2007) Heparanase enhances syndecan-1 shedding: a novel mechanism for stimulation of tumor growth and metastasis. *J. Biol. Chem*. 282(18):13326-13333
16. Escobar Galvis, M., Jia, J., Zhang, X., Jastrebova, N., Spillmann, D., Gottfridsson, E., van Kuppevelt, T., Zcharia, E., Vlodavsky, I., Lindahl, U. and Li, J.-P*. (2007) Transgenic or tumor-induced expression of heparanase upregulates sulfation of heparan sulfate, *Nat. Chem. Biol.* 3(12): 773-778
17. Bode, L., Salvestrini, C., Park, P.W., Li, J.-P., Esko, J.D., Yamaguchi, Y., Murch, S. and Freeze, H.H. (2008) Heparan sulfate and syndecan-1 are essential in maintaining Intestinal Epithelial barrier Function, *J. Clin. Invest.* 118(1): 229-238
18. van den Hoven, MJ., Wijnhoven, TJ., Li, J.-P., Zcharia, E., Dijkman, HB., Wismans, RG., Rops, AL., Lensen, JF., van den Heuvel, LP., van Kuppevelt, TH., Vlodavsky, I., Berden, JH.and van der Vlag, J. (2008) Reduction of anionic sites in the glomerular basement membrane by heparanase does not lead to proteinuria, *Kidney Int.* 73 (3): 278-287
19. Li, J.-P., (2008) Heparin, heparan sulfate and heparanase in cancer: remedy for metastasis? *Anticancer Agents Med Chem*. Jan;8(1):64-76; Review
20. O' Callaghan, P., Sandwall, E., Li, J.-P., Yu, H., Ravid, R., Guan, Z. van Kuppevelt, TH., Nilsson, L., Ingelsson, M., Hyman B.T., Kalimo, H., Lindahl, U., Lannfelt, L. and Zhang, X. (2008) Heparan Sulfate Accumulation with Aβ Deposits in Alzheimer's Disease and Tg2576 Mice is Contributed by Glial Cells; *Brain Pathology* 18(4): 548-561

21. Sandwall, E., Bodevin, S., Nasser, NJ., Nevo, E., Avivi, A., Vlodavsky, I. and Li, J.-P. (2008) "Molecular Structure Of Heparan Sulfate From *Spalax:* Implications Of Heparanase And Hypoxia" *J. Biol. Chem*. Online pub Dec 10.

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IMPLICATIONS OF C5-EPIMERASE IN ANIMAL DEVELOPMENT

Juan Jia, Eva Hjertson

GlcA C5-epimerase catalyzes the conversion of D-GlcA to its C5-epimer, L-IdoA, at the polymer level. The IdoA units are believed to promote binding of HS chains to proteins, due to the marked conformational flexibility of these residues. Therefore, the reaction catalyzed by the C5-epimerase is crucial for many biological functions of HS.

Targeted disruption of the GlcA C5-epimerase gene in mouse resulted in severely disturbed developmental phenotype, in particular renal agenesis, lung hyperplasia and multiple skeletal malformations. The mutant animals die neonatally, likely due to respiratory failure. Analysis of HS isolated from mutant animals revealed a perturbed structure lacking IdoA residues, but with increased N- and 6-O-sulfation contents. We will continue to study embryonic development of the C5-epimerase-deficient mice in general, with the aim to elucidate the functions of selected morphogens that are critically dependent on HS. In collaboration with experts on renal, lung and skeletal development, we will examine embryos at different developmental stages, with particular regard to expression and function of relevant morphogens in each organ. Further, embryonic cell lines established from the mutant animals will be used as a model to probe the functional effects of various growth factors that depend on binding to HS for activity.

Another approach in this project is to generate a conditional GlcA C5-epimerase knockout mouse to enable selectively inactivate the gene at different organ or cells. The construct is designed to eliminate exon1 and the promoter of the gene by inserting this sequence into a site flanked by two LoxP sequences. One upstream sequence of 5 kb and one down stream sequence of 3 kb of the gene are to be inserted into the construct for homologous integration into the genome.

STRUCTURAL STUDIES OF GLUCURONYL C5-EPIMERASE

Jin-ping Li

This project aims at elucidating the molecular structure of GlcA C5-epimerase. The protein is actively expressed in yeast and the purified recombinant enzyme is used for crystallization. The structural information will enable us to understand the catalytic mechanism of C5-epimerase action in biosynthesis of heparin and HS chains, and the mode of epimerase interaction with other enzymes involved in the biosynthetic process. We plan to co-crystallize the enzyme with

oligosaccharide ligands and define the catalytic site through mutations of critical amino acid residues. The mutant constructs will be expressed in a bacterial system and analyzed with regard to structural and functional properties.

CHARACTERIZATION OF HS AND HEPARANASE IN *SPALAX*

Elina Sandwall

HS appeared early in metazoan evolution. It is thought that many biological functions of HS either emerged early in evolution or have depended on the subsequent evolution of protein ligands that interact with the polysaccharide. Evolutionary change is primarily driven by ecological stress. Subterranean mammals adapted to life underground display mosaic evolution of regression, progression, and global convergence across all organizational levels from molecular to organism. The subterranean ecotype is subjected to severe stress due to darkness, energy requirements, infection load, and hypoxia–hypercapnia. It is known that expression of heparanase in *Spalax,* a blind mole rat, is upregulated, which, along with a high expression of vascular endothelial growth factor (VEGF), may be a consequence of adaptation to the hypoxia-hypercapnia condition. As overexpression of heparanase can result in structural changes in HS, it is of interest to characterize the HS structures from this animal. We analyze the structures of HS from two allospecies captured in Israel, the *Spalax galili* (2*n* 52) from the cool-humid Upper Galilee Mountains and *Spalax Judaei* (2*n* 60) from the warm-dry southern regions. Structural analysis reveled a distinguished pattern of *spalax* HS in comparison with HS from mice, which is likely an evolution adaptation. Further, we will investigate the impact of overexpressing *Spalax* heparanase in cells under hypoxic conditions.

ORGANIZATION OF HEPARAN SULFATE BIOSYNTHESIS

Fredrik Noborn, Eva Gottfridsson, Robert Kisilevsky, Ulf Lindahl

Biosynthesis of HS is a complex process; the action of at least 11 different enzymes results in polysaccharide molecules with a high degree of heterogeneity. As the structure of HS is highly tissue/cell specific, it is our primary interest to find out how the biosynthesis is regulated *de novo*. Approaches to understanding the organization of HS biosynthesis involve characterization of the "gagosome", *i.e.* the complex of (largely) membrane-bound proteins in the Golgi that catalyze formation of HS chains in proteoglycan form. Particular attention will be given to the interactions between enzymes (GlcA C5-epimerase and O-sulfotransferases). The experimental approaches are designed using biochemical and biological systems. For biochemical studies, recombinant enzymes (GlcA C5-epimerase, HexA 2-O-sulfotransferase and GlcN 6-O-sulfotransferase) produced in a bacterial expression system will be applied to modify polysaccharide substrates for investigation of 1) substrate specificity of the individual enzymes; 2) interaction/regulation of the enzymes in their separate or concerted action towards various substrates; 3) kinetics of the enzymatic reactions, again with regard to the course of separate or combined processes. In more complex biological systems, tissues, cells or sub-cellular organelles (in particular Golgi fractions) derived from transgenic mice (GlcA C5-epimerase KO, heparanase KO and heparanase overexpression) will be used for identification of enzyme complexes (the "gagosome"), using various analytical approaches.

SUBSTRATE SPECIFICITY OF HEPARANASE AND HS DEGRADATION

Juan Jia, Elina Sandwall and Eva Gottfridsson

Heparanase is a mammalian endoglycosidase that degrades heparin and HS polymers into shorter fragments, by cleaving ß-D-glucuronidic bonds. Heparanase is expressed in most tissues and cells, and is often upregulated in metastatic tissues. In addition to degradation of HS, hence involvement in remodeling of the extracellular matrix, heparanase may regulate angiogenesis, tissue repair and lipid metabolism by releasing HS-bound growth factors and enzymes such as FGFs and lipoprotein lipase. Inhibition of heparanase has been shown to attenuate tumor growth; therefore, developing inhibitors targeting the heparanase has potential therapeutic significance. Our previous work has shown that the enzyme recognizes a broad spectrum of substrate structures. Continued study by testing recombinant heparanase towards tailored oligosaccharides (chemo-enzymatic synthesis) will define the minimal/essential requirements for heparanase recognition; the resulting information will be important for development of heparanase inhibitors, to be potentially applied in therapy against tumor metastasis.

Another line of research is to study the metabolism of HS. We have recently generated a heparanase gene knockout mouse in which the heparanase activity is eliminated. We will use this mouse model, along with a transgenic mouse strain overexpressing heparanase (see below) and corresponding control animals to investigate HS metabolism by analyzing structure and turnover of HS proteoglycans.

HEPARANASE – IMPLICATIONS TO ALZHEIMER'S DISEASE

Elina Sandwall, Ulf Lindahl

HS is consistently found in amyloid deposits of tissues (liver, spleen, kidney, pancreas, brain) affected in various amyloid diseases, including Alzheimer's disease (AD). The amyloid plaque is a hallmark of AD, and amyloidogenesis is considered to be a key element in the onset and development of AD. However, the role of HS in amyloidogenesis is poorly understood. We have recently generated a mouse strain that overexpresses heparanase, resulting in substantially shorter HS chains in various organs in comparison to the control. We have found these mice to be resistant to experimentally induced systemic AA-amyloidosis. The question is whether overexpression of heparanase can also attenuate the cerebral Aß amyloid deposition in AD. Mice overexpressing mutant APP, a precursor protein of Aß peptide, develop amyloid plaques in the brain with aging, and provide a useful model for studying AD. By crossbreeding mice that overexpress APP and heparanase, respectively, we will obtain a novel transgenic mouse that overexpresses both heparanase and mutant APP. We expect that this mouse model will help elucidate the roles played by HS in Aß-amyloid deposition and elimination. Another approach to address the roles of HS in Aß-pathology is to use cell models that are defect in HS biosynthesis or degradation. The toxicity/deposition of Aß will examined by various biochemical techniques.

HEPARAN SULFATE IN AMYLOIDOSIS

Fredrik Noborn, John Ancsin

"Amyloid" consists of deposits of misfolded proteins. Amyloid can accompany or cause a wide range of diseases and hence amyloidosis refers to a clinical condition encompassing a group of ~20 post-secretory protein misfolding diseases. In these disease states, proteins that are normally soluble undergo aggregation to form various intermediates and amyloidogenic species. These species subsequently assemble to generate insoluble fibrils that accumulate in the extracellular space of affected tissues or organs. A common feature of all amyloids is the selective organ deposition of disease-specific fibrillar proteins along with HSPGs. HS and HSPGs appear not to be merely passive components of amyloid deposits but rather play functional roles in the pathophysiology of amyloidosis. Two types of amyloid diseases that have a broad clinical and social impact are Alzheimer's disease (AD) and type 2 diabetes, others are relatively rare, but severe. This project is a part of EU network (EURAMY), and is aimed at illustrating the roles of HS in transthyretin (TTR) and amyloid A (SAA) amyloidoses. Effects of HS in aggregation of the amyloid peptide will be studied using recombinant TTR and SAA and various biochemical tools. Toxic effects of the amyloids will be examined in cell models defective in or overexpressing one or more HS synthesis/degrading enzymes, e.g. GlcA C5-epimerase or heparanase.

METHOD FOR ANALYSIS OF HEPARANASE IN CLINICAL SAMPLES

Lars Lundin

Expression of heparanase is upregulated in most human tumor tissues, correlating with increased metastatic potential, tumor vascularity and poor postoperative survival of cancer patients. We therefore assume that the enzyme together with HS digestion products are promising tumor markers. Heparanase activity can be detected by analysis of products after incubation of the enzyme with HS substrate that is labeled with radioisotopes or fluorescent tags. However, the sensitivity of currently available methods is low, and the analytical procedures are impractical for clinical application. This project is aimed at improving the current methods with regard to sensitivity and adaptation to analysis of clinical samples, such as urine and blood.

PREPARATION OF GYCOSAMINOGLYCAN-DERIVED OLIGOSACCHARIDE LIBRARIES FOR INTERACTIONS WITH PROTEINS

Helena Grundberg

HS functions essentially through non-covalent interaction with various proteins. Such interactions generally involve only limited sequences, typically 8-20 sugar residues, out of the hundreds of monosaccharide units that usually comprise a HS chain. To identify sequences in HS that 'specifically' interact with cognate protein ligands, we generate oligosaccharide libraries composed of oligosaccharides of appropriate size that are variously modified to cover a large sequence space. To this end, oligosaccharide substrates derived either from desulfated heparin/HS, or from *E. coli* K5 capsular polysaccharide (similar in structure to the precursor polymer in HS biosynthesis) are applied as targets for a variety of recombinant enzymes (GlcA

C5-epimerase, N- and O-sulfotransferases) normally committed to HS biosynthesis. The oligosaccharides are chemically or metabolically radiolabeled (^{3}H, ^{35}S, ^{14}C), and applied to interaction studies with selected protein ligands. Captured oligosaccharides are subjected to compositional and sequence analysis.

GLYCOSAMINOGLYCANS – STICKY TENTACLES OR WELL DESIGNED FUNCTIONAL MODULATORS?

Dorothe Spillmann

The wealth of structures in heparan sulfate (HS), and more recently recognized, chondroitin sulfate (CS), provides ideal binding sites for different physiolocially and pathologically important proteins among which we can find morphogens, growth factors, adhesion proteins and enzymes but also microbial adhesion proteins. Based on findings with the protease inhibitor antithrombin that requires a defined sequence in heparin/HS for high affinity binding, we had anticipated that each HS-binding protein will interact with a well-defined sequence for excertion of its HS-related activities. We focused therefore on the question whether it would be possible to design specifically tailored competitors *e.g.* for microbial binders, if we were able identify distinct preferences of different types of ligands. We wanted to hit the pathological binders while not affecting physiologically relevant HS-interactions for instance in a host. After having studied a large number of HS-binding molecules with diverse functions (such as matrix proteins, metabolically active enzymes, growth factors and microbial adhesion proteins) the answer is rather that there is not as much distinct sequence-specificity as we had expected. Nevertheless, our findings have opened the way to develop anti-adhesion strategies for microbes such as *Plasmodium falciparum*.

If not to define for absolute discrimination between different ligands why then do different cells bother to regulate HS and most likely also CS biosynthesis to produce cell and tissue type specific structures? The answers may lay in the set how extracellular triggers are converted into cellular activities by an intricate network of cellular signaling pathways. Our main focus is therefore to link the (mainly) extracellular world of GAG structures with the control of intracellular activities. Thus our goals are to elucidate the underlying mechanisms how HS and CS structures modulate intracellular signaling and how these carbohydrate chains may influence cellular behavior and communication, of critical importance for physiological and pathological processes.

Members of the group during 2008

Anna Eriksson, graduate student (from Oktober 2008)
Nadja Jastrebova, graduate student (PhD May 2008)
Ulf Lindahl, professor emeritus
Rashmi Ramachandra, graduate student
Dorothe Spillmann, project leader

Publications from 2004 to 2008

1. Vogt, A. M, Winter, G., Wahlgren, M., and Spillmann, D.: Heparan sulphate identified on human erythrocytes: a *Plasmodium falciparum* receptor. *Biochem. J.* 381 (2004) 593-597.
2. Ledin, J., Staatz, W., Li, J-P., Götte, M., Selleck, S., Kjellen, L., and Spillmann, D.: Heparan sulfate structure in mice with genetically modified heparan sulfate production. *J. Biol. Chem.* 279 (2004) 42732-42741.
3. Do, A.T., Smeds, E., Spillmann, D., Kusche-Gullberg, M.: Overexpression of heparan

sulfate 6-*O*-sulfotransferases in human embryonic kidney 293 cells results in increase *N*-acetylglucosaminyl-6-*O*-sulfation. *J. Biol. Chem.* 281 (2006) 5348-5356.

4. ten Dam*, G. B., Kurup*, S., van de Westerlo, E. M. Versteeg, E. M., Lindahl, U., Spillmann, D. van Kuppevelt, T. H.: 3-*O*-sulfated oligosaccharide structures are recognized by anti-heparan sulfate antibody HS4C3. *J. Biol. Chem.* 281 (2006) 4654-4662.
5. Vanwildemeersch*, M., Olsson*, A.K., Gottfridsson, E., Claesson-Welsh, L., Lindahl, U., Spillmann, D.: The anti-angiogenic His/Pro-rich fragment of histidine-rich glycoprotein binds to endothelial cell heparan sulfate in a Zn^{2+}-dependent manner. *J. Biol. Chem.* 281 (2006)10298-10304.
6. Kurup, S., Abramsson, A., Li, J.-p., Lindahl, U., Kjellén, L., Betsholtz, C., Gerhardt, H., Spillmann, D.: Heparan sulphate requirement in platelet-derived growth factor B-mediated pericyte recruitment. *Biochem. Soc. Transact.* 34 (2006) 454-455.
7. Spillmann, D., Lookene, A., Olivecrona, G.: Isolation and characterization of low sulfated heparan sulfate sequences with affinity for lipoprotein lipase. *J. Biol. Chem.* 281 (2006) 23405-23413.
8. Jastrebova, N., Vanwildemeersch, M., Rapraeger, A. C., Gimenez-Gallego, G., Lindahl, U., Spillmann, D.: Heparan sulfate-related oligosaccharides in ternary complex formation with fibroblast growth factors 1 and 2 and their receptors. *J. Biol. Chem.* 281 (2006) 26884-26892.
9. de Paz, J. L., Spillmann, D., Seeberger, P. H.: Microarrays of heparin oligosaccharides obtained by nitrous acid depolymerization of isolated heparin. *Chem. Commun.* 29 (2006) 3116-3118.
10. Kreuger, J., Spillmann, D., Li, J.P., Lindahl, U.: Interactions between heparan sulfate and proteins: the concept of specificity. *J. Cell Biol.* 174 (2006) 323-327.
11. Vogt, A. M., Pettersson, F., Moll, K., Jonsson, C., Normark, J., Ribacke, U., Egwang, T. G., Ekre, H. P., Spillmann, D., Chen, Q., Wahlgren, M.: Release of sequestered malaria parasites upon injection of a glycosaminoglycan. *PLoS Pathog.* 2 (2006) 853-863.
12. Abramsson*, A., Kurup*, S., Busse, M., Yamada, S., Lindblom, P., Schallmeiner, E. Stenzel, D., Sauvaget, D., Ledin, J., Ringvall, M., Landegren, U., Kjellén, L., Bondjers, G., Li, J.-p., Lindahl, U., Spillmann, D., Betsholtz, C., Gerhardt, H.: Defective *N*-sulfation of heparan sulfate proteoglycans limits PDGF-BB binding and pericyte recruitment in vascular development. *Genes Dev.* 21 (2007) 316-331.
13. Kurup*, S., Wijnhoven*, T. J., Jenniskens, G. J., Kimata, K., Habuchi, H., Li, J.-p., Lindahl, U., van Kuppevelt, T. H., Spillmann, D.: Characterization of anti-heparan sulfate phage display antibodies AO4B08 and HS4E4. *J. Biol. Chem.* 282 (2007) 21032-21042.
14. Knappe, M., Bodevin, S. Selinka, H. C., Spillmann, D., Streeck, R. E., Chen, X. S., Lindahl, U., Sapp, M.: Surface-exposed amino acid residues of HPV16 l1 protein mediating interaction with cell surface heparan sulfate. *J. Biol. Chem.* 282 (2007) 27913-27922.
15. Escobar Galvis, M. L., Jia, J., Zhang, X., Jastrebova, N., Spillmann, D., Gottfridsson, E., van Kuppevelt, T. H., Zcharia, E., Vlodavsky, I., Lindahl, U., Li, J.-p.: Transgenic or tumor-induced expression of heparanase upregulates sulfation of heparan sulfate. *Nat. Chem. Biol.* 3 (2007) 773-778.
16. Götte*, M, Spillmann*, D., Yip*, G. W., Versteeg, E., Echtermeyer, F. G., van Kuppevelt T. H., Kiesel, L.: Changes in heparan sulfate are associated with delayed wound repair, altered cell migration, adhesion and contractility in the

galactosyltransferase I (bGaltT-7) deficient form of Ehlers-Danlos syndrome. *Hum. Mol. Genet.* 17 (2008) 996-1009.
17. Cébe-Suarez*, S., Grünewald*, F. S., Jaussi, R. Li, X., Claesson-Welsh, L., Spillmann, D., Mercer, A. A., Prota, A. E. , Ballmer-Hofer, K.: Orf virus VEGF-E NZ2 promotes paracellular NRP-1/VEGFR-2 coreceptor assembly via the peptide RPPR. *FASEB J* 22 (2008) 3078-3086.
18. Carlsson*, P., Presto*, J., Spillmann, D., Lindahl, U., Kjellén, L.: Heparin/heparan sulfate biosynthesis: processive formation of N-sulfated domains. *J. Biol. Chem.* 283 (2008) 20008-20014. * shared first author

Other publications by Ulf Lindahl:

1. Chai, W., Leteux, C., Westling, C., Lindahl, U., Feizi, T.: Relative susceptibilities of the glucosamine-glucuronic acid and *N*-acetylglucosamine-gluruconic acid linkages to heparin lyase III. *Biochemistry* 43 (2004) 8590-8599.
2. Bodevin-Authelet, S., Kusche-Gullberg, M., Pummill, P. E., DeAngelis, P. L., Lindahl, U.: Biosynthesis of hyaluronan: direction of chain elongation. *J. Biol. Chem.* 280 (2005) 8813-8818.
3. Kreuger, J., Jemth, P., Sanders-Lindberg, E., Eliahu, L., Ron, D., Basilico, C., Salmivirta, M., Lindahl, U.: Fibroblast growth factors share binding sites in heparan sulphate. *Biochem. J.* 389 (2005) 145-150.
4. Ai, X., Do, A. T., Kusche-Gullberg, M., Lindahl, U., Lu, K., Emerson, C. P. Jr.: Substrate specificity and domain functions of extracellular heparan sulfate 6-*O*-endosulfatases, QSulf1 and QSulf2. *J. Biol. Chem.* 281 (2006) 4969-4976.
5. Lindahl, U.: Heparan sulfate-protein interactions - A concept for drug design? *Thromb. Haemost.* 98 (2007) 109-115.
6. Lindahl, U.: Artificial polysaccharide in contaminated heparin. Acute hypersensitivity reactions explained — but how could it happen? *Läkartidningen* 105 (2008) 24-30.

Agencies that support the work

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HOW DOES HS STRUCTURE INFLUENCE A CELL INTRACELLULARLY....?

Rashmi Ramachandra, Anna Eriksson

HS chains at cell surfaces serve a range of different functions that will all affect the cellular phenotype. Our projects have the ambition to dissect this puzzle and get a mechanistic answer why and how these chains influence cellular activities. In the long run we hope to be able to understand why a cell needs to invest energy to control these structures and to develop models how we could rescue pathological situations where these interactions are hampered.

As our starting model system we have chosen chinese hamster ovary (CHO) cells, for which a collection of mutants with defined defects in GAG biosynthesis are available. We stimulate these cells with growth factors, primarily fibroblast growth factors (FGFs). FGF binds to a tyrosine kinase type receptor and an HS-coreceptor at the cell surface to from a ternary complex. The complex gets activated by transphosphorylation of the receptor kinase domain which in turn triggers a number of signaling pathways. We have shown earlier that FGFs bind in a sulfation degree dependent mode to HS and that also the ternary complex formation is dependent on the sulfation degree of the engaged oligosaccharide. Next, we want to study how exactly the characteristics of HS translate into what happens intracellularly. Our working hypothesis is that structural properties in the carbohydrate chains may fine tune the activation kinetics of different pathways. Therefore, we analyse the activation of different pathways as a function of the HS-structures engaged in the complex, the time of stimulation and dose of growth factor added with the help of antibodies against activated intermediates in different cascades. In parallel we look at the fate of the receptor complex using GFP-tagged receptor molecules.

The HS-carriers, different types of HS-proteoglycans (HSPGs), on the other hand have crucial impact in cellular adhesion and migration and therefore cellular phenotype. A related question is therefore how HS structures influence the functions of their carriers, i.e. their core proteins activities; and how the roles of HS are affected by the different characteristics of the core proteins. These questions we want to approach by following the distribution and function of core proteins in dependence of altered glycosylation.

.... AND WHAT ABOUT CS?!

Anna Eriksson, Rashmi Ramachandra

CS is often associated as the purely architectual, less interesting relative of HS, yet certain CS structures are able to bind growth factors and morphogens as (or almost as) avidly as HS. In cases when HS are missing or defective (*i.e.* poorly sulfated HS) CS seem to replace at least some of the functions of HS. Yet in other cases, CS seem to be the only sulfated GAG in a species and possibly there to excert similar effects as HS. We will therefore use above model system also in respect of CS-replacement studies.

An other example are the invertebra brittle stars (Phylum *Echinoderma*), where we have only been able to identify CS, but not HS and now the question interests whether these CS-chains have similar properties for its carrier as would HS have in a mammalian model. Brittle stars have the ability to regenerate lost limbs after *e.g.* predator impact. We use this model to identify whether their GAGs can assist morphogens and growth factors such as bone morphogenic proteins in determining cell fate and tissue organisation [collaboration with M. Thorndyke, Kristineberg Marine Research Station]. The primary approach is to analyze the CSs found in *Echinoderma* at different stages of limb growth and characterize the binding properties to morphogens engaged in repair processes.

WHEN MICROBES MAKE USE OF HOST GAGS: CAN WE INTERFERE WITH ADHESION?

Dorothe Spillmann

Several types of microbes use surface exposed GAGs to attach to their host or to affect host cell behaviour. We have earlier identified HS and CS motifs that are used by the malaria parasite *Plasmodium falciparum* [collaboration with A. Vogt, Dilafor, J. Norman and M. Wahlgren, KI, Stockholm], herpes virus [collaboration with T. Bergström and E. Trybala, Gothenburg University] and human papilloma virus HPV [collaboration with H.C. Selinka, Johannes Gutenberg University, Mainz and M. Sapp, LSU Health Sciences Center, Shreveport, Louisiana, USA]. As a consequence of our earlier results co-crystallization trials are ongoing for the malaria adhesion protein PfEMP1 and the papilloma capsid protein L1 with defined GAG structures. The longterm goal is to interfere with microbial adhesion in a selective manner by designing competitors for microbial adhesion while avoiding interference with endogenous processes.

THE EVOLUTION OF GAGS AND WHEN ARE GAGS ALTERED?

Lars Lundin, Dorothe Spillmann

We have earlier shown a remarkable tissue specificity of HS structures and looked at the evolutionary development of HS and CS in different tissues [collaboration with Johan Ledin, EBC, UU]. Every cell produces its own glycome, *i.e*. selection of glycan structures including HS and CS. Yet, if GAGs are meant to help in modulation of cellular activities one would assume that different requirements of the body would ask for adaptation of structures in order to fulfill the correct purpose and conversely that in pathological conditions these structures may be changed in a 'wrong' way and thus be reason for altered modulation and cause for pathogenicity. These question we mainly follow by comparing HS and CS structures isolated from different specimen which have either been isolated from normal tissues, pathological biopses or cell and tissue samples that have been exposed to altered growth conditions [collaboration with M. Götte, Univeristy of Münster, Germany, G. Yip, University of Singapore, Singapore]. In order to achieve this purpose we have adapted our high-throughput analysis method to handle also fixed tissue samples.

IMMUNOLOGY

IMMUNE REGULATION

Birgitta Heyman, Jenny Hallgren Martinsson, Kjell-Olov Grönvik

Antibodies have the capacity to specifically up- or down-regulate the immune response, so called antibody feedback regulation. One main objective of our research is to clarify the mechanism behind this regulation, both during a normal response and in autoimmune diseases and allergies. Mechanisms for regulation of mast cells as well as differentiation of T cells into various subpopulations are also studied.

ANTIBODY FEEDBACK REGULATION

Birgitta Heyman

Antibodies in complex with their specific antigen can feedback-regulate antibody responses against this antigen. Depending on antibody class, affinity and type of antigen, complete suppression or 10-1000-fold enhancement of the in vivo immune response can be seen. Both passively administered and actively produced antibodies are effective, suggesting a biological role. One of the most successful clinical applications of modern immunology is Rhesus prophylaxis, where administration of suppressive IgG anti-RhD prevents Rh-immunization in mothers and subsequent hemolytic disease of their newborn. In spite of this, the molecular mechanisms behind feedback regulation are poorly understood. An immune complex is composed of antigen/antibody/complement (if the antibody activates complement) and can bind to the B-cell receptor, Fc- and complement-receptors (CRs). Ligation and co-ligation of these receptors on the B cell surface can negatively or positively regulate the B cell. Increased uptake of complexed antigen by antigen-presenting cells via Fc- or complement-receptors (followed by presentation to T helper cells) or capture of immune complexes by follicular dendritic cells (FDCs) are other possibilities for enhancement. Unlike dendritic cells (DCs), FDCs do not express MHC-II molecules and do not present antigen to T cells. They are interspersed in the B cell follicles of the spleen and lymph nodes and interact closely with B cells. Since FDCs express both FcRs and CRs they may capture immune complexes and act as a concentration device, thereby facilitating antigen recognition by the B cells. A novel interesting function of antibodies seems to be in antigen transport. Using various mouse models, we are trying to understand the molecular mechanisms behind antibody feedback regulation.

Members in the group 2008

Frida Henningson Johnson, postdoc
Annika Hermansson, technician
Birgitta Heyman, professor, group leader
Christian Rutemark, PhD student

Project workers during 2008

Joakim Dahlin

Publications from 2004 to 2008

1. Getahun, A., Dahlström, J., Wernersson, S., and Heyman, B. IgG2a-mediated enhancementof Ab- and T-cell responses and its relation to inhibitory and activating FcγRs. *J. Immunol.172: 5269-5276,* 2004.
2. Getahun A, Hjelm F, Heyman B. IgE enhances antibody and T cell responses in vivo via $CD23^+$ B cells. *J Immunol.* 2005 Aug 1;175(3):1473-82.
3. Heyman, B. Antibody feedback inhibition – a biological principle of immune regulation. *Transfusionmedicin and Hematology*, 2005, 32: 348-354.
4. Hjelm F, Carlsson F, Verbeek S, Heyman B. IgG3-mediated enhancement of the antibody response is normal in Fc gammaRI-deficient mice. *Scand J Immunol.* 2005 Nov;62(5):453-61.
5. Getahun A, Heyman B. How antibodies act as natural adjuvants. *Immunol Lett.* 2006 Apr 15;104(1-2):38-45. Review.
6. Hjelm F, Carlsson F, Getahun A, Heyman B. Antibody-mediated regulation of the immune response. *Scand J Immunol.* 2006 Sep;64(3):177-84. Review.
7. Carlsson F, Hjelm F, Conrad DH, Heyman B. IgE enhances specific antibody and T-cell responses in mice overexpressing CD23. *Scand J Immunol.* 2007 Aug-Sep;66(2-3):261-70.
8. Hjelm, F., Karlsson, M,C.I., and Heyman, B. A novel B-cell mediated transport of IgE immune complexes to the follicle of the spleen. *J. Immunol. 180: 6604-6610,* 2008.

Agencies that support the work

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MECHANISMS FOR COMPLEMENT-MEDIATED REGULATION OF IMMUNE RESPONSES

Birgitta Heyman, Jenny Hallgren Martinsson, Annika Hermansson, Christian Rutemark

Feedback regulation of the immune response occurs when antibodies are delivered to a person or an animal together with the antigen that is recognized by the antibodies. The result is an immune response that can be several hundred times stronger, or inhibited by ninety-nine percent, compared with the response when immunization is done with the antigen alone.

Some of our previous results include the finding that the complement receptor CR2 is an important co-receptor for the production of antibodies in B-cells in response to low doses of antigen, as demonstrated by the fact that CR2-knock-out mice have very poor antibody responses. There are indications that activation of complement via the classical pathway, but not via the alternative or lectin pathways, is what explains the influence of complement on antibody responses. Antibodies, and in particular IgM, are the activators of the classical pathway. We therefore study antibody responses in a knock-in mouse strain which produces IgM with a point-mutation which makes the IgM-molecule unable to activate complement. Using bone marrow chimeras, we are also trying to elucidate which cell in the immune system that must express CR2 in order for a normal antibody response to take place.

MECHANISMS FOR IgE-MEDIATED REGULATION OF IMMUNE RESPONSES

Birgitta Heyman, Joakim Dahlin, Jenny Hallgren Martinsson, Frida Henningson Johnson, Annika Hermansson

Another main project is to try to understand the mechanisms behind IgE-mediated enhancement of antibody responses. We have previously shown that the low affinity receptor for IgE (Fc-epsilon-RII/CD23) on B-cells is required for the ability of IgE to upregulate immune responses. One hypothesis to explain this is that B cells catch IgE/antigen complexes and efficiently present them to the T-helper cells, subsequently leading to an elevated antibody response. However, it is controversial whether B cells are able to activate naïve T cells. In addition, our recent finding that $CD23^+$ B cells rapidly transport IgE/antigen complexes into a special area of the spleen, the follicles, where the interaction between B- and T-cells takes place, would suffice to explain the requirement for $CD23^+$ B cells. Therefore, we are testing an alternative hypothesis, namely that the $CD23^+$ B cells only function to transport the antigen into the follicles where it is captured by the very efficient antigen presenting dendritic cells.

Experiments to test this hypothesis will be done both ex vivo and in vivo in mice carrying a transgenic diphteria toxin receptor coupled to the CD11c promoter (expressed in dendritic cells). By treating the mice with diphteria toxin, it is possible to obtain mice temporarily lacking dendritic cells. Such animals will be immunized with IgE + antigen and their B- and T-cell responses analyzed.

CYTOKINE PROFILES OF ISOLATED $CD4^+$ AND $CD8^+$ MOUSE T CELLS ARE MODULATED BY THEIR DIFFERENTIATION AND BY ANTIGEN PRESENTING CELLS.

Kjell-Olov Grönvik

Stimulated T cells proliferate and differentiate into effector cells, some of which become long-lived memory cells. Dendritic APCs and cytokines are crucial in this process. Naive T cells express low density of the adhesion molecule CD44 whereas high expression is seen in effector/memory T cells.

The aim is to investigate mechanisms for the differentiation of peripheral T lymphocytes into type 1 (TH1), type 2 (TH2) and TH17 immunity as measured by their profile of secreted cytokines which has bearing on the development of efficient vaccines.

Dendritic APCs downregulate IL-2 production of $CD44^{low}$ and $CD44^{high}$ CD4+ as well as CD8+ T cells, and are crucial for the differentiation of CD4+ $CD44^{low}$ cells into secretion of type 2 cytokines and the type 1 cytokine IFN-gamma. $CD44^{low}$ dendritic APCs lack such function. Dendritic APC independent production of type 2 cytokines is selectively enhanced in effector/memory CD4+$CD44^{high}$ T cells. Cytotoxic CD8+$CD44^{high}$ T cells are early producers of the type 1 cytokines IL-2 and IFN-gamma and secrete the type 2 cytokine IL-10.

APC independent production of pro-inflammatory IL-17 occurs early in CD4+ effector/memory T cells whereas naive T helper cells are potent producers when co-stimulated with dendritic APCs. B lymphocytes lack such co-stimulatory capacity.

A FEASIBILITY TRIAL TO TEST THE EFFECT OF CHICKEN IGY RAISED AGAINST INACTIVATED H5N1 INFLUENZA VIRUS TO PROTECT MICE AGAINST LETHAL INFECTION

INTER-INSTITUTIONAL COOPERATION BETWEEN
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St. Jude Children's Research Hospital, Memphis, Tennessee 38105 USA
Michael Wallach
University of Technology, Sydney, AUSTRALIA

The aim is to develop a new form of oral immunotherapy against flu in humans using chicken antibody (IgY) for the control of the pandemic threat from the H5N1 strain of the influenza virus.

The current outbreak initially appeared on chicken farms and in marketplaces in Asia and by March 2008, the disease had occurred in 372 people and caused 235 deaths. Government and industry have worked hard to deal with the threat of the flu pandemic. Obstacles include development of drug resistance, low productivity of H5 antigen with high production costs, and lack of cross-strain protection. We propose an additional approach using passive immunization with chicken egg IgY antibodies. IgY is easy and cheap to produce in large amounts, can be given orally and by injection and is well tolerated in humans.

This antibody can be purified, stored and formulated to provide immediate protection for hundreds of millions if not billions of people world wide.
We have obtained a Swedish and a Vietnamese isolate of H5N1 that were used to vaccinate hens twice with 4 weeks interval. Isolated antibodies from sera and egg yolk were tested for specificity by ELISA, HI and Virus Neutralization tests.

Preliminary results have shown that by using very small quantities of H5 antigen we were able to induce the production of high titer IgY that was cross-strain protective in vitro. When IgY antibodies were administered orally to mice one hour before challenge with infectious H5N1such mice were protected although with some loss of weight.

Members of the group during 2008

Kjell-Olov Grönvik, adjunct professor

Publications from 2004 to 2008

1. Cho-Ngwa, F., Daggfeldt, A., Titanji, V.P.K., and Grönvik, K-O. Preparation and characterization of specific monoclonal antibodies for the detection of adult worm infections in onchocerciasis. *Hybridoma*, 2005, 24: 283-290.
2. Rivera, E., Ekholm Pettersson, F., Inganäs, M., Paulie, S., and Grönvik, K-O. The Rb1 fraction of ginseng elicits a balanced Th1 and Th2 immune response. *Vaccine*, 2005, 23: 5411-5419.

Agencies that support the work

SVA

THE ROLE OF MAST CELLS AND IGE IN ALLERGIC AIRWAY INFLAMMATION (ASTHMA)

Jenny Hallgren Martinsson

Mast cells contribute to many features of asthma and express the high affinity receptor for IgE, FcεRI. Crosslinking of FcεRI-bound IgE with specific antigen degranulates mast cells and release proinflammatory mediators such as tryptase. Mast cells mature in tissues from committed mast cell progenitors, which numbers can be estimated by clonal expansion assays and flow cytometry. The mouse lung contains few mast cell progenitors, but inflammation increases the numbers dramatically. This expands the number of mature lung mast cells and resembles the mast cell hyperplasia that occurs in asthma patients. In asthma, B cells mount an allergen-specific IgE response that can form IgE-allergen immune complexes locally in the lung. We study how the mast cell regulates airway inflammation and the mechanisms behind the mast cell increment in the allergic lung and especially how IgE regulates this process.

Members of the group 2008:
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Project workers

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Publications from 2004 to 2008

1. Hallgren J., Bäckström S., Estrada S., Thuvesson M., Pejler G. Histidines are Critical for Heparin-dependent Activation of Mast Cell Tryptase. J. Immunol. 2004; 173, 1868-1875.
2. Hallgren J., Lindahl S., Pejler G. Structural Requirements and Mechanism for Heparin-dependent Activation and Tetramerization of human βI- and βII-Tryptase. J. Mol. Biol. 2005; 345, 129-139.
3. Zernichow L., Abrink M., Hallgren J., Grujic M., Pejler G., Kolset SO. Serglycin is the major secreted proteoglycan in macrophages and has a role in the regulation of macrophage tumor necrosis factor-alpha secretion in response to lipopolysaccharide.J. Biol Chem. 2006; 281 (37): 26792-801.
4. Abonia JP., Hallgren J., Jones T., Shi T., Xu Y., Koni P., Flavell RA., Boyce JA., Austen KF., Gurish MF. Alpha-4 integrins and VCAM-1, but not MAdCAM-1, are essential for recruitment of mast cell progenitors to the inflamed lung. Blood. 2006; 108 (5): 1588-94.
5. Hallgren J., Pejler G. Biology of mast cell tryptase. An inflammatory mediator. FEBS J. 2006; 273(9):1871-95
6. Hallgren J., Jones T., Abonia JP., Xing W., Humbles A., Austen KF., Gurish MF. Pulmonary CXCR2 regulates VCAM-1 and antigen-induced recruitment of mast cell progenitors, 2007, *Proc. Natl Acad Sci USA*; Dec 10.

7. Hallgren J., Gurish MF. Tracking the routes and roots of the mast cell. *Immunological Reviews*. 2007; 217, 8-1
8. Alcaide P., Jones T., Lord GM., Glimcher LH., Hallgren J, Arinobu Y., Akashi K., Patterson A., Gurish MF., Luscinskas FW. Dendritic Cell expression of the transcription factor T-bet regulates mast cell progenitor homing to the mucosal tissue. *J Exp Med*. 2007; 204 (2): 431-9.
9. Nigrovic PA, Gray DH, Jones T, Hallgren J, Kuo FC, Chaletzky B, Gurish M, Mathis D, Benoist C, Lee DM. Genetic inversion in mast cell-deficient Wsh mice interrupts corin and manifests as hematopoietic and cardiac aberrancy. *Am J Pathol*. 2008, 73(6):1693-701.

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THE ROLE OF MAST CELL TRYPTASE IN ALLERGIC AIRWAY INFLAMMATION (ASTHMA)

Jenny Hallgren Martinsson

The aim of this project is to determine the role of the mouse tryptase, mouse mast cell protease-6 in allergic airway inflammation. Although many of the mast cell mediators are known proinflammatory substances such as histamine and TNF-α little is known of the specific contributions from other mast cell mediators. One such class of mediators is proteaseas with trypsin-like specificity, referred to as tryptases. Tryptases are the most abundant proteins in mast cells and exist in several forms. In humans, the main granule-associated form of tryptase is β-tryptase and the corresponding mouse tryptase is mouse mast cell protease-6 (mMCP-6). Tryptase is linked to allergic inflammatory airway responses through e.g. increased tryptase levels in bronchiolar lavage fluid from asthmatics and through the ability of tryptase inhibitors to block the inflammatory responses in sheep models of allergic asthma. We are using a knock out strain of mMCP-6 on the BALB/c strain in two mast cell dependent models of allergic asthma, one acute and one chronic to study how loss of mMCP-6 effects the outcome of such as inflammation, mucous production, airway hyperresponsiveness and fibrosis.

THE EFFECT OF IgE-IMMUNE COMPLEX FORMATION IN ALLERGIC AIRWAY INFLAMMATION (ASTHMA)

Jenny Hallgren Martinsson, Martin Ivarsson, Annika Hermansson

This project is aimed to study the effect of local IgE-immune complexes in the airways, specifically to explore the possibility that IgE-immune complexes affects the increments of potent effector cells in the lung in allergic asthma such as mast cells, B- and T-cells and determine the mechanism behind. In allergic asthma and antigen-induced airway inflammation in mice, B-cells produce antigen-specific IgE that binds to directly to the

antigen or to IgE-receptors that can be cross-linked and activated by antigen. Previous studies have shown that systemic administration of IgE-immune complexes give a CD23-dependent increment of antibody production and increased T-cell proliferation in the spleen compared to treatment with antigen alone. Moreover, local (intranasal) administration of IgE-immune complexes to antigen-sensitized mice gives a stronger airway inflammation seen as increased IL-4 levels and numbers of eosinophils in bronchiolar lavage. We study the effect of IgE-immune complex in a mouse model where antigen-sensitized mice are given IgE-immune complex or antigen alone.

MOLECULAR AND CELL BIOLOGY

MOLECULAR MECHANISMS REGULATING PLURIPOTENCY AND SELF-RENEWAL OF EMRYONIC STEM CELLS

Cecilia Annerén

Mouse embryonic stem (ES) cells are cell lines derived from the inner cell mass of the mouse blastocyst. They can be maintained and expanded in culture and induced to differentiate into a wide range of cell types. This unique feature is the basis of various applications of ES cell technology such as *in vitro* models of mammalian development and germline transgenesis to make knockout mice. The more recent development of analogous human ES cells raises hope that these cells will provide a resource for cell replacement therapies and drug discovery. Human ES cells are, however, difficult to culture and since there is legitimate fear that the transplanted tissue could cause cancer in the patient one of the most important and challenging issues in stem cell biology right now is to understanding the mechanisms that regulate self-renewal, including the maintenance of growth, survival and the undifferentiated state, of ES cells.

Our aim is to gain a better knowledge of individual genes in the context of self-renewal in both stem cells as well as cancer cells. Although alike, there are differences in these processes that set these cells apart as well, such as the uncontrolled self-renewing machinery and deviant differentiation programs in cancer cells. Hence we will also focus on the identification and exploitation of yet unrecognized molecular differences between these cells, an understanding that might provide new insights into the mechanism of tumorigenesis as well as potential therapeutic targets. We anticipate that the knowledge obtained from this work, will prove to be invaluable in future work on mouse and human ES cells, with respect to *in vitro* propagation and differentiation as well as the use of human ES cells in tissue regeneration and transplantation. We further believe that the results will provide a deeper molecular understanding in tumor cell proliferation and hopefully also generating additional therapeutic targets.

Members of the group during 2008

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Publications from 2004 to 2008

1. Annerén C, Cowan CA, Melton DA. 2004 The Src family of tyrosine kinases is important for embryonic stem cell self-renewal. *J Biol Chem.* 279:31590-8.

2. Welsh M, Welsh C, Ekman M, Dixelius J, Hägerkvist R, Annerén C, Akerblom B, Mahboobi S, Chandrasekharan S, Liu ET. 2004. The tyrosine kinase FRK/RAK participates in cytokine-induced islet cell cytotoxicity. *Biochem J.* 382:261-8.
3. Holmberg J, Genander M, Halford MM, Annerén C, Sondell M, Chumley MJ, Silvany RE, Henkemeyer M, Frisen J. 2006. EphB receptors coordinate migration and proliferation in the intestinal stem cell niche. *Cell.*125:1151-63.
4. Åkerblom B, Annerén C, Welsh M. 2007. A role of FRK in regulation of embryonal pancreatic beta cell formation. *Mol Cell Endocrinol.* 270:73-8.
5. Annerén C, Welsh M, Jansson L. 2007. Glucose intolerance and reduced islet blood flow in transgenic mice expressing the FRK tyrosine kinase under the control of the rat insulin promoter. *Am J Physiol Endocrinol Metab.* 292:E1183-90.
6. Annerén C. 2008. Tyrosine kinase signaling in embryonic stem cells. *Clin Sci.* 115:43-55.

Agencies that support the work

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The Swedish Diabetes Association
Jeanssons' foundations
The Medical Faculty at Uppsala University

THE TEAD2/YAP TRANSCRIPTION FACTOR PATHWAY

Cecilia Annerén, Christoffer Tamm and Miau Wu

The aim of our research is to discover genes and signaling pathways involved in maintenance of self-renewal in mouse and human ES cells. RNA interference and specific inhibitors have been used to study functional consequences of reducing or the expression or activity of gene transcripts that are highly enriched in ES cells relative to differentiated cells. The tyrosine kinase Yes have been found to be enriched in ES cells compared to differentiated cells and to be important for ES cell self-renewal. Signaling pathways downstream of Yes that could be important for the maintenance of the differentiated state are currently being investigated. Preliminary results suggest that YAP/TEAD2-dependent transcription may be activated by Yes and that this pathway is important for maintaining self-renewal of ES cells but more studies are needed. We have generated fusion constructs that will allow us to over-express, constitutively activate, and suppress activation of these genes. By morphological, functional and biochemical studies we will analyze their role in both ES and cancer cell self-renewal and differentiation, but also to elucidate similarities and discrepancies between these cells. We also aim at challenging the gene modified tumor cell lines with known chemotherapy drugs to assess potential efficiency increases. Moreover, in collaboration with a group at the Harvard Stem Cell Institute we have generate TEAD2 conditional knock-out ES cells and the consequences of deleting TEAD2 expression in ES cells will be studied.

INTRACELLULAR PROCESSING OF SECRETORY PROTEINS

Erik Fries

Secretory proteins are cotranslationally transferred into the interior of the endoplasmic reticulum (ER) and are then transported by vesicles to the Golgi complex and from the Golgi complex to the plasma membrane. During this transport the secretory proteins are modified in various ways. Failure of a cell to modify a protein properly will lead to retention of the protein or secretion of a biologically inactive form, which may cause disease. A detailed understanding of the modifying reactions is therefore of medical importance. It is also of commercial relevance, because it is of crucial importance in the production of recombinant proteins that the proteins are properly processed.

My main research interest is the elucidation of mechanisms behind intracellular processing of secretory proteins. In this work I have focused on haptoglobin, bikunin, and the bikunin-containing proteins pre- and inter-α-inhibitor. In the course of this work I have also studied other aspects of these proteins such as their structure and function.

Members of the group in 2008:

Erik Fries, prof
Ieva Vasiliauskeite, undergrad student
Maja Elnerud, undergrad student

Publications from 2004 to 2008

1. Wicher K, Fries, E: (2004) Prohaptoglobin is proteolytically cleaved in the endoplasmic reticulum by the complement C1r-like protein. *Proc Natl Acad Sci*. 101, 14390-14395.
2. Kaczmarczyk A, Blom AM, Alston-Smith J, Sjöquist M, Fries E.: (2005) Plasma bikunin: half-life and tissue uptake. *Mol Cell Bioch*. 271, 61-7.
3. Rugg, MS, Willis, AC, Mukhopadhyay, D, Hascall, VC, Fries, E, Fülöp, C, Milner, CM and Day, AJ: (2005) Characterization of complexes formed between TSG-6 and inter-α-inhibitor that act as intermediates in the covalent transfer of heavy chains onto hyaluronan. *J Biol Chem*. 280, 25674-86.
4. Mahoney DJ, Mulloy B, Forster, MJ, Blundell CD, Fries E, Milner CM and Day, AJ: (2006) Characterization of the interaction between tumor necrosis factor-stimulated gene-6 and heparin: implications for the inhibition of plasmin in extracellular matrix enviroments *J Biol Chem*. 280 27044-55.
5. Wicher KB and Fries E: (2006) Haptoglobin, a hemoglobin-binding plasma protein, is present in bony fish and mammals but not in frog and chicken. *Proc Natl Acad USA* 103, 4168-73.
6. Suder P, Bierczynska-Krzysik A, Kraj A, Brostedt P, Mak P, Stawikowski M, Rolka K, Nyberg F, Fries E and Silberring J: (2006) Identification of bikunin as an endogenous inhibitor of dynorphin convertase in human cerebrospinal fluid. *FEBS J* 273, 5113-20.

7. Forteza R, Casalino-Matsuda, SM, Monzon ME, Fries E, Rugg MS, Milner CM. and Day AJ: (2007) TSG-6 potentiates the anti tissue kallikrein activity of inter-alpha-inhibitor through bikunin release. *Am J Respir Cell Mol Biol* 36, 20-31.
8. Wicher KB and Fries, E: (2007) Convergent evolution of human and bovine haptoglobin: partial duplication of the genes. *J Mol Evol*. 65, 373-9.
9. Asgeirsson, D, Venturoli, D, Fries, E, Rippe, B, and Rippe, C: (2007) Glomerular sieving of three neutral polysaccharides, polyethylene oxide and bikunin in rat. Effects of molecular size and conformation. *Acta Physiol (Oxf)* 191, 237-46.

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EARLY CLEAVAGE OF INTER-ALPHA-INHIBITOR HEAVY CHAINS

Ieva Vasiliauskaite

Just before the secretory proteins leave the cell - in the Golgi complex or in secretory vesicles - many of them are proteolytically cleaved and become biologically active; this cleavage is mediated by a group of enzymes called proprotein convertases. The fact that this activation occurs late during secretion suggests that an earlier expression of activity would be harmful to the cell. There are a few known proteins, however, that are cleaved in the ER. One of these is haptoglobin, and we have identified the cleaving enzyme as a truncated complement protease of otherwise unknown function. Another class of protein that is cleaved in the ER are the (two) heavy chains of inter-alpha-inhibitor. We have previously shown that the specificity of the enzyme that cleaves these proteins is similar if not identical to that of the Golgi proprotein convertases. However, those enzymes have been shown to be inactive during their transport from the ER to the Golgi complex suggesting that a different enzyme mediates the cleavage of the heavy chains. We are currently establishing a simple assay for the cleaving enzyme. With this assay available, we will further characterize the enzyme with the long term goal of its identification.

BINDING OF (CLEAVED) HAPTOGLOBIN TO OTHER PROTEINS

Maja Elnerud, Ieva Vasiliauskaite

As mentioned above, haptoglobin is proteolytically cleaved in the ER. The function of this cleavage is not known - an earlier study has shown that it does not affect the binding to hemoglobin. Haptoglobin has also been shown to bind to other proteins such as apolipoprotein A1 and Mac-1. We are attempting to determine if this binding is affected by cleavage and also where on the haptoglobin molecule those proteins bind.

NEURAL STEM CELL BIOLOGY

Karin Forsberg-Nilsson

The study of neural stem cells increases our understanding of mammalian development and gives a prospect for central nervous system (CNS) repair by stem cell transplantation. Knowledge about neural stem cell proliferation and differentiation will be of equal importance for neuro-oncology, since malignant brain tumors are believed to arise from these cells.

Our objectives are:

- To find new regulators for neural stem cell proliferation and differentiation by gene profiling.
- To identify common pathways regulating pediatric brain tumor cells and neural stem cells.
- To transplant neural stem cells to a mouse model of traumatic brain injury (TBI).
- To develop new 3-dimensional neural stem cell models.

An important aspect of neural stem cell biology is how the balance between growth and differentiation is regulated. Brain tumors are believed to arise as a result of perturbations of this balance. We have shown that platelet-derived growth factor (PDGF), which was previously linked to brain tumor development, supports neural progenitor cell proliferation. PDGF-stimulated neural stem/progenitor cells retain an immature morphology. We have recently shown that neural progenitors regulate their own maturation by endogenous production of PDGF. This may be part of the process of malignant conversion from normal cell to brain tumor. To further explore the regulation of PDGF-stimulation in neural stem cells and to find novel regulators of stem cell growth and differentiation, an expression profiling has been performed. Further analysis and characterization of the regulated genes are currently underway.

The concept of using stem cells for therapeutic purposes is pursued in collaboration with neurosurgeons and material chemists at Uppsala University, using a mouse model for traumatic brain injury. Neural stem/progenitor cells are cultured in a 3D structure, which resembles the extra cellular matrix. Our expectation is that by providing the neural stem/progenitor cells with a suitable structural support, the cell survival will be improved and hopefully this neural stem cell model will function as structural and functional support after brain trauma.

Members of the group during 2008

Tobias Bergström, MSc
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Per-Henrik Edqvist, Ph.D.
Karin Forsberg-Nilsson, Associate professor, Group leader
Jimmy Larsson, Ph.D. Student
Ulrika Wallenquist, Ph.D. Student

Publications from 2004 to 2008

1. Göktürk, C., Nordquist, J., Sugimoto, H., Forsberg-Nilsson, K., Nilsson, J. and Oreland, L. (2004). Induction of diabetes in transgenic mice overexpressing Semicarbazide-sensitive amine oxidase (SSAO), exhibits a feed-back inhibition of the endogenous mouse SSAO gene. *BBRC*, 17, 1013-20.
2. Erlandsson, A., Larsson, J., and Forsberg-Nilsson, K. (2004) Stem cell factor is a chemoattractant and a survival factor for CNS stem cells. *Experimental Cell Research* 301, 201-210.
3. Zhang, X-Q., Afink, G., Hu, X-R., Forsberg-Nilsson, K. and Nistér, M. (2005). Gli1 is not required for Pdgfrα expression during mouse embryonic development. Differentiation. 73, 109-19.
4. Brännvall, K., Sandelin, M., Wallenquist, U., Forsberg-Nilsson, K., Aldskogius, H., and Kozlova, E. N. (2006) Central nervous system stem/progenitor cells form neurons and peripheral glia after transplantation to the dorsal root ganglion. Neuroreport, 17*:* 623-628.
5. Erlandsson, A., Brännvall, K., Gustafsdottir, S., Westermark, B., and Forsberg-Nilsson, K. (2006). Autocrine/Paracrine platelet-derived growth factor regulates proliferation of neural progenitor cells. Cancer Res 66, 8042-8048.
6. Demoulin JB., Enarsson M., Larsson J., Essaghir A., Heldin CH., Forsberg-Nilsson K. (2006) The gene expression profile of PDGF-treated neural stem cells corresponds to partially differentiated neurons and glia. Growth Factors. 24(3): 184-196.
7. Kriz, V., Mares, J., Wentzel1, P., Funa, N., Calounova, G., Zhang, X-Q., Forsberg-Nilsson, K., Forsberg, M., and Welsh, M. (2007) Reduced viability in utero as a consequence of Shb gene inactivation. Developmental Dynamics, 236, 2485-2492
8. Ossipov, D., Brännvall, K., Forsberg-Nilsson, K, and Hilborn, J. (2007) First injectable poly(vinyl alcohol) hydrogel formed by mixing of functional PVA precursors: A model for in situ cell encapsulation. Journal of Applied Polymer Science, 106, 60-70
9. Looman, C., Sun, T., Feinstein, R., Forsberg, H., Hellberg, C., Heldin, C-H., Zhang, X-Q., Forsberg-Nilsson, K., Khoo, N., Fundele, R., and Heuchel, R. (2007) An activating mutation in the platelet-derived growth factor receptor-b causes hyper-proliferation of cells and abnormal morphology of the labyrinthine layer in the mouse placenta. Int. J. Dev. Biol 51, 361-70
10. Göktürk, C, Sugimoto, H., Blomgren, B., Roomans, G., Forsberg-Nilsson, K., Oreland, L. and Sjoquist, M. (2007) Macrovascular changes in mice overexpressing human semicarbazide-sensitive amine oxidase (SSAO) in smooth muscle cells, American Journal of Hypertension 20, 743-50
11. Brännvall, K., Bergman, K., Wallenquist, U., Svahn, S., Bowden, T., Hilborn, J., and Forsberg-Nilsson, K. (2007) Enhanced neuronal differentiation in a 3D collagen-hyaluronan matrix. Journal of Neuroscience Research, 85, 2138-2146
12. Larsson J., Forsberg M., Brännvall K., Zhang X-Q., Enarsson M., Hedborg F. and Forsberg-Nilsson K., (2008) Nuclear Receptor Binding Protein 2 is induced during neural progenitor differentiation and affects cell survival. *Molecular and Cellular Neuroscience* 39, 32-39
13. Brännvall, K., Corell, M., Forsberg-Nilsson, K. and Fex Svenningsen, Å., (2008) Environmental cues from CNS, PNS, and ENS cells regulate CNS progenitor differentiation, *Neuroreport* 19, 1283-1289
14. Forsberg, M., Forsberg-Nilsson K and Ebendal, T,, Genetiskt modifierade försöksdjur (Genetically modified mice), Book chapter in *Försöksdjurskunskap (Animal Experimentation),* Studentlitteratur eds., in press

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Project students 2008

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NOVEL REGULATORS OF NEURAL STEM CELLS AND THEIR CONNECTION TO BRAIN TUMORS

Jimmy Larsson and Per-Henrik Edqvist

Neural stem/progenitor cell (NSPC) proliferation and differentiation is regulated by a variety of endogenous and exogenous factors. NSPCs are propagated in culture with the addition of fibroblast growth factor 2 and/or epidermal growth factor, which are necessary for maintained self-renewal capacity and multipotency. Upon growth factor removal, NSPCs differentiate to neurons and glial cells. During this differentiation process, major changes occur at the RNA and protein level leading to fate determination and formation of the mature cell types of the brain. We have in a previous project used the microarray technique to analyze the transcriptional pattern underlying NSPC proliferation and differentiation (Demoulin et al 2006). From this study we have identified Nuclear Receptor Binding Protein 2 (NRBP2), a gene that is up regulated during NSPC differentiation. In embryonic central nervous system development, NRBP2 was expressed in the walls of the third and fourth ventricles, and in the hippocampus. In the adult brain, Purkinje cells in the cerebellum and neurons in the CA3 region of the hippocampus were main sites of NRBP2 expression. In a pediatric medulloblastoma, NRBP2-positive cells were present in clusters of tumor cells also expressing a neuronal marker. Down regulation of NRBP2 rendered NSPCs more vulnerable to apoptosis, which suggests a role for NRBP2 in progenitor cell survival (Larsson et al, 2008). In addition to NRBP2, we are evaluating several other genes identified by expression profiling, regarding their function in neural stem cells in health and disease

A TRANSGENIC MODEL OF PDGF OVEREXPRESSION IN NEURAL STEM CELLS AND RETINA

Per-Henrik Edqvist and Xiao-Qun Zhang

We have previously shown that platelet-derived growth factor (PDGF) is a mitogen and a survival factor that expands a pool of immature cells from neural stem/progenitor cells (NSPC) in vitro (Erlandsson et al. 2001, 2006). To investigate the role *in vivo* for PDGF during cental nervous system (CNS) development, we generated mice that over express PDGF-B in NSPC under control of the nestin enhancer.

The nestin-PDGFB mice were viable and fertile and stable lines could thus be established. The transgenic expression pattern correlates with that of the endogenous nestin

gene. We have analyzed the cellular composition and brain morphology of embryonic, neonatal and adult transgenic brains by immuno staining with markers to neurons and glia. The major findings were an increased apoptosis of the developing striatum during mid gestation and an enlargement of the lateral ventricles of the brain postnatally. PDGF has been implicated in brain tumor development and we examined if nestin-PDGF-B mice are more prone to develop tumors. No brain tumors were found and we therefore conclude that secondary genetic changes are indeed needed for the malignant conversion. Because PDGF is an important migratory factor, the enhanced cell death at E14 in the ganglionic eminences might be the result of disturbed cell movement in this highly migratory area, due to the deregulated PDGF levels. In collaboration with Professor Björn Vennström's group at the Karolinska Institutet we found that adult male mice showed behavioral aberrations and locomotor dysfunctions. The mechanistic consequences of an increased ventricular volume, and the corresponding impact on behavior remain to be fully understood.

Histological analysis of retinae from nestin-PDGFB mice revealed a gross general disruption in the laminar structure of the retina, accompanied by the apparent lack of mature photoreceptor segments. Upon closer inspection, we also observed severe malformations in the vasculature of transgenic retinae. Currently, work is carried out to extensively characterize this retinal phenotype with emphasis on photoreceptor generation and differentiation, photoreceptor segment formation, cell proliferation, cell death and vasculature development. This work is done partly in collaboration with Professor Finn Hallböök's laboratory at the Department at of Neuroscience, Uppsala University.

USE OF NEURAL STEM CELLS TO RESTORE LOST FUNCTION OF THE CENTRAL NERVOUS SYSTEM

Ulrika Wallenquist and Karin Brännvall

Traumatic brain injury is a major cause of death and disability. Today there is no effective pharmacological treatment for TBI. The aim of this project is to investigate whether transplanted neural stem/progenitor cells (NSPC) can help restore the lost CNS function that follows TBI. In collaboration with Professor Lars Hillered and his group at the Department of Neuroscience, a mouse model that mimics real-life TBI is used. NSPC containing the cell tracer green fluorescent protein are transplanted into the lateral ventricle at various times post trauma. Survival of the grafted cells is analyzed by histology at different time points after transplantation. The inflammation is guiding the transplanted cells to the injured area, and viable cells are found both in the un-injured and in the injured hemisphere up to 12 weeks post transplantation. The majority of the transplanted cells die due to the hostile environment, but green fluorescent protein positive cells that express markers of stem cells, neurons, and glia are found (Wallenquist et al. under revision). By providing the cells with a 3-dimensional structure in the form of a biodegradable matrix we hope to improve the cell viability after transplantation to the injured brain.

EXTRACELLULAR REMODELING IN DEVELOPMENT AND IN BRAIN TUMORS

Tobias Bergström

Invasive behavior is a characteristic feature for several childhood brain tumors and modulation of the extracellular matrix is a prerequisite for cell movement in the brain

parenchyma. Recently, we discovered that culture of early postnatal neural stem cells in a hydrogel composed of collagen/hyaluronic acid promoted adherence and differentiation to neurons (Brännvall et al, 2007). In 3D, 70% neurons formed, compared to 14% neurons on regular fibronectin coating of plastic surfaces. Embryonic and adult neural stem cells, on the other hand, maintained a sphere-like appearance in the 3D system and their ratio of neurons formed was similar to that of 2D culture. The efficient adhesion, spread and generation of neurons caused by culturing postnatal stem cells in a hyaluronan/collagen hydrogel is interesting from a neuro oncology perspecitve. Hyaluronan is one of the major brain extracellular matrix components, and it is increased in primary brain tumors. Fibrillar collagen, on the other hand, is normally not present in the brain parenchyma but in medulloblastoma, collagen 1 is up regulated and may play a role in attracting new vessels to the tumor. We are currently studying the interaction between the present scaffold and the postnatal donor type in order to understand the molecular basis behind the enhanced neuronal differentiation in 3D. Exploration of the mechanism behind this potential difference between stem cells from the embryo and from early postnatal life because of its potential implications for tumors occuring during childhood.

IN VITRO DIFFERENTIATION OF EMBRYONIC STEM CELLS

Maud Forsberg, Katarina Holmborn, Lena Kjellén and Karin Forsberg-Nilsson

In vitro differentiation of embryonic stem (ES) cells is a useful tool for studying specific developmental processes that may be difficult or impossible to assess in the whole organism. The differentiation potential of genetically modified ES cells can be studied and compared to that of wild type ES cells. We have used in vitro differentiation as a method to study ES cells derived from a mouse mutant with an early embryonic lethal phenotype. This mouse mutant carries targeted deletions for two enzymes involved in heparan sulfate (HS) biosynthesis, namely NDST (glucosaminyl N-deacetylase/N-sulfotransferase)1 and NDST2. HS consists of sulfated polysaccharide chains that are mainly found in the extracellular space, bound to core proteins located on the cell surface or in the extracellular matrix. Many proteins, e.g. various growth factors, interact with the HS chains, and these interactions influence growth factor signalling and the generation of morphogen gradients. The NDST enzymes modify the sulfation pattern of the HS chains and this pattern can affect the binding affinity of growth factors to HS. The severe phenotype of the NDST1/2 knockout mouse shows that such HS-modifications are of great importance for normal development. We have analysed the differentiation potential of NDST1/2 double knockout ES cells using established protocols for derivation of osteblasts and adipocytes (through embryoid body formation) as well as monolayer differentiation to neural cells and show that NDST1 and NDST2 are not needed for formation of osteoblasts, but necessary for induction of adipocytes and neural cells. Addition of FGF and heparin completely rescued the differentiation potential to the neural linage. Our data show that heparan sulphate mediated FGF signalling is an early and necessary step in the commitment of undifferentiated ES cells to the neural cell lineage.

MOLECULAR BACTERIOLOGY

VARIABILITY AND STABILITY OF BACTERIAL GENOMES

Dan Andersson

Our research is focused on two areas: A) analysis of the various genetic and environmental factors that affect genome stability and variability in bacteria and B) analysis of the factors that influence the dynamics of the evolution of antibiotic resistance development. We study these problems in several bacterial species using a combination of genetics, physiology and experimental evolution.

A. Genome variability and stability. The long-term goal of this project is to examine the major factors that influence the tempo and mode of bacterial evolution. In particular we are interested in the evolutionary and mechanistic factors that influence genome stability and variability. We use the bacterium Salmonella typhimurium as a model system to: 1. Examine how the extent of genetic variation affects bacterial fitness. 2. Examine if bacteria have evolved physiological mechanisms that ”buffer” against mutations. 3. Examine the role of gene amplification in adaptive responses and in the evolution of novel genes. 4. Experimentally evolve a bacterium with a reduced genome. 5. Experimentally evolve a minimal ribosome. 6. Examine constraints on horizontal gene transfer.

B. Dynamics of the evolution of antibiotic resistance. The overall objective of this project is to understand how antibiotic resistance affects the fitness, virulence and transmission of bacteria and which factors determine how rapidly resistance develops in a bacterial population. Our main aims are to: 1. Determine how various types of resistance mechanisms affect bacterial fitness. 2. Determine how bacteria can compensate for these fitness costs. 3. Determine in animal models and human volunteers how antibiotic resistance affects bacterial transmission. 4. Determine if reduced antibiotic use in community settings may result in a reduced frequency of resistance. 5. Identify mechanisms that confer resistance to antimicrobial peptides and the impact of these mechanisms on bacterial fitness.

Members of the group during 2008

Dan Andersson, Professor
Joakim Näsvall, Postdoc
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Publications from 2004 to 2008

1. Baquero MR, Nilsson AI, Turrientes Mdel C, Sandvang D, Galan JC, Martinez JL, Frimodt-Moller N, Baquero F, Andersson DI. Mutation frequencies in Escherichia coli: emergence of weak mutators in clinical isolates. J Bacteriol. 2004,186:5538-42.
2. Roth JR, Andersson DI. Adaptive mutation: how growth under selection stimulates Lac(+) reversion by increasing target copy number. J Bacteriol. 2004, 186:4855-60.
3. Roth JR, Andersson DI.: Adaptive mutation in Escherichia coli (Foster). J Bacteriol. 2004, 186:4854.
4. Roth JR, Andersson DI.: Adaptive point mutation (Rosenberg and Hastings). J Bacteriol. 2004, 186:4844.
5. Maisnier-Patin S, Andersson DI. Adaptation to the deleterious effects of antimicrobial drug resistance mutations by compensatory evolution. Res Microbiol. 2004, 155:360-9.
6. Roth JR, Andersson DI. Amplification mutagenesis--how growth under selection contributes to the origin of genetic diversity and explains the phenomenon of adaptive mutation. Res Microbiol. 2004, 155:342-51.
7. Mariam DH, Mengistu Y, Hoffner SE, Andersson DI. Effects of rpoB mutations conferring rifampin resistance on fitness of Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2004, 48:1289-94.
8. Starck J, Källenius G, Marklund BM, Andersson DI and Åkerlund T Comparative proteome analysis of Mycobacterium tuberculosis grown under aerobic and anaerobic conditions. Microbiology. 2004, 150:3821-3829.
9. Pettersson M, Andersson DI, Roth JR. and Berg OG. The amplification model for adaptive mutation: simulations and analysis. Genetics. 2004, 169:1105-1115.
10. Nilsson AI, Kugelberg E, Berg OG. and Andersson DI. Adaptation of Salmonella typhimurium to mice. Genetics. 2004, 168:1119-1130.
11. Kugelberg E, Löfmark S, Vretlind B, and Andersson DI. Reduction of the fitness burden of quinolone resistance in Pseudomonas aeruginosa. J Antimicrob Chemother. 2005, 55:22-30.
12. Lundin A, Bjorkholm B, Kupershmidt I, Unemo M, Nilsson P, Andersson DI, Engstrand L. Slow genetic divergence of Helicobacter pylori strains during long-term colonization. Infect Immun. 2005, 73:4818-22.
13. Kugelberg E, Norstrom T, Petersen TK, Duvold T, Andersson DI, Hughes D. Establishment of a superficial skin infection model in mice by using Staphylococcus aureus and Streptococcus pyogenes. Antimicrob Agents Chemother. 2005, 49:3435-41.
14. Sjölund M, Tano E, Blaser MJ, Andersson DI, Engstrand L. Persistence of Resistant Staphylococcus epidermidis after Single Course of Clarithromycin. Emerging Inf Dis, 2005, 11:1389-1393.

15. Wiuff C, Zappala RM, Regoes RR, Garner KN, Baquero F, Levin BR. Phenotypic tolerance: antibiotic enrichment of noninherited resistance in bacterial populations. Antimicrob Agents Chemother. 2005, 49:1483-94.
16. Nilsson AI, Koskiniemi S, Eriksson S, Kugelberg E, Hinton JC, Andersson DI. From the Cover: Bacterial genome size reduction by experimental evolution. Proc Natl Acad Sci U S A (See Commentary). 2005, 102:12112-6.
17. Olofsson SK, Geli P, Andersson DI, Cars O. A pharmacodynamic model to describe the concentration-dependent selection of cefotaxime resistant Escherichia coli. Antimicrob Agents Chemother. 2005, 49:5081-5091.
18. Maisnier-Patin S, Roth JR, Fredriksson Å, Nyström T, Berg OG, Andersson DI. Genomic buffering mitigates the effects of deleterious mutations in bacteria. Nature Genetics. 2005, 37:1376-1379.
19. Nilsson A, Zorzet A, Kanth A, Dahlström S, Berg OG and Andersson DI. Reducing the fitness cost of antibiotic resistance by amplification of initiator tRNA genes. Proc Natl Acad Sci USA. 2006, 103:6976-6981.
20. Andersson DI. The Biological Cost of Antibiotic Resistance: Any Practical Conclusions? Curr Op in Microbiol. 2006, 9:461-465.
21. Roth JR, Kugelberg E, Kofoid E, Reams A, and Andersson DI. Origin of mutations under selection: The adaptive mutation controversy. Ann Rev Microbiol. 2006, 60:477-501
22. Kugelberg E, Kofoid E, Reams A, Andersson DI and Roth JR. Multiple pathways of selected gene amplification during adaptive mutation. Proc Natl Acad Sci USA. 2006, 103:17319-17324.
23. Paulander W, Pennhag A, Andersson DI and Maisnier-Patin S. *Caenorhabditis elegans* as a model to determine fitness and virulence of antibiotic-resistant Salmonella typhimurium. Antimicrob Agents Chemother. 2007, 51:766-769.
24. Wiuff C and Andersson DI. Antibiotic treatment in vitro of phenotypically tolerant bacterial populations. J Antimicrob Chemother. 2007, 59:254-263.
25. Maisnier-Patin S, Paulander W, Pennhag A and Andersson DI. Compensatory evolution reveals functional interactions between ribosomal proteins S12, L14 and L19. J Mol Biol. 2007, 366:207-215.
26. Paulander W, Maisnier-Patin S and Andersson DI. Multiple mechanisms to ameliorate the fitness burden of mupirocin resistance in Salmonella typhimurium. Mol Microbiol. 2007, 64:1038-1048.
27. Bergthorsson U, Andersson DI and Roth JR. Ohno's dilemma: evolution of new genes under continuous selection. Proc Natl Acad Sci U S A. 2007, 104:17004-9.
28. Andersson DI and Hughes D. Effects of antibiotic resistance on bacterial fitness, virulence, and transmission. In: Introduction to the Evolutionary Biology of Bacterial and Fungal Pathogens. Editors: Baquero F, Nombela C, Cassell GH and Gutiérrez JA. ASM Press. 2007, p. 307-318.
29. Lytsy B, Sandegren L, Tano E, Torell E, Andersson DI, Åsa Melhus Å. The First Major Extended-Spectrum ß-Lactamase Outbreak in Scandinavia Was Caused by Clonal Spread of a Multiresistant *Klebsiella pneumoniae* Producing CTX-M-15. APMIS. 2007. In press.
30. Martinez JL, Baquero F, and Andersson DI. Predicting antibiotic resistance. Nature Reviews in Microbiology, 2007, 5:958-965.
31. Löfmark S, Jernberg C, Billström H, Andersson DI and Edlund C. Restored fitness leads to long-term persistence of resistant Bacteroides strains in the human intestine. Anaerobe 2008, 14:157-160.

32. Pränting M, Negrea A, Rhen M, and Andersson DI. Mechanism and fitness costs of PR-39 resistance in Salmonella enterica serovar Typhimurium LT2. Antimicrob Agents Chemother. 2008, 52:2734-2741.
33. Sandegren L, Lindqvist A, Kahlmeter G and Andersson DI. Nitrofurantoin resistance mechanism and fitness cost in Escherichia coli. J Antimicrob Chemother 2008, 62:495-503.
34. Andersson DI. Shrinking bacterial genomes. Microbe. 2008 March.
35. Pettersson M, Sun S, Andersson DI and Berg OG. Evolution of new gene functions: simulation and analysis of the amplification model. Genetica. June 22 Epub.
36. Geli P, Andersson M, Svensson Å and Andersson DI. A Multi-Type Branching Model with Varying Environment for Bacterial Dynamics with Postantibiotic Effect. J Theoretical Biology. 2008. Oct 9 Epub.
37. Lind PA and Andersson DI. Whole-genome mutational biases in bacteria. Proc Natl Acad Sci U S A. 2008. 105:17878-83.

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GENOME REDUCTION THROUGH SPONTANEOUS DELETION FORMATION

Sanna Koskiniemi

A central biological question is how genomes evolve with respect to size and gene content and which factors affect this evolution. Spontaneous deletion formation occurs quite frequently in the bacterial chromosome, even though these deletions rarely become fixed in populations. We are interested in the rate of this spontaneous deletion formation and to find out by which mechanisms this occurs. Our earlier results strongly indicate that deletions are not formed through RecA mediated homologous recombination. Currently we are examining the genetic requirements for deletion formation and which factors affect the rate of deletion formation in exponentially growing cells. Using four different experimental setups we have been able to show that deletion formation in *S. typhimurium* cells is dependent on the presence of active error-prone translesion polymerases. i) Firstly, removal of all four translesion polymerases: PolII (*polB*), PolIV (*dinB*), PolV (*umuDC*) and the PolV homologue SamAB (*samAB*), resulted in a 10-fold decrease in deletion rate in a wild type background, indicating that 90% of all spontaneous deletions are formed by the error-prone translesion polymerases. ii) Second, expression of these polymerases is under control of the DNA damage-inducible LexA regulon (SOS response), and a mutation that de-represses this regulon resulted in a 30-fold increase in deletion rate. This increase was completely dependent on the expression of the four translesion polymerases. iii) Third, over-expression of two of the polymerases PolII and PolIV alone also increased the deletion rate. iiii) Lastly, in a *recBC*$^-$ mutant dsDNA ends are stabilized due to the lack of enzyme capable of processing these ends. In this mutant the deletion rate was increased 20-fold compared to wild type, in a manner completely dependent on the error-prone polymerases. We hypothesize that the translesion polymerases stimulate deletion formation by allowing extension of misaligned single-strands.

CONTROL OF MUTATION RATES BY ERROR_PRONE DNA POLYMERASES AND RPOS

Sanna Koskiniemi

It has been suggested that bacteria can change both the rate and specificity of mutation formation in response to stresses. One model proposes that this putative stress-induced mutagenesis requires both induction of the error-prone DNA polymerase IV under control of the DNA damage-inducible LexA regulon (SOS response) and an increase in levels of the starvation-induced sigma-factor RpoS. Here we have examined this idea by determining whether a simultaneous induction of the LexA-mediated SOS response and an increase in RpoS levels will cause an increase in mutation rates. We studied the impact of these two different stress responses for four different types of mutations in *Salmonella typhimurium*. Constitutive expression of the LexA-regulon conferred by a *lexA*$^-$ mutation caused a 2- to 8-fold increase in mutation rate depending on the mutation class examined. Since it has been suggested that the error-prone DNA polymerases II, IV and V contribute to this mutagenesis we determined if, in a constitutively induced *lexA*$^-$ mutant, inactivation of all three polymerases caused a reduction in the mutation rates. Surprisingly, we did not observe a decrease in the mutation rates when inactivating the three error-prone polymerases in a *lexA*$^-$ mutant for amino acid subtitutions. However, the increased mutation rate seen in a *lexA*$^-$ mutant for frameshift mutations and deletions was completely dependent on the error-prone polymerases. These result show that the increase in mutation rate seen in a *lexA*$^-$ mutant is not primarily due to the error-prone polymerases as has been previously suggested. Furthermore, over-expression of RpoS caused no increase in mutation rate in either a *lexA*$^+$ or *lexA*$^-$ strain. Our results are not compatible with the hypothesis of stress-induced mutagenesis conferred by induction of the error-prone DNA polymerases and RpoS.

CONSTRAINTS ON HORIZONTAL GENE TRANSFER AND WHOLE-GENOME MUTATIONAL BIASES

Peter Lind

Genes acquired through horizontal gene transfer (HGT) make up substantial parts of most bacterial genomes and are important for niche-adaptive variation within a species, pathogenicity and antibiotic resistance. In theory any genetic material can cross species-barriers through transduction, conjugation and transformation. Newly transferred genes can be fixed in a population either if they confer a fitness advantage or by drift if nearly neutral. This project investigates constraints of HGT by measuring the fitness cost of introducing random single mutations in ribosomal protein genes or replacing them with orthologues from other species using *Salmonella typhimurium* as a model organism. Results show that the fitness cost for transfer of closely related orthologous genes is usually small, but that random mutations often confer a fitness cost, even in the case of synonymous mutations. Highly sensitive competition experiments between mutant and wild type strains carrying different variants of GFP are performed using FACS analysis. Compensatory evolution experiments of mutants with orthologous gene replacements suggest that gene amplification is a primary mechanism for the integration of HGT genes into a novel genome.

The genomic base composition of different bacteria varies in ways that deviates from that expected by randomness and this variation is caused by the interplay between biased mutation rates and selection. In this project we studied the specificity and rate of different mutational biases in real time in *Salmonella typhimurium* under conditions of strongly reduced selection and in the absence of the major DNA repair systems involved in repairing common spontaneous mutations caused by oxidized and deaminated bases. The mutational spectrum was determined by whole-genome sequencing and our results suggest that GC content can be rapidly reduced in the absence of the relevant DNA repair systems.

HOW ARE NEW GENES CREATED?

Joakim Näsvall

New gene functions evolve from an initial duplication of a parental gene, followed by functional divergence of the two copies through random mutations. There are several models for this process, differing in the timing of the initial duplication, the appearance of the mutation(s) that lead to the new activity and at which stage natural selection starts. One such model is based on the fact that many proteins have weak secondary biochemical activities. A change in the environment can make one of these minor activities beneficial, leading to a selective pressure to increase its activity. Amplification of the parental gene provides both increased expression of the selected activity as well as a large number of mutational targets. During continuous selection any mutation that improves the trace activity will be favored and further amplification of improved variants and loss of less efficient variants will eventually result in one of the extra copies becoming sufficient to perform the new function without the need for extra copies. If selection for the original function is present during this process, one of the copies will retain the original function while the other copies diverge, and the end result will be a duplication of the original gene, where one of the copies have the new biochemical activity. SerB (phosphoserine phosphatase) and the N-terminal domain of HisB (histidinol phosphatase) are distantly related enzymes in the biosynthesis of the amino acids serine and histidine, respectively. An *E. coli* mutant lacking the *serB* gene can be partially rescued by expression of *hisB* from a high-copy number plasmid, indicating that HisB has a weak serine phosphatase (SerB) activity that may be a starting point for the evolution and divergence of a new gene from its ancestor. We have deleted the *serB* gene in *Salmonella typhimurium* and found that when a culture of the mutant is spread on minimal medium lacking serine,colonies appear within 2 weeks of incubation. One likely mechanism for the slow serine-independent growth in these mutants is amplification of the *his*-operon, which would increase the expression of the HisB enzyme enough to allow its weak serine phosphatase activity to produce enough serine to allow growth. A *gfp* gene will be placed next to the *his*-operon in a *serB* mutant to enable easy detection of amplifications, and any serine-independent clones containing amplifications of the *his*-operon will be passaged on medium lacking serine (and histidine) to select faster growing clones. As possible alternative systems we are also constructing strains for examining if over-expression of SerB can rescue a mutant lacking the histidinol phosphatase domain of HisB, and if strains lacking any of the related HisA, HisF, TrpC or TrpF proteins can be rescued by high-copy number plasmids carrying any of the non-cognate (*hisA*, *hisF*, *trpC* or *trpF*) genes.

A SYSTEMATIC SCREEN FOR MUTATIONS CONFERRING RESISTANCE TO ANTIMICROBIAL PEPTIDES

Maria Pränting

Antimicrobial peptides (AMPs) are important components of innate immunity that have been identified in organisms ranging from bacteria to man. The peptides kill microbes rapidly and have a broad spectrum of activity. These characteristics make them interesting as leads for new pharmaceuticals, and several companies are currently trying to commercialize the use of AMPs in medicine. However, few studies have examined resistance development towards these peptides. In this project, we are examining the evolution of bacterial resistance to AMPs and the resulting effects on bacterial fitness and virulence. To date we have isolated mutants of *Salmonella typhimurium* that are less sensitive to the peptides PR-39 and protamine. The PR-39 mutants appear at a rate of $4x10^{-7}$ per cell per generation, and are as fit as the wild type parental strain as measured by growth rates in culture medium and in mice. The mutants are about 4 times more resistant than the wild type and also show a greatly reduced rate of killing. We have found that mutations in the putative transport protein SbmA are responsible for the observed resistance. The mutation rate to protamine resistance has been calculated to 2×10^{-7} per genome and generation. These mutants are 3 to 30 times more resistant to protamine than the parental strain and the bacteria are also cross resistant to several antibiotics. The resistance mutations have been mapped to different *hem*-genes and are associated with a reduction of fitness of the bacteria. Some of the mutants can evolve to higher fitness by acquiring second site compensatory mutations. In some cases, this compensation seems to involve amplification of the mutated gene followed by an additional mutation in one of the copies after which the amplified array segregates back to one copy.

TRNA GENE COPY NUMBER DYNAMICS IN SALMONELLA

Linus Sandegren

Gene amplification is a common feature of the genomes of prokaryotic organisms. This dynamic genomic state can allow the bacterium to adapt to changing environmental conditions in a rapidly reversible, non-mutational manner. Increased gene dosage provides the capability of over-expression of the genes within the amplified genomic region and may confer the phenotypic advantage needed for survival and adaptation to changes in growth conditions *Salmonella typhimurium* carries an operon with two tandem copies of the initiator-tRNA gene (*metZ* and *metW*). Large amplifications (2-94 kb) of the chromosomal region containing this operon was found to compensate for the fitness cost inferred by mutations that yield resistance to the deformylase inhibitor actinonin, most likely by increasing the expression levels of $tRNA^{iMet}$. These amplifications appear to have occurred in a RecA independent manner without sequence homology at the duplication boundaries. However, direct homologous tandem amplifications of the *metZW* gene pair via the existing sequence identity should theoretically be an efficient way of increasing the gene copy number. No such direct tandem amplifications were found among the growth compensated strains. We have set up a Padlock-probe based system to measure the frequency of different gene copy numbers in bacterial populations and found that such direct amplifications of the *metZW* tandem repeats are common in bacterial cultures and that stable differences in $tRNA^{iMet}$ gene copy number also occurs in clinical Salmonella isolates. We are currently using this system to test copy number

stability and expression pattern of tandemly amplified operons to determine what factors are regulating the number of gene copies in the bacterium.

ESBL-RESISTANCE DEVELOPMENT, SPREAD AND EVOLUTION

Linus Sandegren

Third-generation cephalosporins, or extended-spectrum cephalosporins is one of the major new classes of β-lactam antibiotics and they are a primary choice to treat infections caused by Gram-negative bacteria. However, with each new antibiotic used clinically new variants of β-lactamases with altered specificities have emerged. Because of their increased spectrum of activity, especially against the third-generation cephalosporins, these new enzymes are termed extended-spectrum β-lactamases (ESBLs). Today, ESBLs are an increasingly important cause of multi-drug resistance in Gram-negative bacteria throughout the world.

The first larger outbreak of ESBL-producing bacteria in Scandinavia started at Uppsala University Hospital in the spring of 2005 and took two years to get control over. We have worked together with people at the hospital with the molecular characterization of the outbreak. We have determined the full sequence of the multi-resistance plasmid carrying the ESBL-gene and are now analyzing how the plasmid changes over time during the outbreak and *in vitro*. Spread of the plasmid between different bacteria occurs and what impact this has on the plasmid will be addressed. An important question in this study is if gene amplification of non-ESBL, or ESBL β-lactamase genes occurs as an adaptive response to treatment with extended-spectrum cephalosporins and carbapenems respectively, giving an increased tolerance towards these antibiotics and increasing the likelihood of rare point mutations yielding even more highly resistant enzymes.

GENE AMPLIFICATION AND EVOLUTION OF ANTIBIOTIC RESISTANCE

Song Sun

Intensive use of ß-lactam antibiotics has led to the evolution and global spread of a variety of resistance mechanisms -- including ß-lactamases, a group of enzymes that degrade the ß-lactam ring. The evolution of increased ß-lactams was studied by exposing several independent lineages of *Salmonella enterica* to progressively increasing concentrations of cephalosporin. Each lineage carried a ß-lactamase gene ($bla_{\text{TEM-1}}$) that provided low resistance. In most lineages, the initial response to selection was a 2- to 40-fold increase in copy number of the $bla_{\text{TEM-1}}$ gene by tandem gene amplification. This amplification was followed in some lineages by acquisition of mutations in the *envZ, cpxA* or *nmpC* genes, which further enhanced resistance by reducing expression of the OmpC, OmpD and OmpF porins. Thus, the initial resistance provided by amplification of $bla_{\text{TEM-1}}$ allowed the bacterial population to expand sufficiently that rare point mutations could be realized. Mathematical modeling showed that amplification is the initial response because events that duplicate or further amplify a gene are much more frequent than point mutations. These models also support the importance of the initial population containing gene amplifications in allowing later point mutations. Transient gene amplification in bacterial populations is therefore likely to be a common initial mechanism and an intermediate in adaptive improvement of many pre-existing functions. When later point mutations (allowed by amplification) arise, the amplifications may be taken off selection and lost.

EVOLUTION OF A MINIMAL RIBOSOME

Christina Tobin

The bacterial ribosome contains an impressive number of proteins (r-proteins), the exact functional significance of which is largely unknown. The issues raised and investigated in this project are an attempt to shed some light on these proteins, in terms of their role in protein synthesis and how they may have contributed to ribosome evolution. The overall abundance of RNA at the major functional domains of the particle certainly raises an important question - if RNA is the principal mediator of ribosomal function, then what is the function of the extensive array of proteins and are they all essential? Our main aim is to use experimental evolution as a tool to understand the complexity of the ribosome by removing individual r-proteins and observing the resultant effects on bacterial physiology. Ribosomal gene knockouts have been created in *Salmonella typhimurium* LT2 by means of recombineering. To date, four knockout strains lacking small subunit proteins S6 and S20 and large subunit proteins L1 and L25 have been isolated. These mutants grow significantly slower than the wild-type strain indicating that ribosomal function is impaired. We are currently investigating the fitness cost associated with absence of each protein in terms of ribosomal polypeptide synthetic activity using both *in vivo* and *in vitro* systems. It has now become evident that ribosomes lacking small subunit protein S20 are severely defective in the initiation phase of translation, due at least in part, to a significant reduction in initiator tRNA binding. 30S subunits lacking S20 also display reduced association with 50S subunits. In contrast, absence of large subunit protein L1 does not significantly affect either initiation or dipeptide formation. By means of compensatory evolution we have shown that the fitness costs associated with r-protein loss can be mitigated by second-site compensatory mutations. Identification of these suppressor mutations is ongoing and provides a novel approach to extend our knowledge of the dynamics of protein synthesis, how it may have proceeded in the absence of these proteins, and how the ribosome may have evolved to the complex structure it is today in modern organisms.

COMPENSATING THE FITNESS COSTS OF ANTIBIOTIC RESISTANCE

Anna Zorzet

Antibiotic resistance is a rapidly growing problem in the community and infections that were once easily cured now pose a serious problem because the infecting microbe can be resistant to virtually every available antibiotic. However, resistance does not come without a price and resistance mutations often confer a fitness burden on the bacteria, typically displayed as a reduced growth rate and/or virulence. For many types of resistance mechanisms and for different bacterial species, it has been shown that the organism can reduce this loss of fitness by secondary compensatory mutations. Sometimes these intra- or intergenic compensatory mutations can restore fitness to wild type levels without loss of resistance. The focus of this study is to understand the mechanism of compensation of actinonin resistant mutants by extragenic mutations in *S. typhimurium*. We have previously found compensated resistant mutants with a 5- to 40-fold amplification of the *metZW* genes encoding initiator tRNA. Recently, we also found compensatory mutations in *infB*, encoding initiation factor 2 (IF2). We have expressed the mutant proteins and used them in an *in vitro* translation assay to

investigate the mechanism of compensation. Also, we are investigating the compensatory mechanisms of actinonin-resistant mutants of *S. aureus*, which seem to be quite different when compared to *S. typhimurium*.

A NEW DIAGNOSTIC METHOD FOR URINARY TRACT INFECTIONS

Anna Zorzet

Urinary Tract Infection (UTI), one of the most common infections among women 15 years old and older, have been diagnosed in the same way for many years and account for approximately 5% of all antibiotic prescriptions in adults. Diagnosis is made by culturing urine samples and testing for antibiotic susceptibility which usually takes at least two days for an accurate diagnosis. Empirical management is also a common way to diagnose a patient, but this method promotes unnecessary antibiotic use. In this project, we have been developing a new method for quick and accurate diagnosis of UTIs combining a genotypic and a phenotypic test simultaneously. The aims with developing this new diagnostic method have been to reduce the time needed for accurate diagnosing from days to hours and also to create a method that provides information about the pathogen's antibiotic susceptibility. The phenotypic part of the test is comprised of culturing the pathogen in urine in presence and absence of antibiotics. This will give information about the pathogens antibiotic susceptibility pattern and guidance in the choice of treatment. Characterization of the pathogen is performed genotypically by applying species-specific padlock probes, targeting species-specific regions the 16S rRNA in the bacteria, isolated from the cultured samples. The padlock probes that have bound to DNA are then amplified by rolling circle amplification and detected in a sandwich-type assay. By adding substrate, a colour change will report if the particular bacterium is present or not. Rolling circle amplification (RCA) is a very sensitive method with the capacity of amplifying very small amounts of starting material up to detectable levels, meaning that the urine samples only need to be cultured for a few hours to reach a detectable amount of cells, instead of overnight which is the case for the currently used methods. In the future, our goal is to transform the work of this project into a commercial kit, with a high throughput capacity of diagnosing urinary tract infections, which can be used in clinical laboratories. Also, in a wider perspective we aim at finding other areas where the basic technique of our method can be applicable.

THE INTERPLAY BETWEEN PATHOGENIC BACTERIA AND EPITHELIAL CELLS.

Helena Aro

Pathogenic microorganisms represent a serious threat to human health. Our group aim to identify the molecular mechanisms of how microbes exploit the host environment and how host responses occur as a consequence of microbial attack. The goal is to characterize factors of the pathogen and host that contribute to the severity of bacterial disease, especially caused by pathogenic *Neisseria*. By increasing our knowledge of the bacterial behaviour, we will have the better understanding the right targets for treatments. Our research projects covers the process of microbial infection, from initial adherence of the pathogen to long-term cellular response. In future, our research will contribute to new ways of developing vaccine candidates towards the goal of preventing bacterial disease.

Gonorrhoea is caused by the bacteria *Neisseria gonorrhoeae* is one of the most common sexually transmitted disease in the world after HIV/AIDS. It causes urogenital infections. Infected women also can pass gonorrhoea to their newborn infants during delivery, causing eye infections (conjunctivitis) in their babies (which if left untreated, can cause blindness). Between 30–60% of people with gonorrhoea are asymptomatic. This is lead to easy spread of the pathogen and long term infections that may lead to pelvic inflammatory disease (PID), infertility, ectopic pregnancy, and chronic infections.

Neisseria are gram negative diplococci, which only infects humans. The adhesion to epithelial cells is mediated via Type IV pili that recognize the human receptor CD46 on the cell surface. This interaction release intracellular calcium levels and opens up a link in a major G-protein- and Rho- mediated cross talk between the bacterium and the target cell. CD46-transgenic mice are now a model for *Neisseria* infections. Little is known about the intracellular life of the bacterium within target epithelium. We have shown that the infection decreases cell proliferation by reduceng levels of Cyclin B in infected cells, a good strategy to remain colonised for a longer period of time. Long-term infections may predispose tumor development. We attempt to develop a mice model for such chronic infections to further study the effect in cell proliferation and regulation upon infection. In addition, a new project has emerged during 2008. We have shown that there is a striking difference between different clinical *Lactobacillus* strains in interacting with and preventing gonococcal adhesion. This project is continued with great enthusiasm.

Members of the group during 2008

Helena Aro, group leader
Katarina Vielfort, PhD student
Sonja Löfmark, postdoc

Project students during 2008

Gabriella Bering, medical student
Sanna Hessel, biomedical student
Charlotta Hansson, biomedical student

International exchange during 2008

Gesine Kaiser, Berlin

Publications from 2004 to 2008

(Aro´s maiden name was Källström)

1. Vielfort, K., Sjölinder, H., Roos, S., Jonsson, H., and Aro, H. (2008) Adherence of clinically isolated lactobacilli to human cervical cells in competition with *Neisseria gonorrhoeae*. Microbes and Infection, doi: 10.1016/j.micinf.2008.07.032
2. Lövkvist, L., Sjölinder, H., Wehelie, R., Aro, H., Norrby-Teglund, A., Plant, L., and Jonsson, A-B. (2008) CD46 Contributes to the Severity of Group A Streptococcal Infection. Infect. Immun. 76(9):3951-3958.
3. Sjölinder, H., Lövkvist, L., Plant, L., Eriksson, J., Aro, H., Jones, A., and Jonsson, A-B. (2008) The ScpC Protease of Streptococcus pyogenes Affects the Outcome of Sepsis in a Murine Model. Infect. Immun. 76(9):3959-3966
4. Sjölinder, H., Eriksson, J., Maudsdotter, L., Aro, H., and Jonsson, A-B. (2008) Meningococcal outer membrane protein NhhA is essential for colonization and disease by preventing phagocytosis and complement attack. In press Infect. Immun.
5. Jones, A., Jonson, A-B., and Aro, H. (2007) *Neisseria gonorrhoeae* índuces Cyclin down regulation and early G1 arrest in human epithelial cells. FASEB J. 2007 21(2): 345-55.
6. Källström, H., Lindqvist, A., Pospisil, V., Lundgren, A., Karlsson-Rosenthal, C. (2005) Cdc25A Localisation and Shuttling: Characterisation of Sequences Mediating Nuclear Export and Import. Exp. Cell Res. 303(1): 89-100.
7. Lindqvist, A., Källström, H., Lundgren, A., Barsum, E., and Karlsson Rosenthal, C. (2005) Cdc25B cooperates with Cdc25A to induce mitosis but has a unique role in activating Cyclin B1-Cdk1 at the centrosome. J. Cell. Biol. 171: 35-45.
8. Kähler, A.K., Persson, A-S, Sánchez, F., Källström, H., Apt, A.S., Schurr, E., and Lavebratt, C. (2005) A new coding mutation in the Tnf-a leader sequence in tuberculosis-sensitive I/St mice causes higher secretion levels of soluble TNF-a. *Genes and Immunity* 6(7): 620-627.
9. Lindqvist, A., Källström, H., and Karlsson Rosenthal, C. (2004) Characterisation of Cdc25B localisation and nuclear export during the cell cycle and in response to stress. J. Cell Sci. 117; 4979-4990.
10. Rytkönen, A., Albiger, B., Hansson-Palo, P., Källström, H., Olcén, P., Fredlund, H., and Jonsson, A-B. (2004). Neisseria Meningititdis undergoes PilC phase variation and PilE sequence variation during invasive disease. J. Inf. Dis. 189; 402-409.

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THE HOST CELL CYCLE CONTROL AND REGULATION IN BACTERIAL INFECTIONS

Sonja Löfmark, Katarina Vielfort, Gabriella Bering

Microbial pathogens have developed a variety of strategies to manipulate host cell functions, presumably for their own benefit. The effects resulting in a cell cycle arrest, checkpoint response, and apoptosis is for me of particular interests. Passage through the cell cycle is mainly regulated by cyclins and cyclin dependent kinases (cdk's). Cell cycle checkpoints exist to stop the cell cycle in the event of unreplicated or damaged DNA, allowing time for repair or apoptosis. Bacteria can gain considerable advantage by arresting the cell cycle by causing cellular phenotypic changes in the host cell, possibly resulting in increased bacterial survival. We have shown that *N. gonorrhoeae* arrests the host cell in early G1 phase by down regulating the expression of cyclins.

To further dissect pathways involved in microbial pathogenesis and the control of mammalian cell cycle, we analyze the host cell cycle progression during *N. meningitidis* and *H. pylori* infection. We determine whether bacterial infections modify the cyclins and the cell cycle. Then, we search for the bacterial protein responsible for the cell cycle arrest, and find the pathway it uses for interfering with the cell cycle by staining with cell cycle markers, amphiregulin, p21, p27, p53 antibodies, checkpoint proteins and phosphorylations, and use inhibitors and activators of the Rho/Ras signalling pathway. Also, We further investigate the early G1 arrested cell phenotype. These cells have difficulties in exit mitosis and undergo cytokinesis.

THE BACTERIAL INDUCED PREDISPOSITION OF CELLULAR MALIGNANCIES

Helena Aro, Katarina Vielfort

Gonococcal infection in women is often asymptomatic and the bacteria can colonise a live at the site of infection for a long period of time. During this time, major damage can be made on the female reproductive system with even infertility as a consequence. There is growing recognition that infection with pathogenic bacteria can play a role in cancer risk and induction of tumour development. Cancer of the uterine cervix has been associated with sexually transmitted infections and a history of gonorrhoea was shown to be significantly associated with the risk of cancer in cervix, bladder and prostate.

We investigate the cellular response and damage made from long-term colonisation of pathogenic *Neisseria* in animals. The possible role of *Neisseria* predisposing cellular malignancies is fully examined. Since N. gonorrhoeae is an obligate human pathogen, we use the humanCD46 transgenic mouse model to set up a long-term infection of *N. gonorrhoeae* in the lower genital tract of female mice and the urethra of male mice. Preliminary results of

infection show that the gonococci can colonize. Analysis of organs and tissues will be performed to search for cellular abnormalities such as altered morphology, tumour antigen expression, cell cycle regulators and checkpoint events, p53, and the number of mitotic cells in the tissue.

THE FUNCTION OF OUR NORMAL FLORA IN PROTECTING AND PREVENTING BECTERIAL DISEASE

Katarina Vielfort, Sanna Hessel, Gesine Kaiser

Lactobacilli are acidic bacteria known to dominate the colonisation of the vaginal mucosa. The involvement of Lactobacilli and *Neisseria* infections are poorly understood, but studies suggest Lactobacilli protect the host against gonorrhea since clinical trials have shown that women suffering from endometritis have less Lactobacilli present. We analyse several *Lactobacillus* strains in their ability to inhibit *Neisseria* infections in human endocervical cell lines. In a recent published publication we show the interactions between lactobacilli and gonococci and investigated how they compete for adherence to human epithelial cervical cells. We show that lactobacilli adhere at various levels and that the number of adherent bacteria does not correlate to the level of protection against gonococcal infection. Protection against gonococcal adhesion varied between *Lactobacillus* species. *L. crispatus*, *L. gasseri* and *L. reuteri* were capable of reducing gonococcal adherence while *L. rhamnosus* was not. *Lactobacillus* strains of vaginal origin had the best capacity to remain attached to the host cell during gonococcal adherence. Further, we show that gonococci and lactobacilli interact with each other with resultant lactobacilli incorporation into the gonococcal microcolony. Hence, gonococci bind to colonised lactobacilli and this complex frequently detaches from the epithelial cell surface, resulting in reduced bacterial colonisation. We will continue with analysing the interplay between Lactobacilli and gonococci. We will set up a live cell imaging system with a continuous flow of a mixture of different lactobacilli species, to try to mimic the true situation in the human body.

THE BACTERIAL LIFE AND TRAVELING WITHIN THE HUMAN CELL

Helena Aro

During 15-20 hours after initial infection, the bacteria are localized inside the host cell. The intracellular compartment, in which the *Neisseria* resides and travels in during the passage through the epithelial cell layer, is still fairly unknown. It is yet not known where the bacteria reside in the cytoplasm and how the bacteria survive for 20 hours inside the cell. There is also much to learn about the bacterial growth and antigenic variation inside the host cell.

We focus the experiments to bacterial localization, survival and motility within the infected host cell. The intracellular localization of *Neisseria* within ME-180 cells and HEC-1B is studied. Also, a repertoire of cellular markers such as dsRED-Tubulin, CFP-Golgi, EYFP-Histone 2B, EYFP-coupled to Cyclin B and Cyclin A, is transiently transfected into the cell of interest. The cells are montaged in a live cell imaging system for advanced time-lapse and total internal reflection fluorescence (TIRF) imaging.

BACTERIAL PATHOGENESIS AND CELL SIGNALLING

Ann-Beth Jonsson

We study molecular mechanisms of the interaction and cross-talk between pathogenic bacteria and human target cells. Pathogenic bacteria use intricate strategies to exploit the host environment, such as induction of signal transduction pathways upon attachment to host epithelial cells, extensive antigenic variation of surface components, and interaction with regulators of complement activation.

Our research focuses on bacterial pathogenesis and modulation of host cell responses during life-threatening bacterial diseases caused by *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Streptococcus pyogenes* and *Helicobacter pylori*. How do bacteria manipulate the host defence mechanisms to survive within the host and cause disease? Understanding bacterial survival mechanisms are important and will open up alternative ways to prevent bacterial infections. We also study how long-term infections affect the cellular machinery and hope to find ways to prevent bacteria induced cell damages that lead to malignancies.

The first step in the process of establishing mucosal infection is adherence of the bacteria to target cells. These processes must involve complex interactions between adhesins and corresponding receptors in each membrane. Adherence of bacteria to epithelial cells may trigger and induce host cell signalling, such as calcium fluxes and phosphorylation of receptor proteins. Thus, an important function of bacterial adhesins may be to send a signal into the cell, initiating an upregulation and/or modification of the host cell surface that would lead to tight and stable adherence. Initially the bacteria must communicate with the host, and then exploit existing signal transduction networks to intimately attach and enter into the target cell. When the immune system fails to battle an infection or if the infection remains untreated, the disease may develop into a chronic infection. Bacterial modulation of host cells during long-term infections can lead cell damages and cancer.

The main goal is to better understand bacterial adherence, cell signalling, invasion, and immunity. We aim to dissect critical steps of bacteria-host cell interactions, and thereby contribute to development of novel appropriate therapies and vaccines against bacterial diseases.

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Publications from 2004 to 2008

1. Rytkönen, A., Albiger, B., Hanson-Palo P., Källström, H., Olcén, P., Fredlund, H., and Jonsson, A-B. (2004) *Neisseria meningitidis* undergoes PilC phase variation and PilE sequence variation during invasive disease. J. Infect. Dis, 189: 402-409.
2. Plant L., Asp, V., Lövkvist. L., Sundqvist, J., and Jonsson, A-B. (2004) Epithelial responses induced upon adherence of pathogenic *Neisseria*. Cell. Microbiol. 6: 663-670.
3. Bergman, P., Johansson, L., Asp, V., Gudmundsson, G., Jonsson A-B., and Agerberth., B. (2005) *Neisseria gonorrhoeae* downregulates expression of the antimicrobial peptide LL-37. Cell. Microbiol. 7:1009-1118.
4. Gorringe A.R., Reddin, K. M., Funnell, S. G., Johansson L., Rytkönen, A., and Jonsson A-B. (2005) Experimental disease models for the assessment of meningococcal vaccines. Vaccine, 23:2214-2217.
5. Bergman, P., Termén, S., Johansson, L., Nyström, L, Arenas, E., Jonsson, A-B., Hökfelt, T, Gudmundsson, G. H., and Agerberth, B. (2005) The cathelicidin rCRAMP is present in the central nervous system of the rat. J. Neurochem, 93: 1132-1140.
6. Jonsson, A-B., Normark, S., Rhen, M. (2005) Fimbriae, pili, flagella and bacterial virulence. Contrib Microbiol. 12:67-89. Review.
7. Johansson, L., Rytkönen, A., Wan, H., Bergman, P., Plant, L., Agerberth, B., Hökfelt, T., and Jonsson A-B. (2005) Human-like immune responses in CD46 mice. J. Immunol. 175: 433-440.
8. Bergman, P., Johansson, L., Wan, H., Gallo, R. L., Gudmundsson, G. H., Hökfelt, T., Jonsson, A-B., and Agerberth, B. (2006*)* The antimicrobial peptide mouse CRAMP reduces bacteremia and is induced in the blood brain barrier and meninges after meningococcal infection. Infect. Immun**.** 74: 6982-6991.
9. Linden, M., Tuohimaa, T., Jonsson A-B., Wallin, M. (2006) Brownian motors in a ring and type IV pilus retraction. Phys. Rev. E, Stat. Nonlin. Soft Matter Phys. 74(2 Pt 1):021908.
10. Plant, L., Wan, H., and Jonsson A-B. (2006) MyD88-dependent signalling affects the development of meningococcal sepsis by nonlipooligosaccharide ligands. Infect. Immun. 74: 3538-3546.
11. Plant, L. J., and Jonsson, A-B. (2006) Type IV pili of *Neisseria gonorrhoeae* influence the activation of human $CD4^+$ T cells. Infect. Immun. 74: 442-448.
12. Plant, L., Sundqvist, J., Zughaier, S., Lövkvist, L., Stephens' D., and Jonsson, A-B. (2006) Lipooligosaccharide structure contributes to multiple steps in the virulence of *Neisseria meningitidis*. Infect. Immun. 74: 1360-1367.
13. Plant, LJ, Wan, H., and Jonsson A-B. (2007) Non-lipooligosaccharide mediated signalling via toll-like receptor 4 causes fatal meningococcal sepsis in a mouse model. Cell Microbiol, 9: 645–656.
14. Jones, A., Jonsson, A-B, and Aro, H. (2007) *Neisseria gonorrhoeae* causes a G1 arrest in human epithelial cells**.** FASEB J. 21: 345-355.
15. Sjölinder, H., and Jonsson A-B. (2007) Imaging of disease dynamics during meningococcal sepsis. PLoS ONE. Feb 21;2:e241.

16. Sjölinder, H., Eriksson, J., Maudsdotter, L., Aro, H., and Jonsson, A-B. (2008) Meningococcal outer membrane protein NhhA is essential for colonization and disease by preventing phagocytosis and complement attack. Infect. Immun. 76:5412-5420.
17. Sjölinder, H., Mogensen, T. H., Kilian, M., Jonsson, A-B*., and Paludan, S. P.* (2008) Important role for TLR-like receptor 9 in host-defense against meningococcal sepsis. Infect. Immun. 76: 5421-5428. *contributed equally
18. Lövkvist, L., Sjölinder, H., Plant, L., Eriksson, J., Aro, H., Jones, A., Norrby-Teglund, A., and Jonsson, A-B. (2008) The ScpC protease of Streptococcus pyogenes affects the outcome of sepsis. Infect. Immun. 76:3959-3966.
19. Lövkvist, L., Sjölinder, H., Wehelie, R., Aro, H., Norrby-Teglund, A., Plant, L., and Jonsson, A-B. (2008) CD46 contributes to the severity of group A streptococcal infection. Infect. Immun. 76: 3951-3958.

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MENINGOCOCCAL DISEASE

Hong Sjölinder, Mari Karched, Björn Lindström, Ann-Beth Jonsson

Acute meningococcal sepsis is a rapid and life-threatening disease with overwhelming inflammatory responses and high mortality. Many bacterial pathogens, such as pathogenic *Neisseria*, cause disease only in humans, and have no other ecological niche. The absence of appropriate animal models has hindered development of therapies and stopped a rapid evaluation of potential vaccine candidates against disease. In order to develop an experimental model system for meningococcal disease, we used mice expressing human CD46 in potential target tissue. CD46 mice were susceptible to meningococcal disease, providing an important animal model for studying pathogenesis as well as for development of efficient meningococcal vaccines. To study disease development, we have monitored spread of bioluminescent bacteria in CD46 transgenic mice by a real-time biophotonic camera system. These studies demonstrated waves of bacterial clearance and growth, which selected for bacteria expressing the phase-variable virulence factor Opa, indicating the importance of this protein. In these imaging studies we also discovered that *N. meningitidis* accumulated in the thyroid gland, while thyroid hormone T4 levels decreased. The molecular mechanisms for these results will be investigated.

In order to analyze host recognition of meningococci, we infected TLR2, TLR4 and MyD88 knock-out mice, and showed that sepsis is mediated by LPS via TLR4 as well as by a non-LPS ligand that signals via MyD88 and mediates a strong proinflammatory response. We will identify and characterize this novel TLR4 interacting component(s) of meningococci. In addition, we have also started analysis of the response in TLR9 knockout mice recognizing CpG DNA. We will determine the role of TLR9 in meningococcal-activated inflammatory signal transduction and elucidate the mechanism of the TLR9-dependent anti-bacterial activity. Such studies will assist in generation of therapeutic and protective tools against bacterial disease.

BACTERIAL MODULATION OF ANTIMICROBIAL PEPTIDES

Allison Jones, Sonja Löfmark, Lisa Maudsdotter, Sara Eriksson, Ann-Beth Jonsson

In mammals, antibacterial peptides represent an important first line defence against invading pathogens. LL-37 belongs to the cathelicidin antimicrobial gene family and is expressed on mucosal cell surfaces and in neutrophils. This project involves studies of how bacteria modulate LL-37 in order to gain advantage and survive within the host. We have shown that pathogenic *Neisseria* down-regulate LL-37 in human epithelial cells, and we are currently characterizing the molecular mechanisms responsible for this effect. The long-term goal is to identify ways to make pathogenic bacteria more sensitive to LL-37, or to increase LL-37 expression in host cells, thus generating novel ways to fight bacterial infections.

To study the role of LL-37 during *N. meningitidis* infection, we used *Neisseria* microarrays to identify changes in the bacteria upon contact with LL-37. Bacteria show a rapid response to LL-37, with changes in transporter systems, capsular gene expression, and defined sets of outer membrane proteins. We identified 35 differentially regulated genes not previously reported to respond to external stimuli, many of which encode membrane proteins. Selected genes will be further analyzed and this will shed light on the mechanisms of evasion of innate defences and will help to develop drugs that enhance efficiency of innate immunity. Detailed understanding of the effector molecules in innate immunity and their induction might in the future lead to alternative treatments, which is of importance as antibiotic resistant bacterial strains are evolving with enormous costs for health care.

NOVEL VIRULENCE FACTORS OF NEISSERIA

Hong Sjölinder, Asaomi Kuwae, Ann-Beth Jonsson

By using two-dimensional gel electrophoresis, we have identified a number of bacterial proteins that are upregulated after attachment to epithelial cells. One protein identified was GNA992, a surface exposed lipoprotein conserved among a broad range of meningococcal strains. We have shown that this protein is essential for successful colonization of the nasopharyngeal mucosa and that the protein contributes to biofilm formation. Further, GNA992 protects bacteria from being phagocytosed by macrophages, but does not affect cytokine or chemokine responses. Interestingly, the protein also confers extensive serum resistance enabling the bacteria to survive in blood and to cause lethal disease. We show that GN992 directly protects bacteria from complement killing by preventing deposition of the membrane attack complex (MAC). This study reveals a multivalent impact of GNA992 during meningococcal disease, indicating that this protein is an important target for therapeutic treatments as well as for vaccine development.

We will further dissect the pathways for the function of GNA992 by identifying receptor interactions and characterize cell signalling. Also, we will purify the GNA992 protein, immunize CD46 transgenic mice, and subsequently challenge the mice with *N. meningitidis* to check for protection.

BACTERIAL IMMUNE EVASION OF GROUP A STREPTOCOCCI

Hong Sjölinder, Allison Jones, Ann-Beth Jonsson

Streptococcus pyogenes is a human pathogen that causes a wide range of diseases from pharyngitis to more severe streptococcal toxic shock syndrome and necrotizing fasciitis. The streptococcal M-proteins are important adhesins that mediate interaction with a range of host cell receptors, such as the soluble factor H and the cell surface glycoprotein CD46. *S. pyogenes* incubated with human cells for various time periods induced changes in host cell receptor expression. We have shown that *S. pyogenes* induces shedding of the complement regulator CD46 from host cells. The bacteria bind released soluble CD46 and this leads to increased survival in the presence of phagocytic cells. The protective role of CD46 was confirmed in a CD46 transgenic mouse model. *S. pyogenes* induced higher level of bacteremia and more severe disease in CD46 transgenic mice than in nontransgenic mice. This is an interesting finding that will be further evaluated, and the bacterial factor(s) that interact with CD46 will be identified. Further, the cell signalling mediated by CD46 after interaction with *S. pyogenes* will be investigated.

S. pyogenes secrets an IL-8 degrading protease called ScpC, which seems to be important for development of necrotizing fasciitis. We have shown that ScpC has an opposite role after blood infection where ScpC mutants caused more severe sepsis, higher bacteremia levels, and stronger induction of IL-6 and C5a compared to the wild-type strain. It is possible that the level of ScpC expression contributes to the diversity of diseases caused by *S. pyogenes* strains, and we will further analyze this.

CELLULAR MOTILITY AND MICROBIAL MOLECULAR MOTORS

Jens Eriksson, Miriam Geörg, Tim Sarkissian, Ann-Beth Jonsson

Molecular motors are biological machines that are responsible for most forms of movement we encounter in the cellular world. Three types of eukaryotic cytoplasmic motors are known: myosins, which move on actin filaments, and dyneins and kinesins, which use microtubules as tracks. Although a considerable amount of analysis have been done with the cellular motors above, not much is known about biological motors in smaller living cells, such as bacteria.

Movement of bacteria can for example be mediated by rotation of the bacterial flagella, or by twitching motility resulting from extension and retraction of the bacterial type-IV pilus. Type IV pili are polymeric structures found on a wide range of bacterial species, such as pathogenic *Neisseria*, *Pseudomonas*, *E. coli* and many more. They play a crucial role in bacterial movement and they mediate adhesion to surfaces, DNA uptake, biofilm formation, and even evoke signalling responses in other cells. A characteristic feature of Type IV pili is that these pili are able to generate considerable force by retraction. A membrane associated ATPase called PilT is required for pilus retraction, force generation, and movement of bacteria on surfaces. The mechanism they use to convert chemical energy into mechanical work is ATP hydrolysis that causes a small conformational change that is translated into movement. PilT deficient bacteria are unable to retract their pili leading to hyperpiliation and loss of motility. Further, PilT deficient bacteria are unable to enter into target cells. Pilus retraction is critical for host-cell responses and movement of bacteria through viscous membranes.

We study the molecular mechanisms of pilus-mediated motility and pilus retraction. The overall aim of this project is to characterize important aspects of motility of living cells. Deeper understanding of this biological motor system opens the possibility to inhibit pilus retraction. Interference with pilus retraction will block tight adhesion of bacteria to host cells, as well as bacterial invasion and spread. Further, it is likely that inhibitors of pilus retraction could act against many different bacterial species, since PilT is strictly conserved.

CHRONIC BACTERIAL INFECTION

Rahma Wehelie, Elin Wallin, Hasanthi Karunasekera, Helena Aro, Ann-Beth Jonsson

Microbial pathogens have developed a variety of strategies to manipulate host cell functions, presumably for their own benefit. Interference with the host cell cycle progression is likely beneficial for bacteria and may lead increased survival and spread of the invading pathogen. The cell cycle is divided into the phases: gap1 (G1), synthesis (S), gap2 (G2), and mitosis (M). Progression of the cell cycle is regulated by a complex and fine tuned array of processes. Many cells reside in a non-dividing G0 phase and can be activated to reenter the G1 phase. Passage through the cell cycle is mainly regulated by cyclins and cyclin dependent kinases (cdk's). Proper control ensures that initiation of each cell cycle step occurs only after successful completion of the previous step. Cell cycle checkpoints exist to stop the cell cycle in the event of unreplicated or damaged DNA, allowing time for repair or apoptosis. We are currently examining the detailed mechanisms of how *Helicobacter pylori* and pathogenic *Neisseria* interfere with cell cycle progression and how these processes contribute to development of disease, cell damage and malignancies. Further, we study the molecular mechanisms of how *H. pylori* manipulate the host cells to persist and survive at mucosal surfaces. For this purpose we use both *in vitro* and *in vivo* experimental model systems. Our data demonstrate that certain *H. pylori* strains interact with CD46. This will be further evaluated, and the bacterial factor(s) that interact with CD46 will be identified. Further, the cell signalling mediated by CD46 and *H. pylori* will be investigated. The aim is to characterize how bacteria manipulate and interfere with human cells in order to find alternative ways to prevent immune evasion and cell damage caused by bacteria.

RECOMBINATIONAL SPREAD OF ANTIBIOTIC RESISTANCE

Lars Sundström

Lateral DNA transfer is an important evolutionary strategy for genetic expansion in bacteria. In the newly transferred DNA pool antibiotic resistance genes are frequent. Plasmids and phage are self-transmissible between different bacteria but to be operational in gene spreading they must either pick up or eliminate genes. Integrons give an illustrative solution to this problem by allowing directed and precise gene module rearrangements through site-specific, tyrosine-based recombination. In being located both on plasmids and chromosomes, the integrons are strategic for communicating genes between transferable plasmids and constins on one hand, and stationary genomes on the other. The recombinational behaviour of integrons differs from most comparable integrating elements in the lack of capacity for transposing the integron to new genetic postions. Instead the recombination activity is devoted entirely to internal reorganization of the integron through site-specific integration or excision of internal segments called cassettes. Each cassette is mobile due to a 3' site, attC, and most of its length is occupied by a gene or pro-gene (ORF). Chromosomal integrons are widely occurring in microorganisms of environmental habitats including many examples of food chain bacteria. Plasmid borne integrons by contrast, are typically carrying 0-5 fully functional antibiotic resistance genes, while chromosomal integrons may carry 200 cassettes. The largest proportion of cassettes appear to originate from protein-coding sequences but have commonly deteriorated by mutations to a level they no longer yield a functional product.

Members of the group 2008

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Publications from 2004 to 2008

1. Johansson C, Kamali-Moghaddam M, Sundström L. 2004. Integron integrase binds to bulged hairpin DNA. Nucleic Acids Res. 32:4033-4043.
2. Grape M, Farra A, Kronvall G and Sundström L. 2005. Integrons and gene cassettes in clinical isolates of co-trimoxazole-resistant Gram-negative bacteria. Clin Microbiol Infect, 11: 185-192
3. Johansson, C, Samskog J, Sundström L, Wadensten H, Björkesten L, Flensburg J. 2006. Differential expression analysis of Escherichia coli proteins using a novel software for

relative quantitation of LC-MS/MS data. Proteomics. 6:4475-4485.
4. Grape M, Sundström L, Kronvall 2007. Two new dfr genes in trimethoprim-resistant integron-negative Escherichia coli. Antimicrob Agents Chemother. 51: 1863-1864.

REGULATED AND ORGANIZED GENE TRANSFER

Lars Sundström, Daniel Bajka

The interplay and regulation of integrons and commonly neighbouring mobile elements, especially IS91-like mobile elements called ISCRs, are studied. Several resistance cassettes have recombination sites indicating origins in a chromosomal integron of an environmental species and even examples of direct ancestry have been found. The IntI-proteins belong to the tyrosine recombinase family proteins such as lambda phage integrase or the Cre protein of phage P1 but differ by a regional structure used to bind cassette sites in a hairpin conformation. This structural feature allows lower sequence constraints than the classical mechanism. This operation on single-stranded DNA overcomes recognition problems raised by the wide variation among *attC* sites. In single-stranded DNA, *attC*, gets opportunity to form the recognized hairpin structure with bulging, critically positioned extrahelical bases. The handedness of the bulged harpins is opposite for either strand and explains why only the bottom strand is bound and cleaved in the initial phase of the recombination. Mutated *attC* sites as well as mutated forms of the IntI recombinases were assayed for in vitro binding of purified recombinase IntI1, by monitoring the electrophoretic retardation of complexes. Structural data recently published by other groups and modeling data, further explain the specificity aspects and how cleavage and rejoining of DNA are likely to occur in the process of recombination. Clarification of the complete cassette integration mechanism awaits further study. We also study regulation of integron recombination that may be boosted through single-stranded DNA recognition e.g. at stress-induced lesions. Several antibiotics are reported to cause inducing lesions in the DNA.

ORGANIZED GENE MOBILITY, THE MOBILOME

Lars Sundström, Emma Larsson

Integrons on plasmids are known to attract antibiotic resistance gene cassettes in a combinatorial fashion and curiously, integrons are commonly found adjacent to a second gene clustering mechanism, IS*CR*, related to the rolling circle replicating IS-transposon, IS*91*. The functional crosstalk between integrons and IS91-like elements is under study.

Plasmid integrons seem to have taken up their cassettes from chromosomal integrons that occur in some pathogens such as *V. cholerae* as well as from many plant pathogens, environmental species and organisms present in food or its production chains. The chromosomal integrons constitute a natural gene library with hundreds of cassettes per integron. This immense pool of floating genetic elements is available for horizontal tranfer to other bacteria via plasmid-borne integrons. The distribution, variation and function of chromosomal integrons in clinical, food chain isolates and environmental microorganisms are compared. The work is made in collaboration with Henning Sørum, Oslo.

Transposons connected with integrons borne on plasmids may operate in concert with integrons to form a network of DNA-transfer events. The probably largest group of bacterial transposons uses transposases with a metal-binding motif, DDE, which appears in related

structure contexts in different transposases and retroviral integrases. The Mu-like DDE transposon Tn*5090*/Tn*402* is the primary carrier of class 1 integrons. The terminal sequences of the Tn*5090*/Tn*402* element are organized in multiple repeats of 19 bp on either end. Upon binding to these repeats the transposase assembles into its active form. The transposases of all Mu-like elements need support from one or several auxilliary factors including an ATP-binding switch protein supposed to confer transposon immunity. Tn*5090*/Tn*402* in addition uses the cryptic protein, TniQ. In the case of Tn*5090*/Tn*402* transposition as a rule yields DNA fusions that need to be resolved by the site-specific resolvase, TniC. A remarkable property of Tn*5090* /Tn *402* is its insertion specificity for serine recombinases (other than TniC) bound to their sites. The molecular background of this unusual targeting effect is studied. The transposon work is done in collaboration with Mark Toleman, Cardiff.

MUTATIONS AND GENETIC TRANSFER CONTRIBUTE TO EVOLUTION AND STABLE PERSISTENCE OF DRUG RESISTANT MICROORGANISMS

Göte Swedberg

Asexually reproducing microorganisms mainly rely on mutations for genetic variation. However, bacteria have evolved a variety of genetic transfer mechanisms that enhance genetic exchange and evolution of new traits like antibiotic resistance. My main interest has been sulfonamide drugs that act by inhibition of folate synthesis, thereby interfering with biosynthesis of nucleotides and some amino acids. Clinically, sulfonamides are used in very small amounts for treatment of bacterial infections, therefore sulfonamide resistant bacteria are not selected and are good models for stable persistence of drug resistance. We have detected specific point mutations that can explain resistance but also found evidence for horizontal transfer of resistance genes. In malaria treatment, sulfonamides are still very important for combination therapy. The rapid development of resistance to antimalarial drugs in Africa is a serious problem and we follow the development by field studies. One aim of the project is to explain the evolution of drug resistance on a molecular level and hopefully point the way towards design of better inhibitors both for bacterial and malarial infections. This is done by biochemical analysis of the target enzyme, dihydropteroate synthase, and the biochemical pathway, folate biosynthesis, where this enzyme has its function.

Members of the group during 2008

Göte Swedberg, associate professor
Amany Kheir, PhD student
William Buwembo, PhD sudent (Makerere University, Kampala, Uganda)
Woraphol Ratanachuen, PhD student (Mahidol university, Bangkok, Thailand)
Kristina Lundberg, technician

Project workers during 2008

Izla Baravdish: Effects of amino acid deletions in malarial hydroxymethylpterin pyrophosphokinase.
Fatemeh Gheysari: Methods for detection of polymorphism in the PfATP6 gene.
Nizar Enweji: Construction of control plasmids for mutation detection in the PfATP6 gene.

International exchange during 2008

William Buwembo, Makerere University, Uganda, worked in the lab from May 15 to July 31.
Hamza Babiker, Sultan Qaboos, University, Oman, visited the lab from July 15 to August 2.
Göte Swedberg visited Makerere University and Bugando Medical College, Mwanza, Tanzania in November, one week each place.

Publications from 2004 to 2008

1. Eriksen J, Mwankusye S, Mduma S, Kitua A, Swedberg G, Tomson G, Gustafsson, LL, Warsame M. 2004. Patterns of resistance and DHFR/DHPS genotypes of *Plasmodium falciparum* in rural Tanzania prior to the adoption of sulfadoxine—pyrimethamine as first-line treatment. Transactions of the Royal Society of Tropical Medicine and Hygiene 98: 347—353.
2. Jönsson M, Qvarnström Y, Engstrand L, Swedberg G. 2005. Clarithromycin treatment selects for persistent macrolide-resistant bacteria in throat commensal flora. Int J Antimicrob Agents 25:68-74.
3. Jönsson M, Swedberg G. 2005. Hydroxymethyldihydropterin pyrophosphokinase from *Plasmodium falciparum* complements a folK-knockout mutant in *E. coli* when expressed as a separate polypeptide detached from dihydropteroate synthase. Mol Biochem Parasitol140:123-5.
4. Sendagire H, Kyabayinze D, Swedberg G, Kironde F. 2005. *Plasmodium falciparum*: higher incidence of molecular resistance markers for sulphadoxine than for pyrimethamine in Kasangati, Uganda. Trop Med Int Health 10: 537-43.
5. Arakere G, Nadig S, Swedberg G, Macaden R, Amarnath SK, Raghunath, D. 2005. Genotyping of methicillin-resistant *Staphylococcus aureus* strains from two hospitals in Bangalore, South India. J Clin Microbiol 43: 3198-202.
6. Sendagire H, Kaddumukasa M, Ndagire D, Aguttu C, Nassejje M, Pettersson M, Swedberg G, Kironde F. 2005. Rapid increase in resistance of *Plasmodium falciparum* to chloroquine-Fansidar in Uganda and the potential of amodiaquine-Fansidar as a better alternative. Acta Tropica 95: 172-82.
7. Jönsson M, Swedberg G. 2006. Macrolide resistance can be transferred by conjugation from viridans streptococci to *Streptococcus pyogenes*. Int J Antimicrob Agents. 28:101-3
8. Qvarnström Y, Swedberg G. 2006. Variations in gene organization and DNA uptake signal sequence in the *folP* region between commensal and pathogenic *Neisseria* species. BMC Microbiol. 2006 Feb 17;6:11.
9. Eriksen J, Mwankusye S, Mduma S, Veiga MI, Kitua A, Tomson G, Petzold MG, Swedberg G, Gustafsson LL, Warsame, M. 2008 Antimalarial resistance and DHFR/DHPS genotypes of *Plasmodium falciparum* three years after introduction of sulfadoxine-pyrimethamine and amodiaquine in rural Tanzania. Trans R Soc Trop Med Hyg 102: 137-42.

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RESISTANCE TO ANTIFOLATE DRUGS IN TREATMENT OF MALARIA AND EVALUATION OF NEW DRUG TARGETS

Erasmus Kamugisha, Izla Baravdish, Woraphol Ratanachuen, Nizar Enweji, Fatemeh Gheysari

Several antimalarial drugs act on the folate metabolism affecting synthesis of DNA precursors, especially dTTP. Examples are Fansidar, which is a combination of pyrimethamine and sulfadoxine, and LapDap, which is a combination of chlorproguanil and dapsone. This project

involves further characterization of one already known drug target, the bifunctional enzyme HPPK-DHPS. An expression clone giving good amounts of bifunctional enzyme has been constructed. The plasmodial enzyme contains long stretches of amino acids that do not align with the corresponding bacterial enzymes. We are generating deletions in these stretches and have found both sequences that can be removed without losing enzyme activity as well as sequences that seem to be necessary for function. The enzyme kinetic analysis is carried out by Woraphol Ratanachuen at Mahidol University, Bangkok, Thailand.

Another approach is to evaluate different treatment strategies by genotyping of malaria parasites exposed to antifolate drugs. This is done by PCR-based analysis of parasite DNA in blood samples from patients undergoing anti-malaria therapy. The project is based on a collaboration with Makerere University, Kampala, Uganda and Bugando Medical College in Mwanza, Tanzania. Both countries are now switching from using antifolates to a drug combination with artemisinin and lumefantrine (coartem). The project is aimed at analysing the genetic changes in the parasites that results from this change in drug use. In vitro analysis of developing resistance to coartem are set up in Kampala and Mwanza, while improved methods for mutation detection are developed in Uppsala. The work during 2008 has mostly focused on methods for detection of emerging resistance to artemisinin coumpounds.

EVOLUTION OF DRUG RESISTANT *PLASMODIUM FALCIPARUM* IN EASTERN SUDAN

Amany Kheir, Nizar Enweji

The project addresses two related questions:
1) Are drug resistant *Plasmodium. falciparum* genotypes in Sudan independent lineages or similar to those originally selected in south east Asia and later appearing in east and southern Africa?
2) Do mutant *P. falciparum* genotypes that persist during the lengthy dry and anti-malarial drug free period have lower fitness (ability to multiply and produce transmissible stages), compare to drug sensitive ones?

So far a number of patients with PCR positive samples from a full year has been recovered and they will form the basis for continued analysis of stability of parasite markers.

In a pilot study we identified a major drug resistant *dhfr* haplotype connected to a specific microsatellite pattern among parasites that existed during the transmission season in Asar village in eastern Sudan, which differs in its genetic background to those identified in South Africa and Tanzania, as well as in Kenya. Our findings supports the notion that *dhf*r resistant haplotypes probably develop independently in different countries rather than due to migration and gene flow. In future work we wish to build on the above findings to investigate the origin of drug resistant *P. falciparum* in Sudan and examine persistence and gametocyte production (fitness) of drug resistance *P. falciparum* haplotypes and microsatellite patterns, in presence and absence of anti-malarial drug usage. The hypothesis we wish to test is that the evolution of drug resistance is limited by the lower fitness of resistant parasites in the absence of drug pressure.

ESTABLISHMENT OF A DRUG-RESISTANT BACTERIAL FLORA IN HUMANS

Göte Swedberg, William Buwembo

The use of antibacterial drugs exerts a selection pressure for drug resistant bacteria. Most important for the long term use of antibiotics is the impact of drug use on the establishment of drug resistant bacteria in the flora of commensal bacteria, that persist in the human body. Transfer of resistance determinants from commensal streptococci to *Streptococcus pyogenes* has been detected and one vehicle of transfer has been identified as a recently described defective conjugative transposon. In cases where we detect transfer, the defective transposon is inserted into a larger conjugative element, which is only partly characterized. Work during 2008 has been targeted towards further characterization of this conjugative element, which is partly related to a transferable element found in *Streptococcus thermophilus,* but some functions linked with excision and insertion of the element are more similar to elements found in *Streptococcus equi* subsp *zooepidemicus*.

William Buwembo has been investigating drug resistance in oral streptococci. Earlier he established that the majority of isolates of *Strep pneumoniae* and related viridans streptococci are resistant to sulfonamides and trimethoprim, and that the mechansim of resistance is in both cases mutational changes in the target enzymes dihydropteroate synthase and dihydrofolate reductase. In contrast, *Strep mutans* and *Strep sobrinus* from general dentistry patients show very low levels of resistance to these drugs. However, HIV-positive patients taking co-trimoxazole prophylaxis have elevated levels of resistant isolates. A number of these have now been analysed and there are no detectable changes in the target enzymes in resistant isolates. Besides, most resistant isolates rapidly loose resistance when cultivated in the lab. We will now investigate other possible resistance mechanisms.

MOLECULAR VIROLOGY/VIRAL MODEL SYSTEMS

ADENOVIRUS IN BASIC AND MEDICAL RESEARCH

Göran Akusjärvi

Viruses typically encode for a few potent regulatory proteins that have the capacity to rapidly and efficiently inactivate host cell gene expression, resulting in a selective synthesis of virus specific gene products in infected cells. The great advantage with viruses is that they are small and therefore offer a simple genetic system that is easy to manipulate in vitro. Further, the mechanisms discovered in viral model system often recapitulates what life does in general. Thus, there have been, and still are, several lessons to be learned from studies of our viruses. Our current work is focused around six areas covering basic mechanisms in gene expression and medical applications of viral vectors.

We study:
- The remodeling of the host cell RNA splicing machinery during an adenovirus infection.
- The role of the viral proteins L4-33K and L4-22K in virus-specific splicing and transcription
- The structure and function of the miRNAs encoded by adenovirus.
- The mechanisms in, and significance of, adenovirus suppression of RNA interference (RNAi).
- Construction of novel recombinant adenovirus systems for efficient siRNA delivery in cancer gene therapy.
- Significance of protein kinase A (PKA) in constitutive and alternative RNA splicing

Members of the group during 2008

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Heidi Törmänen, PhD student
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Project workers during 2008

Khalid Saaed, cloning of cellular targets to mivaRNAs

Publications from 2004 to 2008

1. Lee, T.W.R, Dauksaite, V., Blair, G.E., Akusjärvi, G. and Matthews, D.A. (2004). Precursor of adenovirus core polypeptide Mu targets the nucleolus, modulates the expression of E2 proteins and alters splice site selection fo E1A pre-mRNA in vitro. J. Gen. Virol. 85, 185-196.
2. Dauksaite, V. and Akusjärvi, G. (2004). The second RNA binding domain of human splicing factor ASF/SF2 is the critical domain controlling adenovirus E1A alternative 5' splice site selection. Biochemical J 381, 343-350.
3. Barbu, A.R., Akusjärvi, G., Welsh, N. (2005). Adenoviral-mediated transduction of human pancreatic islets; importance of adenoviral genome for cell viability and association with a deficient antiviral response. Endocrinology 146, 2406-2414.
4. Lützelberger, M. Backström, E. and Akusjärvi, G. (2005). Substrate dependent differences in U2AF requirement for splicing in adenovirus-infected cell extracts. J. Biol.Chem. 280, 25478-25484.
5. Andersson, G., Haasnoot, J., Xu, N., Berenjian, S., Berkhout, B. and Akusjärvi, G. (2005). Suppression of RNA interference by adenovirus VA RNA. J. Virol. 79, 9556-9565.
6. Mühlemann, O. and Akusjärvi, G. (2006). Preparation of soluble extracts from adenovirus-infected cells for studies of RNA splicing. In Methods in Molecular Biology. Ed. W.S.M. Wold. Humana Press Inc., Totowa, New Jersey. 131, 33-46.
7. Berenjian, S. Akusjärvi, G. (2006). Binary AdEasy vector systems designed for Tet-ON or Tet-OFF regulated control of transgene expression. Virus Res. Virus Res. 115, 16-23.
8. Andersson, G., Xu, N. and Akusjärvi, G. (2006). In vitro methods to study RNA interference in adenovirus-infected cells. In Methods in Molecular Biology. Ed. W.S.M. Wold. Humana Press Inc., Totowa, New Jersey. 131, 47-61
9. Öhrmalm, C. and Akusjärvi, G. (2006). The cellular splicing/transcription regulatory protein p32 represses adenovirus major late transcription unit and causes RNA polymerase II hyperphosphorylation. J. Virol. 80, 5010-5020.
10. Törmänen, H., Backström, E., Carlsson, A. and Akusjärvi, G. (2006). L4-33K, an adenovirus encoded alternative RNA splicing factor. J. Biol. Chem. 281, 36510-36517.
11. Kvissel, A.K., Orstavik, S., Eikvar, S., Brede, G., Jahnsen, t., Collas, P., Akusjärvi, G. and Skålhegg, B. S. (2007). Involvement of the catalytic subunit of PKA and HA95 in pre-mRNA splicing. Submitted Exp. Cell Res. 313, 2795-2809.
12. Akusjärvi, G., Kreivi, J-P. and Petersen-Mahrt, S. (2007). Messenger RNA in eukaryotes. In: ENCYCLOPEDIA OF LIFE SCIENCES. John Wiley & Sons, Ltd: Chichester http://www.els.net/ [10.1002/9780470015902.a0000875.pub2. (Invited paper subjected to peer review).
13. Xu, N. Zhou, X. Segerman, B. and Akusjärvi, G. (2007). Adenovirus VA RNAII derived small RNAs are efficiently incorporated into RISC and associate with polyribosomes. J. Virol. 81, 10540-10549.
14. Ruuskanen, O., Meurman, O., Metcalf, J. and Akusjärvi, G. (2007). Adenoviruses. In Clinical Virology, (ed. D. Richnmond, R. Whitley, F. Hayden). ASM Press, Washington. (invited review). In press.
15. Akusjärvi, G. (2008). Temporal regulation of adenovirus alternative RNA splicing. Front Biosci., 13, 5006-5013.
16. Dyachok, O., Idevall-Hagren, O., Sågetorp, J., Tian, G., Wuttke, A., Arrieumerlou, C., Akusjärvi, g., Gylfe, E. and Tengholm,m A. (2008). Glucose-idnuced cyclic AMP oscillations regulate pulsatile insulin secreation. Cell Metab. 8, 26-37

17. Sol, E., Sargsyan, E., Akusjärvi, G. and Bergsten, P. (2008). Glucolipotoxicity in INS-1E cells is counreracted by carnitine palmitoyltransferase 1 over-expression. BBRC 375, 517-521.
18. Somberg, M., Rush, M., Fay, J., Ryan, F., Lambkin, H., Akusjärvi, G. and Schwartz, S. (2008). Adenovirus E4ORF4 induces HPV-16 late L1 mRNA production. Virology in press.

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TO SPLICE OR NOT TO SPLICE THAT IS THE DIFFERENCE BETWEEN MAKING A SPLICING FACTOR OR A TRANSCRIPTION FACTOR

Ellenor Backström

Expression of adenoviral late genes is regulated both at the level of transcription and alternative 3' splice site usage. We are studying how gene products from the adenoviral L4 region is involved in this highly regulated process.

The L4-22K and L4-33K proteins are highly related and have the same 106 N-terminal amino acids. They differ from each other in that the L4-33K protein is translated from the mRNA that is internally spliced, that changes the translational reading frame and gives the two proteins distinct C-terminal sequences.

Previously, we showed that L4-33K functions as a virus-encoded alternative RNA splicing factor that preferentially activates splicing of transcripts with weak 3' splice sites: A hallmark of many late adenoviral pre-mRNAs. We are investigating the mechanism of L4-33K alternative 3' splice site activation. Preliminary data indicates that, in contrast to our results from the *in vitro* system, L4-33K may not be a sequence and/or context dependent factor *in vivo*, but rather acts as a distal splice site activator.

After the onset of viral genome replication, a complex of viral protein IVa2 and a protein expressed from the L4 region has been suggested to function as a transcription factor activating major late transcription. Reports from two different groups claim the L4 product to be L4-33K and L4-22K, respectively. We show that the L4-22K protein functions as the viral protein activating major late transcription whereas the L4-33K is a virus encoded RNA splicing factor.

DEVELOPMENT OF A NEW GENERATION OF shRNA EXPRESSING ADENOVIRUS VECTORS POTENTIALLY USEFUL IN CANCER GENE THERAPY.

Sofia Gkountela

Adenoviruses are generally associated with mild infections in humans. The fact that they are stable, can infect a broad range of human cells, including those of lung, liver, blood vessels, brain and that they don't integrate into the host chromosome make them particularly attractive as gene transfer vectors for gene therapy. In our lab we study human adenovirus type 5. This virus encodes two approximately 160 nt long non translated RNA polymerase type III

transcripts, the virus associated (VA) RNAs, VA RNAI and VA RNA II. During the late infection VA RNAI accumulates at 10^8 copies / cell.

In this work we wanted to examine the efficiency of RNAi induced by expression cassettes driving shRNA synthesis using the adenovirus VA RNAI promoter but also the commonly used H1-small RNA promoter. We constructed a 29 bp short hairpin RNA designed to target the BCR-ABL fusion protein expressed in CML. We have selected CML as a model system in our studies since this tumor type critically depends on the expression of the BCR-ABL fusion protein. Both types of promoter constructs were reconstructed into the AdEasy adenovirus vector system. As a reporter gene we used a plasmid expressing a part of the breakpoint region of the BCR-ABL fused to E-GFP and also constructed a viral vector expressing the same reporter gene. Results show that shRNA delivery via a viral vector system is more effective compared to plasmid DNA transfection and further produced a silencing effect that was more rapid compared to transfection. Furthermore Northern blot analysis reveals that the sh/siRNA accumulating in infected cells is significantly more compared to the transfected cells.

INVOLVEMENT OF THE CATALYTIC SUBUNIT OF PROTEIN KINASE A IN PRE-MRNA SPLICING

Anne-Katrine Kvissel

Protein kinase A (PKA) is involved in multiple signalling pathways regulating a vast number of cellular processes, such as metabolism, gene expression, cell growth and differentiation. In its inactive form PKA is a holoenzyme consisting of two catalytic (C) subunits bound to a regulatory (R) subunit dimer. Upon cAMP-stimulation, the enzyme dissociates and the C subunits are released to phosphorylate target substrates in its vicinity, but it is also known that a proportion of the C subunits translocates to the nucleus. Apart from transcriptional regulation, little is known about C subunit functions in the nucleus.

We have recently shown that a subpopulation of the C subunit is constitutively localized to splicing factor compartments. Using an adenovirus E1A *in vivo* splicing assay we found that catalytically active C subunits promote distal 5' splice site activation. Surprisingly, cAMP stimulation alone did not affect the E1A splicing pattern, which could indicate that PKA regulates alternative splicing through a cAMP-independent mechanism. We have also shown that the PKA C subunit phosphorylates several members of the SR-protein splicing factor family *in vitro*, and more specifically, preliminary results indicate that PKA phosphorylates ASF/SF2 on S119 located within the RRM2 domain.

To get further insight into how PKA regulates alternative splicing, we will investigate to what extent the C subunits are able to regulate splice site selection *in vitro*. For these experiments we have generated stable cell lines expressing either Ca1 or the kinase-inactive mutant Ca1K73M. We are also in the process investigating the significance of PKA phosphorylation for ASF/SF2 function both *in vitro* and *in vivo*. In addition, our preliminary experiments show that PKA phosphorylates the adenoviral L4-33K protein and also causes a suppression of adenovirus IIIa pre-mRNA splicing. Since the L4-33K protein is believed to be the key regulator of IIIa splicing PKA may have a natural function as a factor controlling the early to late shift in adenovirus replication.

CHARACTERIZATION OF SPLICEOSOME ASSEMBLY IN ADENOVIRUS-INFECTED NUCLEAR EXTRACTS

Heidi Törmänen, Gunnar Alkemar

Alternative splicing is temporally regulated during an adenovirus infection to produce two transcripts from the L1 unit, the 52,55K and IIIa mRNAs. The 52,55K mRNA (proximal 3' splice site) is produced both early and late after infection whereas production of the IIIa mRNA (distal 3' splice) is confined to the late phase of infection. The two key elements regulating IIIa 3'-splice site selection is the IIIa repressor element (3RE) and the IIIa virus-infection dependent splicing enhancer element (3VDE) that acts as a molecular switch regulating IIIa splicing. We have shown that the adenoviral L4-33K protein activates IIIa splicing via the 3RE/3VDE elements and thus imitates the splicing activation observed during a virus infection. Further, L4-33K also activates the early to late switch in L1 alternative splicing both in vivo and in vitro. It activates splicing via non-consensus 3' splice sites. L4-33K stimulates an early step in spliceosome assembly and appears to be the only viral protein necessary to convert a nuclear extract prepared from uninfected cells to an extract with splicing properties very similar to a nuclear extract prepared from adenovirus late infected cells.

We are currently studying how the adenoviral L4-33K protein remodels the host cell RNA splicing machinery in uninfected HeLa cell nuclear extract supplemented with L4-33K. The work aims at finding the cellular proteins that interact with L4-33K in order to be able to mechanistically explain how L4-33K stimulates spliceosome assembly. MS sequencing of affinity purified spliceosomes will be used to establish the protein composition of spliceosomes assembled in HeLa cell nuclear extract supplemented with L4-33K as well as in adenovirus-infected cells.

ADENOVIRAL CONTROL OF THE RNAI/MIRNA PATHWAYS IN HUMAN CELLS

Ning Xu, Khalid Saeed

It is well documented that RNAi is an antiviral mechanism in plants and insects, whereas it is still unclear whether RNAi naturally limit viral infections in vertebrates.

In our previous work we have shown that human adenovirus inhibits RNAi by blocking the activity of Dicer and the RNA-induced silencing complex (RISC). The virus-associated RNAs, VA RNAI and VA RNAII bind Dicer through their terminal stems and are cleaved by Dicer into functional siRNA, which are incorporated into RISC. By cloning and sequencing small RNAs we have shown that approximately 80% of Ago2-containing RISC immunopurified from late infected cells is associated with VA RNA-derived small RNAs (mivaRNAs). VA RNAII appears to be the preferred substrate for Dicer and accounts for approximately 60% of all small RNAs in RISC. Collectively, our results suggest that the mivaRNAs are efficiently used for RISC assembly in late-infected cells. Potentially they function as miRNAs regulating translation of cellular mRNAs.

It has been reported that human adenovirus type 5 VA RNAI has two transcription initiation sites, which produces two clusters of VA RNAI with 3nt difference at their 5' end. We have observed that this heterogeneity contributes to the strand bias of VA RNAI incorporating into RISC. We are currently characterizing the mechanism(s) controlling 3' or 5' strand incorporation.

Our previous results have shown that the efficiency of RNAi is enhanced in a cell line stably over expressing the Ago2 protein, a result that suggests that Ago2 is a limiting factor for RNAi. Adenovirus is an important viral vector system that has been designed for short hairpin RNA delivery to target cells. We speculate that by increasing the amount of the Ago2 protein in target cells, the efficacy of RNAi should be improved. At present, we are testing this hypothesis by constructing a recombinant adenovirus, which expresses the Ago2 protein from an inducible promoter. This virus may be beneficial for the therapeutic application of RNAi.

CHARACTERIZATION OF STRUCTURE AND FUNCTION OF THE ADENOVIRAL PROTEINS L4-22K AND L4-33K

Sara Östberg

The adenovirus L4 region encodes for two proteins of interest to our lab, the L4-33K and L4-22K. These proteins are highly related and share the 106 N-terminal amino acids, but differ by having unique C-terminal sequences. Previous work has shown that L4-33K acts as a virus-encoded RNA splicing factor that preferentially activates alternative splicing of transcripts with weak 3' splice sites, a hallmark of many adenoviral late mRNA's. The addition of L4-33K to an uninfected HeLa cell nuclear extract is necessary and sufficient to give it the same splicing properties as a nuclear extract prepared from adenovirus late-infected HeLa cells. This generates evidence of L4-33K being a key player in the early to late switch of adenoviral protein production.

The function of L4-22K is currently ambiguous. Published data have implicated the protein as a virus assembly factor and as a transcription factor stimulating adenovirus major late promoter (MLP) activity. However, there has also been data suggesting that L4-33K is the transcription factor activating major late transcription. Our results (see Backström) points to L4-22K being the sole transcriptional activator of the MLP.

Our aim is to structurally and functionally dissect the functions associated with the L4-33K and L4-22K proteins, and also investigate the structural motifs of the L4-33K pre-mRNA. We will also investigate the distribution of these proteins in the cell, by transient transfection assays and immunofluorescence staining.

REPLICATION AND GENETIC STABILITY OF POLYOMAVIRUS

Göran Magnusson

The Polyomavirus family consists of closely related viruses that infect mammals, including humans, and birds. Infection of so-called permissive cells leads to massive virus production and cell death. In contrast, infection of cells that do not sustain virus replication occasionally results in malignant transformation. In vivo, infection of the natural host species leads to life-long, inapparent infections. Under natural conditions, induction of tumours is exceedingly rare. However, in animal experiments polyomavirus causes tumours in newborn rodents at a high frequency. In immunocompromised individuals, large amounts of virus are produced that eventually leads to disease. In humans, two conditions with polyomavirus activation are particularly problematic. One is a consequence of immunosuppressive treatment after kidney transplantation. The other occurs in AIDS.

Members of the group during 2008

Göran Magnusson, professor
Lena Möller, technician

GENOME INSTABILITY OF MOUSE POLYOMAVIRUS LINKED TO DISORDERD INITIATION OF DNA SYNTHESIS

Göran Magnusson and Lena Möller

DNA viruses are genetically stable and, in general, evolve at rates similar to those of their hosts. However, there are parts of the viral genomes that mutate at much higher frequencies. To facilitate replication of viral DNA, polyomaviruses are able to induce the infected cell to enter the cell division cycle. This capability is the basis for the tumorigenic potential of polyomaviruses. When infected cells enter the S-phase of the cell cycle, viral and cellular DNA synthesis is carried out by the same mechanism, except for the initiation step. Although cellular and viral origins of replication are similarly organized, the polyomavirus ori^{rep} is recognized and activated by a viral DNA helicase. In addition to the presence of binding sites for the viral helicase, an adjacent transcriptional enhancer, rendering cell specificity to the replication process, influences the activity of ori^{rep}. Importantly, the enhancer of polyomavirus genomes is prone to rearrange if the enhancer has been disabled by genetic manipulation, or when the virus is grown in cell types, where the enhancer has a suboptimal function.

Our data show that the enhancers of two mouse polyomaviruses, a mutant murine polyomavirus (MPyV) and wild-type murine pneumotropic virus (MPtV), are hypervariable. The hypermutation phenomenon can be observed because the structural changes lead to an increase of reproductive fitness of the viral genomes. Since the occurrence of a mutation and its establishment in a population of genomes are separate events, polyomavirus offers a useful model for the study of replication-induced mutations in the cellular genome.

The observed mutations at the viral ori^{rep} did not represent a random sample of sequence variation. PCR analysis showed of viral DNA showed that structural variation occurred exclusively close to ori^{rep}, and that many more types of sequence variants were present in the

infected cells than in propagated virus. Thus, the variants that became established in the population must have undergone functional selection. Analysis of replicating DNA molecules showed that the appearance of duplications and deletions in viral genomes was linked to the presence of abnormal structures in replicating DNA molecules. These abnormalities were common only in genomes with a disabled enhancer. Moreover, inhibition of ATM and ATR, two DNA damage-induced protein kinases, increased the occurrence of the abnormal replicative intermediates. This result corroborates our working hypothesis that the duplication events in the MPyV enhancer arise by non-homologous recombination following repair of double-strand breaks. These breaks probably arise during resolution of improperly initiated replication forks. Further experiments will be directed to investigation of the coupling of enhancer disability and collapse of replication forks.

VIRAL GENE REGULATION

Stefan Schwartz

Human papillomavirus type 16 (HPV-16) is a very common small DNA tumour virus that is intimately linked to cervical cancer in women. The vast majority of all infections are subclinical and are efficiently cleared by the immunocompetent host. However, HPV-16 can establish persistence in rare cases. These persistent infections may cause dysplasias, premalignant lesions that increase the risk of progression to cancer. At the molecular level, these lesions are characterised by the continuous production of the early viral proteins, in particular E6 and E7 that inhibit the functions of p53 and pRb, whereas the late genes encoding the viral structural proteins L1 and L2 are silent. Normally, L1 and L2 are produced in the upper layers of the infected epithelium, in the terminally differentiated cells. This adaptation of the virus to the host cell probably reflects the high immunogenicity of L1 and L2. One may speculate that inhibition of late gene expression is a prerequisite for cancer progression. It is therefore of interest to determine how HPV-16 L1 and L2 late gene expression is regulated.

Human immunodeficiency virus type 1 (HIV-1) infection results in AIDS in the infected host. Over 50 million people have been infected with HIV-1, but preventive vaccines are still not available. Antiviral drugs have been successfully developed, but are haunted by the appearance of drugs-resistant variants of HIV-1. There is a constant demand for new antiviral drugs. Here we study HIV-1 splicing regulation to identify splicing regulatory elements in HIV-1 that are functionally important in context of the viral genome. Splicing factors that affect the HIV-1 splicing program will be identified. We wish to identify small molecules and antisense molecules that interfere with the major, essential splicing elements on HIV-1.

Influenza A virus has an amazing ability to rapidly change its properties. We believe that pathogenic properties of an influenza virus could be determined also by differences in influenza virus RNA sequence, in addition to genetic changes that affect the sequence of viral proteins. The immediate goal of this project is to investigate if naturally occurring RNA sequence variations in various influenza virus isolates with different pathogenic properties cause differences in influenza virus RNA structure and function. The long-term goal of this project is to determine if the influenza virus RNA sequence itself, independently of effects on viral protein sequence, affects viral pathogenesis and tropism.

Hepatitis C virus (HCV) is a hepatotropic RNA virus that causes persistent infections that may result in liver cancer. This virus has been difficult to study as it does not replicate in vitro. More than 180 million people are chronically infected with HCV around the world. The HCV prevalence is particularly high in Egypt. We have initiated a collaboration with researchers in Egypt to identify cellular RNA binding proteins that may be required for efficient replication of HCV. This information may be used to establish a cell culture system for propagation of HCV in vitro.

Members of the group during 2008

Stefan Schwartz, group leader
Xiaoze Li, project student
Sofia Lindström, graduate student
Samir Abdurahman, post doctoral Researcher
Monika Somberg, graduate student
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International visitors during 2008

Maha Adel El Demellawy, professor, visiting from Genetic Engineering and Biotechnology Research Institute, Alexandria, Egypt

Publications from 2004 to 2008

1. Rollman, E., Arnheim, L., Collier, B., Öberg, D., Hall, H., Dillner, J., Pastrana, D. V., Buck, C. B., Wahren, B. and Schwartz, S. 2004. HPV-16 L1 genes with inactivated negative RNA elements induce potent immune responses. Virology, 322:182-189.
2. Zhao, X., Rush, M., Schwartz, S. 2004. Identification of an hnRNP A1-dependent splicing silencer in the HPV-16 L1 coding region that prevents premature expression of the late genes. J. Virol., 78:10888-10905.
3. Schwartz, S., Zhao, X., Öberg, D., Rush, M. 2004. Regulation of papillomavirus late gene expression. *In* Recent Research Developments in Virology, 6: 29-45.
4. Zhao, X., Öberg, D., Rush, M., Fay, J., Lambkin, H., Schwartz, S. 2005. A 57 nucleotide upstream early polyadenylation element in human papillomavirus type 16 interacts with hFip1, CstF-64, hnRNP C1/C2 and PTB. J. Virol., 79:4270-4288.
5. Öberg, D., Fay, J., Lambkin, H., Schwartz, S. 2005. A downstream polyadenylation element in human papillomavirus type 16 L2 encodes multiple GGG-motifs and interacts with hnRNP H. J. Virol., 79:9254-9269.
6. Rush, M., Zhao, X., Schwartz, S. 2005. A splicing enhancer in the E4 coding region of HPV-16 is required for early mRNA splicing and polyadenylation, and inhibition of premature late gene expression. J. Virol., 79: 12002-12015.
7. Zhao, X., Rush, M., Carlsson, A., Schwartz, S. 2007. The presence of inhibitory RNA elements in the late 3'-untranslated region is a conserved property of human papillomaviruses. Virus Research., 125: 135-144.
8. Zhao, X., Fay, J., Lambkin, H., Schwartz, S. 2007. Identification of a 17-nucleotide splicing enhancer in HPV-16 L1 that counteracts the effect of multiple hnRNP A1-binding splicing silencers. Virology, 369: 351-363.

9. Schwartz, S., Somberg, M., Zhao, X., Rush, M., Öberg, D., Cassidy, H., Fay, J., Lambkin, H. 2007. Regulation of HPV Gene Splicing and Expression. *In* "HPV Gene regulation and transformation", Ed: B. Norrild, Research Signpost.
10. Schwartz, S. 2008. HPV-16 RNA processing. *In* "Protein-RNA interactions in viral RNA processing", Ed: T. Zheng, Frontiers in Bioscience, 13:5880-5891.
11. Zhao, X. and Schwartz, S. 2008. Inhibition of HPV-16 L1 expression from L1 cDNAs correlates with the presence of hnRNP A1 binding sites in the L1 coding region. Virus Genes, 36: 45-53.
12. Somberg, M., Zhao, X., Fröhlich, M., Evander, M., Schwartz, S. 2008. PTB induces HPV-16 late gene expression by interfering with splicing inhibitory elements at the major late 5'-splice site SD3632. J. Virol., 82:3665-3678.
13. Somberg, M., Rush, M., Fay, J., Ryan, F., Lambkin, H., Akusjärvi, G. and Schwartz, S. 2008. Adenovirus E4orf4 induces HPV-16 late L1 mRNA production. Virology, in press.
14. Fay, J., Kelehan, P., Lambkin, H., and Schwartz, S. 2008. Increased expression of cellular RNA-binding proteins in HPV-induced neoplasia and cervical cancer. J. Med. Virol., in press.

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CELLULAR AND VIRAL TRANS-ACTING FACTORS AND VIRAL CIS-ACTING ELEMENTS THAT REGULATE HPV-16 GENE EXPRESSION

Monika Somberg

We are attempting to identify cellular and viral trans-factors that are functionally involved in the regulation of HPV-16 gene expression by knocking down or over expressing cellular or viral RNA binding or regulatory proteins. Initially we are studying the hnRNP and SR protein families and we have shown that proteins including hnRNP I and SRp30c alter the HPV-16 gene expression program, most notably by inducing late gene expression. This induction occurs by different mechanisms and turns on either HPV-16 L1 or L2 expression. We are identifying the target sequences on the HPV-16 genome as well as mechanism of action. Interestingly, we have found that HPV-1, which is a benign HPV type never found in cancer, respond differently to overexpression of the cellular factors than HPV-16, indicating that regulation of HPV gene expression may be linked to the biological properties of the HPV type. In collaboration with Dr. Magnus Evander in Umeå, Sweden, we have studied the effect of these factors on HPV-16 gene expression in infected cells.

The SR protein family is involved in RNA splicing, nuclear export, stability and translation and the various processes are tightly linked to the phosphorylation status of the SR protein. We have found that overexpression of the adenovirus E4orf4 protein, which dephosphorylates SR proteins, induces HPV-16 late gene expression. In addition, we have found that SR proteins are downregulated in terminally differentiated cells in cervical epithelium; the same cells that express HPV-16 L1 and L2 in infected epithelium. On the other hand, we have seen that SR proteins are overexpressed in dysplasias and cancer, suggesting a

model in which inactivation of one or several SR proteins leads to induction of HPV-16 late gene expression. These experiments are performed in collaboration with Drs. Helen Lambkin Fergus Ryan at the Dublin Institute of Technology, Ireland, in which we look at the expression levels of primarily RNA binding proteins that we know interact with HPV mRNAs in relation to the differentiation level of the epithelial cell, HPV infection, HPV expression levels, viral persistence and clinical symptoms of the infection.

HPV-16 SPLICING AND POLYADENYLATION IN VITRO

Xiaoze Li

Regulation of HPV-16 gene expression is complex and involves alternative splicing and polyadenylation. To determine the mechanism of function of the RNA elements and transacting factors identified in the project described above, we are studying these elements and factors using in vitro splicing and in vitro polyadenylation methods. These experiments will determine the exact mechanism of action of the various RNA regulatory elements on the viral mRNAs, as well as the functions of the proteins that induce HPV-16 late gene expression.

CIS-ACTING RNA ELEMENTS AND RNA BINDING PROTEINS AS TARGETS FOR ANTIVIRAL THERAPY AGAINST HIV-1

Anna Tranell

The HIV-1 genome encodes only one promoter but expresses more than 15 functional proteins (p17gag, p24gag, p6gag, p7gag, PR, RT, IN, gp41, gp120, vif, vpr, tat, rev, nef and vpu). mRNAs generated to produce the viral proteins are therefore undergoing extensive alternative splicing. This process is precisely controlled by cellular and viral factors to ensure that all mRNAs are made and that the relative HIV-1 mRNA levels are correct. This process must at the same time be very vulnerable and should be highly sensitive to small changes in the concentrations of the splicing regulatory factors involved in the process. As a first steps towards finding novel targets for antiviral therapy against AIDS, we are using subgenomic HIV-1 clones to identify viral regulatory RNA sequences that are essential for generation of correct levels of viral mRNAs in the context of the viral genome. In other experiments, the levels of known RNA binding proteins are altered by different methods in cells expressing subgenomic HIV-1 plasmids to identify cellular RNA binding proteins that are involved in regulation of HIV-1 mRNA processing. In collaboration with prof. Eva Maria Fenyö, Lund University, we also investigate if the identified RNA elements and trans-acting factors identified here are needed for efficient production of infectious virus. Antisense oligonucleotides that bind to regulatory RNA elements may alter the RNA processing pathway and redirect the splicing machinery to cause expression of selected viral genes. We are currently identifying regulatory RNA elements in the HIV-1 genome that would be optimal targets for antisense oligonucleotides.

STUDYING INFLUENZA VIRUS RNA PROCESSING USING AN INFLUENZA VIRUS POLYMERASE ASSAY

Samir Abdurahman

Influenza virus produces 8 unspliced mRNAs that encode eight different proteins. Two of these mRNAs encoding M1 and NS1 proteins can be spliced to generate at least two more mRNAs encoding M2 and NS2, respectively. Since influenza is an RNA virus that is transcribing RNA into mRNA using its own polymerase, we have established a transient transfection-influenza virus RNA polymerase assay in living cells. Using this assay, we can study regulation of influenza virus RNA splicing under natural conditions. We are using site-directed mutagenesis to identify regulatory RNA elements and transient over-expression of regulatory RNA-binding proteins to identify cellular factors that are regulating influenza virus RNA splicing. The final goal is to investigate if mutations that affect efficiency of influenza virus RNA splicing, also affects the biological and pathogenic properties of the virus.

DIFFERENCES IN SPLICING OF M AND NS mRNAs OF INFLUENZA A VIRUSES WITH DIFFERENT PATHOGENIC PROPERTIES

Sofia Lindström

While there are many studies in which one has tried to identify a specific influenza virus protein that is responsible for the pathogenic properties of highly pathogenic influenza such as Spanish flu and the highly pathogenic bird flu H5N1, it appears that the determinants of pathogenicity are multifactorial. These studies have been heavily focused on virus proteins function. However, since it is actually the RNA sequence of the virus that changes, and these changes do not always affect protein sequence, we wish to investigate if RNA sequence heterogeneity between influenza virus isolates with different pathogenic properties affects the function of the viral RNAs. We have focused on one RNA processing event in particular: the splicing of the pre-mRNAs of influenza virus segments 7 and 8. The results obtained within this project will reveal if natural RNA sequence variation between different influenza virus strains with different pathogenic properties affect splicing of the viral RNAs. The results will also reveal if these differences correlate with the biological or pathogenic properties of the virus isolates from which the sequences were derived.

ADENOVIRUS INDUCED REPROGRAMMING OF HOST CELL ACTIVITIES

Catharina Svensson

There is a profound overlap between the deregulatory programs elicited by a virus infection and the changes occurring during progression of normal cells into tumour cells. During an infection, virus needs to reprogram the host cell for mainly two reasons. First, optimal conditions for viral replication must be established to ensure efficient production of progeny virus. Secondly, the virus must be able to evade the anti-viral activities of the host for sufficiently long time to allow for escape and spread of progeny virus. Adenovirus expresses a multitude of regulatory proteins (E1-E4) to achieve these goals. The immediate early E1A gene encodes two primary regulators essential for transcriptional activation and forced entry of the host cell into the S-phase of the cell cycle. Through detailed molecular analyses, E1A has been shown to be highly conserved between different adenovirus serotypes and to interact with cell cycle regulators, transcriptional activators, co-activators and co-repressors. During infection of tissue culture systems, the highly oncogenic adenovirus type 12 is slower, less productive and shows a reduced cytopathic effect compared to an ad2 infection. The inability of ad12 to completely evade the first line antiviral defence might explain the relatively low virulence of this virus compared to ad 2, but an inadequate expropriation of the biosynthetic machinery of the host cell is likely to play an important role.

Members of the group during 2008

Catharina Svensson, associate professor
Hongyan Xiu, post doctoral fellow

International Exchange

Norbert Hochstein

Publications from 2004 to 2008

1. Dorn, A., Zhao, H., Granberg, H., Hösel, M., Webb, D., Svensson, C., Pettersson, U. and Doerfler, W. (2005). Identification of specific cellular genes up-regulated late in adenovirus type 12 infection. J. Virol. 79: 2404-2412.
2. Rosalind M Glasspool, R. M., Burns, S., Hoare, S. F., Svensson, C. and Keith, W. N., (2005). The hTERT and hTERC telomerase gene promoters are activated by the second exon of the adenoviral protein, E1A, identifying the transcriptional co-repressor CtBP as a potential repressor of both genes. Neoplasia, 7, 614-622.
3. Johansson, C., Zhao, H., Bajak, E., Granberg, F., Pettersson, U. and Svensson, C. (2005). Impact of the interaction between adenovirus E1A and CtBP on host cell gene expression Virus Res, 113. 51-63

4. Fredrik Granberg, Catharina Svensson, Ulf Pettersson, and Hongxing Zhao (2005) Modulation of host cell gene expression during onset of the late phase of an adenovirus infection is focused on growth inhibition and cell architecture. Virology 343, 236-245
5. Granberg F, Bojie H, Svensson C. Pettersson U. and Zhao H. (2006) Adenovirus-induced alterations in host cell gene expression prior to the onset of viral gene expression. Virology, 353, 1-5.

PROTEIN STRUCTURE/FUNCTION

Pia Ek, Åke Engström, Per Jemth, Sophia Schedin Weiss, Birgitta Tomkinson

The amino acid composition of a protein dictates its structure. The precise positioning of atoms, within that structure, in combination with any chemical modifications, defines the range of interactions that a protein can form with other molecules (e.g. other proteins, DNA, oligosaccharides, substrates). These interactions are the basis of biological activity. The Structure/Function group comprises several laboratories with diverse biological interests but share a common philosophy in understanding these phenomena. Namely, in order to understand function, we need to establish the identity, composition and structure of the protein of interest and thoroughly characterize its interactions with binding partners.

REGULATION BY PHOSPHORYLATION

Pia Ek

Protein phosphorylation is a central mechanism of signal transduction in eukaryotic cells involved in all inter- and intracellular functions. The phosphorylation is performed by specific protein kinases, which transfer the γ-phosphate from ATP to an acceptor group of the selected amino acid residue in target proteins. Protein phosphatases make this process reversible. A prediction is made that the number of protein kinases in the human genome is almost 1000, which may cover 3 % of all the genes. So far, only a few of these enzymes have been characterised on the protein level, and the physiological roles of most protein kinases have not yet been identified. We are presently further characterizing the phosphohistidine phosphatase, PHPT1 that was found and purified by us 6 years ago.

Members of the group during 2008

Ulla Beckman-Sundh, graduate student
Pia Ek, Assoc. Professor
Åsa Haglund, Dr. Med. Sci em
Elvy Netzel, Biomedical scientist
Gunilla Pettersson, Biomedical scientist
Örjan Zetterqvist, Professor em

Publications from 2004 to 2008

1. Loog, M., Ek, B., Oskolkov, N., Narvanen, A., Järv, J., Ek, P. (2005) Screening for the optimal specificity profile of protein kinase C using electrospray mass-spectrometry. *J Biomol Screen 10,* 320-8.
2. Loog, M., Oskolkov, N., O´Farrell, F., Ek, P., Järv, J. (2005) Comparison of cAMP-dependent protein kinase substrate specificity in reaction with proteins and synthetic peptides. *Biochim Biophys Acta 1747,* 261-6.

3. Ek, T., Ek, P. (2005) Laboratoriediagnostikens historia – med fokus på utvecklingen i Sverige, del 1. *Laboratoriet 3,* 14-18.
4. Ek, T., Ek, P. (2005) Laboratoriediagnostikens historia – med fokus på utvecklingen i Sverige, del 2. *Laboratoriet 4,* 10-14.
5. Ek, T., Ek, P. (2005) Laboratoriediagnostikens historia – med fokus på utvecklingen i Sverige, del 3. *Laboratoriet 5,*
6. Ma, R., Kanders, E., Beckman Sundh, U., Geng, M., Ek, P., Zetterqvist, Ö., Li, J.-P. (2005) Mutational study of human phosphohistidine phosphatase: Effect on enzymatic activity. *Biochim Biophys Res Commun 337,* 887-891.
7. Marknell DeWitt, Å., Andersson, K., Peltre, G., Lidholm, J. (2006) Cloning, expression and immunological characterization of full-length timothy grass pollen allergen Phl p 4, a berberine bridge enzyme-like protein with homology to celery allergen Api g 5. *Clin Exp Allergy 36(1),* 77-86.

Agencies that support the work

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CHARACTERIZATION OF MAMMALIAN 14-kDa PHOSPHOHISTIDINE PHOSPHATASE

Ulla Beckman-Sundh

Protein histidine phosphorylation accounts for about 6 % of the total protein phosphorylation in eukaryotic cells; still details concerning histidine phosphorylation and dephosphorylation are limited compared to that of serine/threonine and tyrosine. The major reason for this may have been methodological difficulties: phosphohistidine, which exists as one of two isomeric forms (3-phosphohistidine and 1-phosphohistidine) is labile at acid and neutral pH. Histidine phosphorylation would therefore easily escape detection, for instance with standard SDS-PAGE.

In the present project, a mammalian 14-kDa phosphohistidine phosphatase, also denominated PHPT1, that we found 6 years ago by probing pig liver extracts with a phosphohistidine-containing peptide, is further investigated. The localization of PHPT1-mRNA by Northern blot analysis revealed high expression in heart and skeletal muscle. The main object of the study was to determine the PHPT1 expression on protein level in mouse tissues. Tissue samples from adult mice and 14.5 days old mouse embryos were processed for immunostaining using a PHPT1-specific polyclonal antibody. The same antibody was also provided to the Swedish human protein atlas project (HPR) (www.proteinatlas.org/index.php). The results from both studies were essentially consistent with the previously reported expression of mRNA of a few human tissues. In addition, several other tissues, including testis displayed a high protein expression. A salient result of the present investigation was the ubiquitous expression of the PHPT1-protein and its high expression in continuously dividing epithelial cells.

Ongoing work is a search of natural substrates for PHPT1. In the literature, phosphorylation of histone H4 has been described, and histone H4 histidine kinase has been

isolated from different eukaryote cells; yeast, slime mould and from mammalian cells. Protein histidine phosphorylation has been described in cells that are highly proliferative and in regenerating cells. In pilot experiments in our laboratory we have phosphorylated recombinant histones H1 (one type), H2A, H2B, H3 and H4 on histidine, using a chemical approach. We observed that the histones were dephosphorylated by PHPT1 and some of them more rapidly than the peptide succinyl-Ala-His(P)-Pro-Phe-*p*-nitroanilide, the peptide that was used in previous activity studies in the laboratory. Besides the recombinant histones, we have used histone H4 purified from commercially available sources. The corresponding phosphorylated H4 was dephosphorylated at a similar high rate. We plan to continue to work with histone H4, since a mammalian kinase has been described for this type of histone. We are also in the process of preparing chromatine from a fetal cell line for preparation and purification of histones for further studies on histidine phosphorylation and dephosphorylation with the aim to get closer to *in vivo* conditions.

PROTEOMICS RESOURCE CENTER

EXPRESSION PROTEOMICS

Åke Engström and Eva Andersson

This laboratory gives the scientific community an opportunity to at a low cost make use of recent developments in techniques and instrumentations for the analysis and identification of proteins. The facility for Expression Proteomics is equipped with 2-D electrophoresis systems, systems for post or pre-gel labeling of proteins, visible and UV light scanners, software for image analysis, spot picking systems, semi automated spot processing, and MALDI-TOF/TOF and MS/MS instrumentation. The facility has expertise for 2-D analysis, mass, spectrometry, image analysis, data base searches and general protein chemistry.

Our area of work is analysis and comparison of proteomes, identification of proteins in protein spots/bands by mass spectrometry, analysis of expressed proteins for quality control and analysis of proteins for post-translational modifications. The facility is open for all scales of problem solving or analysis, although the capacity for 2D gels might be a limiting factor for very large undertakings.

The service is primarily intended for identification of proteins from species with large numbers of genes or proteins characterized. The facility has in addition a limited capacity for de novo sequencing of proteins from any species. The service includes straightforward methods for characterization of expressed recombinant proteins. Considering the low cost for analysis this is highly recommended to avoid the potential risk of doing experiments with the "wrong" or modified protein. An MS analysis of intact expressed protein and a peptide mapping with MS give much better confidence than a simple SDS-gel analysis. If suitable for our techniques and knowledge we provide analysis of any type of sample

Publications from 2004 to 2008

1. Bajtner E, Nandakumar KS, Engstrom A, Holmdahl R., Chronic development of collagen-induced arthritis is associated with arthritogenic antibodies against specific epitopes on type II collagen. Arthritis Res Ther. 2005;7(5):R1148-57
2. Zavialov AV, Engstrom A., Human ADA2 belongs to a new family of growth factors with adenosine deaminase activity, Biochem. J. (2005) 391 (51–57)
3. Burkhardt H, Sehnert B, Bockermann R, Engstrom A, Kalden JR, Holmdahl R., Humoral immune response to citrullinated collagen type II determinants in early rheumatoid arthritis, Eur J Immunol. 2005 May;35 (5):1643-52.
4. Kocher T, Engstrom A, Zubarev RA., Fragmentation of Peptides in MALDI In-Source Decay Mediated by Hydrogen Radicals, Anal. Chem.; 2005; 77(1) pp 172 - 177;
6. Gianni, S., Engström, Å., Larsson, M., Calosci, N., Malatesta, F., Eklund, L., Ngang, C. C., Travaglini-Allocatelli, C., and Jemth, P. (2005) The kinetics of PDZ domain – ligand interactions and implications for the binding mechanism. J. Biol. Chem. 280, 34805-34812.

7. Forsberg J, Strom J, Kieselbach T, Larsson H, Alexciev K, Engström Å and Åkerlund H-E. Protease activities in the chloroplast capable of cleaving an LHCII N-terminal peptide, Physiologia Plantarum 123: 21^29. 2005
8. Chi CN, Engstrom A, Gianni S, Larsson M, Jemth P., Two Conserved Residues Govern the Salt and pH Dependencies of the Binding Reaction of a PDZ Domain.J Biol Chem. 2006 Dec 1;281(48):36811-36818
9. Burkhardt H, Huffmeier U, Spriewald B, Bohm B, Rau R, Kallert S, Engstrom A, Holmdahl R, Reis A., Association between protein tyrosine phosphatase 22 variant R620W in conjunction with the HLA-DRB1 shared epitope and humoral autoimmunity to an immunodominant epitope of cartilage-specific type II collagen in early rheumatoid arthritis. Arthritis Rheum. 2006 Jan;54(1):82-9.
10. Barman, J., Acharya, S., Zhou, C., Chatterjee, S., Engstrom, A. and Chattopadhyaya, J. Non-identical electronic characters of the internucleotidic phosphates in RNA modulate the chemical reactivity of the phosphodiester bonds. Org. Biomol. Chem. 2006 4, 928-941.
11. Gianni, S., Walma, T., Arcovito,A., Calosci,N., Bellelli,A., Engstrom,A., Traviaglini-Allocatelli,C., Brunori,M., Jemth,P. and Vuister,G.W. Demonstration of long-range interactions in a PDZ domain by NMR, kinetics, and protein engineering. Structure. 2006 12, 1801-9.
12. Trulson, A., Byström, J., Engström, Å., Larsson,R. and Venge, P. The functional heterogeneity of eosinophil cationic protein is determined by a gene polymorphism and post-translational modifications. Clinical and Experimental Allergy. 2007 37, 208-218.
13. Chi,C.,N., Elfström, L., Shi, Y., Snäll, T., Engström, Å. and Jemth, P. Reassessing a sparse energetic network within a single protein domain. Proc. Natl. Acad. Sci. U.S.A. 15:105 (12): 4679- 84, 2008.
14. Sun, W., Parry, S., Panico, M., Morris, H.R., Kjellberg, M., Engström, Å., Dell, A., and Schedin-Weiss, S. N-glycans and the N terminus of protein C inhibitor affect the cofactor-enhanced rates of thrombin inhibition. J.Biol. Chem. 4:283(27), 18601-11, 2008.
15. Bergström-Lind, S., Molin, M., Savitski, M.M., Emilsson, L., Åström, J., Hedberg, L., Adams, C., Nielsen, M.L., Engström, Å., Elfineh, L., Andersson, E., Zubarev, R.A. and Pettersson, U. Immunoaffinity enrichments followed by mass spectrometric detection for studying global protein tyrosine phosphorylation. J. Proteome Res. 7(7): 2897-910, 2008.
16. Snir, O., Widhe, M., von Spee, C., Lindberg, J., Padyukov, L., Lundberg, K., Engström, Å., Venables, P.J., Lundeberg, J., Holmdahl, R., Klareskog, L. and Malmström, V. Multiple antibody reactivities to citrullinated antigens in sera from rheumatoid arthritis patients - association with HLA-DRB1 alleles. Ann Rheum Dis. 2008 Jul 17.
17. Calosci, N., Chi, C. N., Richter, B., Camilloni, C. Engström, Å, Eklund, L., Travaglini-Allocatelli, C., Gianni, S.*, Vendruscolo, M.* and Jemth, P.* (2008) Comparison of successive transition states for folding reveals alternative early folding pathways of two homologous proteins.. *Proc. Natl Acad. Sci.* USA. 105 (49), 19241 - 6.

STRUCTURE-FUNCTION RELATIONSHIPS OF PROTEINS

Per Jemth

The ultimate goal of our research is to better understand fundamental structure-function and structure-reactivity relationships in proteins. Our research focuses on the nature and specificity of protein-ligand and protein-protein interactions, and also on protein folding, stability and allostery. We use protein engineering and biophysics to dissect the chemical reactions of proteins.

One of the systems we are working on is PDZ domains and their interactions with various ligands. The PDZ domains are present in numerous proteins where they function as adaptors, governing binding to other proteins and thereby modulating, for example, signal tranduction and scaffolding. We are also looking at the E6 and E7 proteins of human papillomavirus. Certain strains of human papillomavirus cause cancer, for example cervival cancer, and this discovery was awarded the Nobel prize in physiology or medicin in 2008. The E6 and E7 proteins are so-called oncogenes, and the main culprits in the carcinogenesis. We want to understand the molecular details of their interactions with cellular proteins. Also check our web page: http://www.anst.uu.se/pje13912/.

Members of the group during 2008

Celestine Chi, PhD student
Lisa Elfström, postdoc
Raza Haq, PhD student
Wang Huiqun, visiting scholar
Per Jemth, Associate professor

Publications from 2004 to 2008

1. Jemth, P., Gianni, S., Day, R., Li, B., Johnson, C. M., Daggett, V., and Fersht, A. R. (2004) Demonstration of a low energy on-pathway intermediate in a fast folding protein by kinetics, protein engineering and simulation. Proc. Natl. Acad. Sci. USA 101, 6450-6455.
2. Kreuger, J., Jemth, P., Sander-Lindberg, E., Eliahu, L., Ron, D., Basilico, C., Salmivirta, M., and Lindahl, U. (2005) Fibroblast growth factors share binding sites in heparan sulfate. Biochem. J. 389, 145-150.
3. Jemth, P., Gianni, S, Day, R., Khan, F., Allen, M. D., Daggett, V., and Fersht, A.R. (2005) The structure of the major transition state for folding of an FF domain. J. Mol. Biol. 350, 363-378.
4. White, G. W. N., Gianni, S., Grossman, J. G., Jemth, P., Fersht, A. R., and Daggett, V. (2005) Simulation and experiment conspire to reveal cryptic intermediates and the slide from the nucleation-condensation to framework mechanism of folding. J. Mol. Biol. 350, 757-775.
5. Knauth, K., Bex, C., Jemth, P., and Buchberger, A. (2005) Renal cell carcinoma risk in type 2 von Hippel-Lindau disease correlates with defects in pVHL stability and HIF-1 alpha interactions. Oncogene 25, 370-377

6. Gianni, S., Engström, Å., Larsson, M., Calosci, N., Malatesta, F., Eklund, L., Ngang, C. C., Travaglini-Allocatelli, C., and Jemth, P. (2005) The kinetics of PDZ domain – ligand interactions and implications for the binding mechanism. J. Biol. Chem. 280, 34805-34812.
7. Gianni, S., Geierhaas, C. D., Calosci, N., Jemth, P., Vuister, G. W., Travaglini-Allocatelli, C., Vendruscolo, M., and Brunori, M.* (2006) A PDZ domain recapitulates the unifying mechanism for protein folding. Proc. Natl Acad. Sci. USA 104, 128-133
8. Chi, C. N., Engström, Å., Gianni, S., Larsson, M. and Jemth, P.* (2006) Two conserved residues govern the salt and pH dependencies of the binding reaction of a PDZ domain. J. Biol. Chem. 281, 36811-36818
9. Gianni, S., Walma, T., Arcovito, A., Calosci, N., Bellelli, A., Engström, Å., Travaglini-Allocatelli, C., Brunori, M., Jemth, P.*, and Vuister, G. W.* (2006) Demonstration of long-range interactions in a PDZ domain by NMR, kinetics and protein engineering. Structure 14, 1801-1809
10. Ivarsson, Y., Travaglini-Allocatelli, C., Jemth, P., Malatesta, F., Brunori, M., and Gianni, S.* (2007) An on-pathway intermediate in the folding of a PDZ domain. J. Biol. Chem. 282, 8568-8572
11. Chi, C. N., Gianni, S.*, Calosci, N., Travaglini-Allocatelli, C., Engström, Å., and Jemth, P.* (2007) A conserved folding mechanism for PDZ domains. FEBS Lett. 581, 1109-1113
12. Gianni, S.*, Ivarsson, Y., Jemth, P., Travaglini-Allocatelli, C., and Brunori, M. (2007) Identification and characterization of protein folding intermediates. Biophys. Chem. 128, 105-113 (review)
13. Jemth, P.* and Gianni, S.* (2007) PDZ domains: folding and binding. Biochemistry 46, 8701-8708 (current topics/perspectives)
14. Jemth, P., Johnson, C. M., Gianni, S., and Fersht, A. R.* (2008) Demonstration by burst phase analysis of a robust folding intermediate in the FF domain. Prot. Eng. Des. Sel. 21, 207-214
15. Chi, C. N., Elfström, L., Shi, Y., Snäll, T., Engström, Å, and Jemth, P.* (2008) Reassessing a sparse energetic network within a single protein domain. Proc. Natl Acad. Sci. USA. 105, 4679-4684.
16. Bach, A., Chi, C. N., Olsen, T. B., Pedersen, S. W., Clausen, R. P., Jemth, P., and Strømgaard, K.* (2008) Peptidomimetic inhibitors of the PSD-95/NMDA receptor interaction. J. Med. Chem. 51, 6450-6459.
17. Calosci, N., Chi, C. N., Richter, B., Camilloni, C. Engström, Å, Eklund, L., Travaglini-Allocatelli, C., Gianni, S.*, Vendruscolo, M.* and Jemth, P.* (2008) Comparison of successive transition states for folding reveals alternative early folding pathways of two homologous proteins. *Proc. Natl Acad. Sci.* USA. 105 (49), 19241 - 6.

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Agencies that support the work

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PROTEINS: FOLDING, STABILITY, INTERACTIONS AND ALLOSTERY

Celestine Chi, Lisa Elfström, Raza Haq

It is clear that many protein-ligand interactions involve structural rearrangements of the protein. These conformational changes are not restricted only to proteins with several subunits such as the classical example haemoglobin. Structural changes have also been detected in single domains and one important question is how signals are transmitted through a protein. Another crucial question is the actual role of the often subtle perturbations of proteins that are detected upon binding. Other perturbations are not minor and the most extreme case of a conformational change would be folding of the protein. Indeed, many proteins are even unfolded until they meet their binding partner.

One of the systems we are working on is PDZ domains and their interactions with other proteins. The PDZ domains are present in numerous proteins where they function as adaptors, governing binding to other proteins and thereby modulating, for example, signal tranduction and scaffolding. One question we're addressing is how inter- and intramolecular conformational changes affect protein-ligand interactions. Another phenomenon we are interested in is proteins that fold only in the presence of their ligand. These so-called intrinsically unstructured proteins are very common in the eukaryotic proteome, yet very little is known about them. Another area of research is the biochemistry of proteins encoded by human papillomavirus (see below), which in fact tie everything together; these proteins interact with PDZ domains and they contain regions of structural disorder.

HUMAN PAPILLOMAVIRUS AND CANCER: PROTEIN LIGAND INTERACTIONS

Celestine Chi, Wang Huiqun

The implication of human papillomavirus (HPV) in cancers of the uterine cervix has been firmly established biologically and experimentally and this discovery was awarded the Nobel prize in physiology or medicin in 2008. Most cancers of the vagina and anus are caused by HPV as are a number of cancers of the vulva, penis and oropharynx. HPV's are classically divided into two groups: "low risk" and "high risk". The mucosal HPV's are considered "low risk" while the "high risk" is based on their prevalence ratio in cervical cancer and its precursors. About 99% of the cervical cancers contain DNA of the high-risk types with HPV16 being the most prevalent, followed by types 18, 45, 13 and 33.

HPV utilises a number of ways to establish its pathogenic potential; one of them includes targeting the tumour suppressor p53 and SAP97 (synapse associated protein 97) for degradation. A lot has been done in trying to understand the mechanism of p53 interaction with HPV. However, little is known about the molecular mechanism with SAP97. During pathogenesis, it is known that an early protein known as E6 is secreted, which binds to the PDZ2 domain of SAP97. Other early proteins such as E4 and E7 are also implicated in the pathogenesis through their interaction with cell cycle proteins cyclin A/E, retinoblastoma tumour supressor and cdk's.

Understanding the molecular principles that govern HPV infection is a glorious goal, since this will probably improve the chances of rapid pharmaceutical intervention. We are looking at the molecular mechanisms of the interactions between HPVE6/SAP97, and E7/Retinoblastoma tumour supressor.

MOLECULAR MECHANISMS BEHIND THE ACTIVITIES OF MULTIFUNCTIONAL SERINE PROTEASES AND THEIR INHIBITORS IN BLOOD

Sophia Schedin Weiss

We study blood proteins that can regulate more than one physiologically important process. The identification of factors that label multifunctional blood proteins to choose one particular task out of several possible ones is medically valuable, since it may lead to more specific medications than those that exist today. The focus is on the serine proteases and the serpin superfamily of proteins, which are comprised of several proteins found in blood that regulate important processes, such as blood coagulation, fibrinolysis, inflammation, angiogenesis and tumor formation. Effects of posttranslational modifications, including *N*- and *O*-glycosylation, proteolytical processing and conformational changes on the functions of these proteins, are studied. We have access to a wide range of biophysical, biochemical and cell biological techniques that we use for studying molecular interactions. The results are further evaluated in *in vivo* models. Since the proteins that we study function by regulating physiologically very important processes, our results may lead to discoveries of high medical importance. In the extension, they may lead to novel medications against diseases such as cardiovascular disorders and different forms of cancer.

Members of the group during 2008

Sophia Schedin Weiss, associate professor, group leader
Susanne Tingsborg, post doc
Wei Sun, PhD student

Project workers during 2008

Anna Eriksson, Masters student
Noëlle Nickolaus, Erasmus student

Publications from 2004 to 2008

1. Schedin-Weiss, S., Desai, U.R., Bock, S.C., Olson, S.T. and Björk, I. (2004) Roles of N-terminal region residues Lys11, Arg13 and Arg24 of antithrombin in heparin recognition and in promotion and stabilization of the heparin-induced conformational change. *Biochemistry*, 43:675-683.
2. Turunen, M. and Schedin-Weiss, S. (2007) Defect in fatty acid esterification of dolichol in Niemann-Pick type C1 mouse livers in vivo. *Biochim. Biophys. Acta*, 1771: 506–513.
3. Hjelm, R. and Schedin-Weiss, S. (2007) High affinity interaction between a synthetic, highly negatively charged pentasaccharide and α- or β-antithrombin is predominantly due to nonionic interactions. *Biochemistry*, 46: 3378–3384.

4. Hjelm, R. (2007) Molecular mechanisms behind the antiangiogenic properties of antithrombin. Licentiate thesis in medical biochemistry at the Department of Medical Biochemistry and Microbiology, Uppsala University. Supervisor: Sophia Schedin Weiss.
5. Richard, B., Swanson, R, Schedin-Weiss, S., Ramirez, B, Izaguirre, G., Gettins, P.G.W. and Olson, S.T. (2008) Characterization of the conformational alterations, reduced anticoagulant activity, and enhanced antiangiogenic activity of prelatent antithrombin. *J. Biol. Chem.,* 283:14417–14429.
6. Sun, W., Parry, S., Panico, M., Morris, H.R., Kjellberg, M., Engström, Å., Dell, A. and Schedin-Weiss, S. (2008) N-glycans and the N-terminus of protein C inhibitor affect the cofactor-enhanced rates of thrombin inhibition. *J. Biol. Chem.,* 283: 18601-18611.
7. Schedin-Weiss, S., Richard, B., Hjelm, R. and Olson, S.T. (2008) Antiangiogenic forms of antithrombin specifically bind to the anticoagulant heparin sequence. *Biochemistry,* in press.
8. Sun, W., Eriksson, A.S. and Schedin-Weiss, S. (2008) Heparin enhances the inhibition of factor Xa by protein C inhibitor in the presence but not in the absence of Ca^{2+}. *Biochemistry,* under revision.

Agencies that support the work

Magnus Bergvall Foundation
The Medical Faculty, Uppsala University
The Research Council Medicine

ROLES OF POSTTRANSLATIONAL MODIFICATIONS ON THE FUNCTIONS OF PROTEIN C INHIBITOR

Wei Sun, Anna Eriksson, Noëlle Nickolaus

Due to the low selectivity of protein C inhibitor for proteases, we are investigating how posttranslational modifications affect the functions of this protein. Protein C inhibitor isolated from human blood is largely heterogenous and we have shown that the heterogeneity is fully explained by differences in N-glycosylation occupancy, N-glycan structures and the length of the N-terminus. Consequently, we are studying how he N-glycans and the N-terminus affect the ability of protein C inhibitor to inactivate its target proteases, including active protein C, thrombin and factor Xa, in the absence or presence of Ca2+ and cofactors, such as heparin and thrombomodulin.

EFFECTS OF DISEASED CONDITIONS ON THE N-GLYCAN STRUCTURES OF ANTITHROMBIN

Wei Sun, Susanne Tingsborg

Inflammatory disorders are often accompanied by altered glycosylation patterns and hypercoagulability states. Since antithrombin is a vital plasma anticoagulant and the levels of antithrombin are reduced in inflammatory conditions, this project aims at investigating:
1) How the N-glycosylation pattern of antithrombin is altered in inflammatory animal models.

2) How the observed changes in N-glycan structures affect the functional activities of antithrombin, i.e. the strength of the interaction with heparin and the rate of inactivation of blood clotting proteases.

BINDING OF HEPARINS AND SYNTHETIC SACCHARIDES TO CONFORMATIONAL- AND GLYCOSYLATION VARIANTS OF ANTITHROMBIN

Susanne Tingsborg, Sophia Schedin Weiss

Antithrombin exists in different conformations. The native conformation is a vital plasma anticoagulant, by being capable of efficiently inhibiting blood coagulation proteases. Latent and reactive site-loop cleaved antithrombins, on the other hand, are potent antiangiogenic agents. The heparin binding-site of antithrombin is important for the anticoagulant as well as the antiangiogenic properties of this protein. The interaction of native antithrombin with heparin has been extensively characterized but in this project we study the interaction between the antiangiogenic antithrombin variants and heparin. We also study how the degree of glycosylation affects the anticoagulant and antiagiogenic properties of antithrombin, since two N-glycosylation variants of antithrombin exist in human blood plasma, one with four and one with three N-linked glycans, denoted α- and β-antithrombin, respectively.

STRUCTURE, FUNCTION AND PHYSIOLOGICAL ROLE OF TRIPEPTIDYL-PEPTIDASE II

Birgitta Tomkinson

Intracellular protein degradation is as important for regulating the concentration of specific proteins in the cell as protein synthesis, but much less well characterized. Protein degradation is malfunctioning in a number of diseases such as cancer, muscle wasting and Alzheimers disease. Tripeptidyl-peptidase II (TPP II) is an important player in intracellular proteolysis, and the ultimate goal of our work is to determine the specific physiological role of the enzyme in this process. TPP II is a huge enzyme complex with a widespread distribution in eukaryotic cells and the ability to cleave oligopeptides into tripeptides. Our main focus is a biochemical characterization of TPP II, in order to investigate how its substrate specificity is determined and how oligomerization is regulated. We are also investigating how expression of this enzyme is regulated. This type of investigations will provide a basis for construction of e.g. specific inhibitors of TPP II. Since TPP II appears to be important for inactivation of the neuropeptide cholecystokinin and also for tumour progression, it is a potential drug target.

Members in the group during 2008:

Birgitta Tomkinson, associate professor
Sandra Eriksson, Ph. D. Student
Linnéa Lundholm, technician (3 months)

Agencies that support the work

Carl Tryggers Stiftelse för Vetenskaplig Forskning
O.E. och Edla Johanssons Vetenskapliga stiftelse
Department of Biochemistry and Organic chemistry
Medical Faculty

Publications from 2004 to 2008

1. Tomkinson, B.: Tripeptidyl-peptidase II (2004) in *Handbook of Proteolytic Enzymes* (2nd ed., Barrett, A. J., Rawlings, N. D. & Woessner, J. F. eds), pp. 1882-1885 Academic Press, London
2. Stavropoulou, V., Xie, J., Henriksson, M., Tomkinson, B., Imreh, S., & Masucci, M.: Mitotic infidelity and centrosome duplication errors in cells overexpressing the oligopeptidase tripeptidyl-peptidase II (2005) *Cancer Res*. 65 (4), 1361-1368
3. Tomkinson, B. & Lindås, A.-C.: Tripeptidyl-peptidase II: A multi-purpose peptidase (2005) *Int. J. Biochem. Cell. Biol*. 37 (10), 1933-1937
4. Lindås, A.-C. & Tomkinson, B.: Identification and characterization of the promoter for the gene encoding human tripeptidyl-peptidase II (2005) *Gene* 345, 249-257

5. Radu, D., Tomkinson, B., Zachrisson, O., Weber, G., de Belleroche, J., Hirsh, S. & Lindefors, N.: Overlapping regional distribution of CCK and TPPII mRNAs in Cynomolgus monkey brain and correlated levels in human cerebral cortex (BA 10). (2006) *Brain Res*. 1104(1), 175-182
6. Lindås, A.-C. (2006) Tripeptidyl-peptidase II – Structure, function and gene regulation. Acta Universitatis Upsaliensis. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology, 243, 52 pp, Uppsala
7. Lindås, A.-C., & Tomkinson, B.: Characterization of the promoter of the gene encoding human tripeptidyl-peptidase II and identification of upstream silencer elements (2007) *Gene* 393, 62-69
8. Lindås, A.-C., Eriksson, S., Jozsa, E. & Tomkinson, B.: Investigation of a role for Glu-305 and Glu-331 in substrate binding of tripeptidyl-peptidase II (2008) *Biochim. Biophys. Acta* 1784, 1899-1907

INVESTIGATION OF STRUCTURE AND FUNCTION OF TPP II

Sandra Eriksson

This project focuses on the relationship between structure and function in TPP II. These studies are important not only for understanding the physiological role of the enzyme, but also in designing drugs targeting TPP II. One of the current studies has demonstrated the presence of a molecular ruler in TPP II, which ensures that the products are tripeptides and not longer or shorter peptides. This molecular ruler consists of at least one glutamate residue, which positions the N-terminus of the substrate so that there are three amino acid residues to the active site serine, where the peptide is cleaved. In another study, we have identified regions of high evolutionary conservation and created signatures that can be used in annotation. What is more, these regions are very interesting for future studies, as they most likely have an impact on structure and function.

The major focus of the structure/function project in the future will be on the endopeptidase activity of the enzyme, which has been reported to be slow compared to the exopeptidase activity (i.e. the release of tripeptides), but still comparable to other major proteases, e.g. the proteasome. The substrate specificity in particular will be studied, as it seems to diverge quite significantly from that of the exopeptidase activity. Further studies include the pH-dependence of TPP II from three species with two different substrates. The results so far have given some insights into the structure of the active site, and will be expanded with experiments on point mutations and/or a specific substrate for the endopeptidases activity.

CHARACTERIZATION OF TRIPEPTIDYL-PEPTIDASE II AND INVESTIGATION OF ITS POTENTIAL AS A TUMOUR MARKER

Linnea Lundholm, Sandra Eriksson, Birgitta Tomkinson

TPP II activity is increased in some cancer cells e.g. Burkitt´s lymphoma, and an overexpression of TPP II increases the risk for chromosomal damage since TPP II appears to protect tumour cells from apoptosis. Therefore TPP II could be a potential marker for malignant tumours. In order to investigate this a screening method for the expression of TPP

II will be developed. The first step in this process is to get access to pure TPP II to use as control material. Pure enzyme is also a prerequisite for the catalytic characterization of TPP II. During this part of the project, the purification method for TPP II has been improved, mainly by applying conventional chromatographic steps. We have also managed to purify dTPP II from an *E. coli* expression system and will continue to exploit this expression system also for expression of mTPP II.

TUMOR BIOLOGY

MECHANISMS OF TISSUE VASCULARIZATION

Pär Gerwins

We have developed a novel in vivo angiogenesis assay and discovered a potentially important and novel mechanism of tissue vascularization. Tensional forces generated by myofibroblast-mediated contraction of wounds mediate and direct translocation of neovessels which are pulled from the pre-existing vasculature as loops with functional circulation within the expanding tissue. This mechanism and additional questions are further explored by the following experiments. 1. Is vascularization reduced if tissue contraction is inhibited? 2. Can neovascularization be inhibited by targeting myofibroblasts? 3. What is the role of individual growth factors in angiogenesis and in maintaining the neovasculature? 4. In vivo time-lapse imaging of neovascularization using in vivo models. 5. Mechanisms of lymphangiogenesis particularly in relation to the novel mechanism of vascularization. 6. Discovery of novel genes that regulate vascularization using our new in vivo assay and cDNA microarray. 7. Establishment of a tissue bank with biopsies from patients with wounds that do not heal. 8. Analysis of human wound tissue arrays with antibody based proteomics to understand why some wounds have impaired healing and potentially develop new treatment options. Angiogenesis is an integrated part of tissue growth and remodeling and has been suggested to be a suitable therapeutic target for the treatment of a number of human diseases. The mechanism of angiogenesis that we have discovered might provide novel therapeutic options.

Members of the group during 2008

Pär Gerwins, group leader
Ludvig Petersson, PhD student
Peder Fredlund Fuchs, PhD student

Publications from 2004 to 2008

1. Lennmyr F, Gerwins P, Akterin S, Johansson L, Ahlström H, Ericsson A and Terént A. (2004). Src family kinase-inhibitor PP2 reduces focal ischemic brain injury. Acta Neurol Scand. 110(3):175-9.
2. Kilarski W, Jura N and Gerwins P. (2005) An ex vivo model for functional studies of myofibroblastss. Lab Invest, 85(5):643-654.
3. Liu M, Kilarski WW, Gerwins P, Oberg K, Zhou Y. (2006) Efficient Human Interferon-alpha Gene Transfer to Neuroendocrine Tumor Cells with Long-Term and Stable Expression. Neuroendocrinology. 82(5-6):264-273
4. Larsson A, Peng S, Persson H, Rosenbloom J, Abrams WR, Wassberg E, Thelin S, Sletten K, Gerwins P and Westermark P. (2006) Lactadherin binds to elastin - a starting point for medin amyloid formation? Amyloid, 13(2):78-85.

5. Rennel E, Mellberg S, Dimberg A, Petersson L, Botling J, Ameur A, Westholm JO, Komorowski J, Lassalle P, Cross MJ, Gerwins P. (2007) Endocan is a VEGF-A and PI3K regulated gene with increased expression in human renal cancer. Exp Cell Res. 313(7):1285-94.
6. Peng S, Persson A, Wassberg E, Gerwins P, Thelin S and Westermark P. (2007) Role of aggregated medin in the pathogenesis of thoracic aortic aneurysm and dissection. Lab Invest, 87(12): 1195-1205.

EFFECT OF GRWOTH FACTOR INHIBITION ON ANGIOGENESIS

Ludvig Petersson

Tissue vascularization/angiogenesis is regulated by a variety of growth factors. We are investigated the relative importance of endogenous VEGF, PDGF, FGF and TGFβ in FGF-2 and PDGF-BB induced vascularization of a provisional matrix implanted on the chick chorioallantoic membrane. Neovascularization induced by FGF-2 or PDGF-BB was dependent on the presence of VEGF, since inactivation of VEGF receptors with PTK787 or sequestration of VEGF with VEGF-trap reduced angiogenesis. We are currently investigating the role of FGF receptors and TGFβ signalling by using inhibitors. The necessity of continuous presence of growth factors to maintain neovessels was analyzed by using the vasculature of the chick chorioallantoic membrane as model. At concentrations where growth factor induced neovascularization was inhibited no effect was seen on pre-existing vessels. However, if the concentrations of VEGF or PDGF inhibitors were increased vessels regressed. PDGF inhibition can cause pericytes to detach from vessels. Finally we are exploring if the inhibitory effect of inhibitors on neovascularization and vessel regression could be overcome by adding exogenous growth factors. In summary it seems that there is a substantial interdependence among different growth factors during formation of neovessels and for maintaining the neovasculature.

BIOMECHANICAL REGULATION OF TISSUE VASCULARIZATION AND THE CONTRIBUTION OF GROWTH FACTORS

Peder Fredlund Fuchs

Preliminary results from our group suggest that tissue vascularization during wound healing is regulated by biomechanical forces. These forces can be generated by myofibroblast-mediated contraction of the fibrin clot that is formed in wounds. We are now exploring if biomechanical forces generated by other mechanisms can also induce neovascularization. Vacuum assisted wound closure (VAC) is a widely used treatment of wounds that do not heal. VAC treatment is based on application of a negative pressure over the wound through an air tight dressing. The treatment has been shown to accelerate wound healing and generation of granulation tissue through unknown mechanisms. Our new model suggests that the mechanism could be that the negative pressure by “pulling” would mediate tissue expansion. We are exploring this possibility using the CAM model. It is also possible that biomechanical forces created by “pushing” might cause a neovascular tissue to be formed. This will be tested by implanting small tissue expanders in mouse ears and in the skin. Whole mount stainings will then be used to determine the mechanism of neovascularization associated with tissue expansion.

We postulate that the myofibroblast is an important mediator of mechanical forces which in turn mediates and directs neovascularization. Myofibroblasts are found in the provisional fibrin matrix and we are investigating if degradation products of fibrin (FDPs) regulate the myofibroblast functions. Preliminary findings suggest that FDPs have the ability transform fibroblasts into myofibroblasts by inducing expression of αSMA. In addition the pro-migratory effects of FDPs are under investigation.

Although mechanical forces might be important during angiogenesis it is clear that growth factors have important functions as well. The cornea assay that we have used allows detailed analysis of vessel growth by whole mount stainings and determination of perfusion of individual vessels. We have found that selective inhibition of VEGF-receptor-2 does not prevent vascularization but blocks formation of endothelial sprouts. We will next compare the effects of selective inhibition of VEGF-receptor-1, VEGF-receptor-2 and VEGF-receptor-3 on angiogenesis and on ingrowth of lymphatic vessels.

ADHESION-DEPENDENT CELL SIGNALING

Staffan Johansson

Adhesion of cells to specific proteins in the extracellular matrix contributes to the organization of tissues. In addition, it provides the cells with information about the surrounding environment, which is important for their migration, differentiation, and proliferation. As the main adhesion and migration receptors of cells, integrins are potential targets for regulation of several adhesion-related events of clinical relevance, such as wound healing, angiogenesis, thrombus formation, leukocyte extravasation to inflammatory sites, and tumor metastasis. Our work is focused on questions of how integrins transfer signals across the plasma membrane, and their interactions with components inside the cells.

Members of the group during 2008

Rajesh Gupta, postdoc, since December
Staffan Johansson, professor
Anjum Rjesh, PhD student, since December
Anne Stefansson, postdoc, until July
Birgitta Wärmegård, technician
Kathrin Zeller, PhD student

Publications 2004 to2008

1. Armulik, A., Velling, T., Johansson, S. (2004). " The integrin b1 subunit transmembrane domain regulates PI3K-dependent tyrosine phosphorylation of CAS". *Mol. Biol. Cell*, 15, 2558-2567.
2. Stefansson, A,. Armulik, A., Nilsson, I-M. von Heijne, G., Johansson, S. (2004). "Determination of N- and C-terminal borders of the transmembrane domain of integrin subunits". *J. Biol. Chem.* ,279, 21200-21205.
3. Velling, T., Nilsson, S., Stefansson, A., Johansson S. (2004). "b1-Integrins induce phosphorylation of PKB/Akt on serine 473 independently of focal adhesion kinase and Src family kinases". *EMBO Reports*, 5, 901-905.
4. Kzhyshkowska J, Gratchev A, Martens JH, Pervushina O, Mamidi S, Johansson S, Schledzewski K, Hansen B, He X, Tang J, Nakayama K, Goerdt S. (2004) Stabilin-1 localizes to endosomes and the trans-Golgi network in human macrophages and interacts with GGA adaptors. *J Leukocyt Biol.,* 76, 1151-1161.
5. McCourt PA, Hansen B, Svistuonov D, Johansson S, Longati P, Schledzewski K, Kzhyshkowska J, Goerdt S, Johansson S, Smedsrod B. (2004) The liver sinusoidal endothelial cell hyaluronan receptor and its homolog, stabilin-1 – Their roles (known and unknown) in endocytosis. *Comp Hepatol.* Jan 14;3 Suppl 1:S24.
6. Hansen B, Longati P, Elvevold K, Nedredal GI, Schledzewski K, Olsen R, Falkowski M, Kzhyshkowska J, Carlsson F, Johansson S, Smedsrød B, Goerdt S, McCourt P, Johansson S. (2005). "Stabilin-1 and stabilin-2 are both directed into the early endocytic pathway in hepatic sinusoidal endothelium via interactions with clahtrin/AP-2 independent of ligand binding". *Exp Cell Res.*, 303, 160-73.

7. Lannergård J, Flock M, Johansson S, Flock J-I , Guss B. (2005). "Studies of fibronectin-binding proteins of *Streptococcus equ*". *Infect. Immun*. 75, 7243-7251
8. Nilsson, S., Caniovska, D., Brakebush, C., Fässler, R., Johansson S. (2006). "Threonine-788 in integrin subunit b1 regulates integrin activation." *Exp Cell Res*., 312, 844-853.
9. Velling T, Stefansson A, Johansson S. (2008). "EGFR and b1-Integrins utilise different signalling pathways to activate Akt". *Exp Cell Res*., 314, 309-316.

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REGULATION OF PI3K, AKT, AND ACTIN POLYMERIZATION BY INTEGRINS

Anne Stefansson, Birgitta Wärmegård and Kathrin Zeller

During cell adhesion to extracellular ligands integrins generate intracellular signals. A key step in integrin-mediated signaling is the activation of PI3 kinase, a reaction required for the anti-apoptotic effect of adhesion as well as for several other signaling pathways. We found this reaction to occur independently of FAK, src-family kinases, and the receptor tyrosine kinases EGFR/PDGFR, and identified the membrane proximal region of the cytoplasmic β1 domain as responsible for PI3K activation. We are currently investigating which isoform of the complex PI3K family that is activated by β1 integrins as well as the mechanism for the activation.

Akt, a central regulator of cell survival and metabolism, is activated by several receptors in strictly PI3K-dependent manners. We have found that Akt phosphorylates different subsets of target proteins after integrin stimulation and EGF receptor stimulation. The variation in substrate specificity may be caused by the difference in tyrosine phosphorylation on Akt that we have observed after the two types of cell stimuli. We aim to identify the integrin-specific Akt targets by mass spectroscopy.

Actin polymerization is required for cell migration. Based on TIRF microscopy, we have developed an assay to analyze the mechanism by which integrins trigger the polymerization reaction. Inhibition of PI3K activity with chemical inhibitors or siRNA suppressed the polymerization rate in this assay. With similar approaches we will further characterize the pathway to nucleation of actin polymerization after adhesion via β1 integrins.

THE ROLE OF PROTEOGLYCANS IN ANGIOGENESIS

Johan Kreuger

Angiogenesis is the process whereby new blood and lymph vessels develop from already existing vessels. We aim to better understand how secreted and cell bound signaling molecules regulate angiogenesis, and we are in particular interested in how different proteoglycans impact angiogenesis. The rationale for our research is that increased understanding of the basic mechanisms of developmental and pathological angiogenesis will constitute a foundation for new approaches to treat pathological vessels found for example in tumors.

Proteoglycan (PG) core proteins carrying polysaccharide side chains of heparan sulfate (HS) or chondroitin sulfate (CS) type play important roles as modulators of the activity of secreted signaling molecules. The HS and CS chains differ with regard to the composition of the polysaccharide backbone, but both HS and CS are similar in that they are rich in negative charge due to abundant sulfation. The sulfate groups form clusters of negative charge along the HS and CS chains that mediate binding to patches of positively charged amino acids on the surface protein ligands, such as the angiogenic growth factors VEGFA and FGF2. PGs are abundantly expressed at the cell surface of all types of cells, including endothelial cells, and function as co-receptors contributing to the formation of complexes between growth factors and their high affinity receptors. Several studies demonstrate that PGs also impact the transport and turnover of morphogens and growth factors in developing tissues. The expression of PGs as well as their polysaccharide side chains is spatially and temporally regulated during development. However, little is known about how this regulation is achieved. The precise roles of different PGs in angiogenesis also remain to be defined.

We use spheroids of differentiating embryonic stem cells, denoted embryoid bodies, as a model system for vascular development. The embryoid bodies are particularly well suited for loss-of-function analysis of genes and miRNAs required for early embryogenesis. In addition, we use several mouse and zebrafish models to study angiogenesis in vivo, together with a novel microfluidic cell migration assay to study the effects of growth factor gradients on vascular development.

Members of the group

Sébastien Le Jan, Postdoc
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Eduar Hejll, Ph.D. student
Irmeli Barkefors, Ph.D. student
Johan Heldin, project student

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Publications from 2004 to 2008

1. Kreuger, J., Perez, L., Giraldez, A. and Cohen, S. (2004) Opposing activities of Dally-like glypican at high and low levels of Wingless morphogen activity. Developmental Cell, 4, 503-12.
2. Kreuger, J., Jemth, P., Sanders-Lindberg, E., Ron, D., Liat Eliahu Basilico, C., Salmivirta,M., and Lindahl, U. (2005) Fibroblast growth factors share binding sites in heparan sulfate. Biochem. J., 389, 145-50.
3. Kreuger J., Nilsson I. , Alitalo K. , Kerjaschki D., and Claesson-Welsh, L. (2006)Early lymph vessel development from embryonic stem cells. Arteriosclerosis, Thrombosis and Vascular Biology, 26, 1073-1078
4. Jakobsson, L., Kreuger, J., Holmborn, K., Lundin, L., Eriksson, I., Kjellen, L. Claesson Welsh, L. (2006) Heparan sulfate in trans potentiates VEGFR-mediated angiogenesis. Developmental Cell, 10, 625-34.
5. Hufnagel, L., Kreuger, J. , Cohen, S., and Shraiman, B.I. (2006) On the role of glypicans in the process of morphogen gradient formation. Developmental Biology, Dec 15;300(2):512-22.
6. Olsson AK, Dimberg A, Kreuger J and Claesson-Welsh, L. (2006) Vascular endothelial growth factors, biology and signal transduction. Nature Reviews Molecular Cell Biology, 7, 359-371.
7. Kreuger, J., Spillmann, D., Li, JP., and Lindahl, U. (2006) Interactions between heparan sulfate and proteins – the concept of specificity. Journal of Cell Biology, 174(3):323-7.
8. Jakobsson, L., Kreuger J., and Claesson-Welsh, L. (2006) Building blood vessels; stem cell models in vascular biology Journal of Cell Biology, 177(5):751-5.
9. Saetre, P., Emilsson, L., Axelsson, E., Kreuger, J., Lindholm, E., and Jazin, E. (2007) Inflammation-related genes upregulated in schizophrenia brains. BMC Psychiatry. 2007 Sep 6;7:46.
10. Barkefors, I., Le Jan, S., Jakobsson, L., Hejll, E., Carlson, G., Johansson, H., Jarvius, J., Park, J.W., Jeon, N.L., and Kreuger, J. (2008) Endothelial cell migration in stable gradients of VEGFA and FGF2: Effects on chemotaxis and chemokinesis J. Biol. Chem, May 16;283 (20):13905-12.
11. Barkefors, I., Thorslund, S., Nikolajeff, F., and Kreuger, J. (2008). A fluidic device to study directional angiogenesis in complex tissue and organ culture models. Manuscript accepted for publication. *Lab on a chip,* DOI:10.1039/B814691H.

Patent application

A US provisional patent application (US 61/115,124), having the title **"FLUIDIC ORGAN CULTURE SYSTEM"** and covering a novel organ culture chamber where stable and controllable gradients can be generated to direct complex processes such as angiogenesis, was filed on November 17, 2008.

PROTEOGLYCANS REGULATING BLOOD VESSEL DEVELOPMENT

Sébastien Le Jan, Katarina Holmborn Garpenstrand

HSPGs have recently been shown to be critical for vascular development, with capacity to modulate and potentiate VEGF-receptor mediated angiogenesis. In addition, HS has been implicated in recruitment of pericytes, a special type of supporting cell, to small vessels by allowing formation of PDGF gradients. We focus our study on the capacity of HSPGs to activate PDGF-receptor beta (PDGFRβ) to allow for subsequent development of pericytes and capillary structures from clusters of differentiating stem cells. Blood vessel formation is studied in single or mixed cultures of cells lacking either PDGFRβ or functional HS (deficient in either Exostosin-1 or N-deacetylase/N-sulfotransferase 1 and 2). Preliminary results indicate that other proteoglycans than HSPGs may support angiogenesis and pericyte recruitment to vessels, and that the PGs do not necessarily need to be expressed by the endothelial cells in order to modulate angiogenesis.

IDENTIFICATION OF GENE REGULATORY NETWORKS INVOLVED IN ANGIOGENESIS

Peder Fredlund-Fuchs, Johan Heldin

We have identified genes and miRNAs expressed at high levels by actively sprouting blood vessels in order find gene regulatory networks that promote angiogenesis. We are now developing tools for efficient knock down of individual genes and miRNAs in embryonic vessels in differentiating stem cell cultures, to identify their roles for the formation, patterning and pathfinding of growing blood vessels.

VASCULAR DEVELOPMENT AND PATHFINDING IN RESPONSE TO GROWTH FACTOR GRADIENTS

Irmeli Barkefors

Directed migration of endothelial cells is important for angiogenesis in both normal development and disease progression. Our goal is to better understand the basic mechanisms behind endothelial cell chemotaxis. We are developing several new in vitro models based on microfluidic technology that enable real time imaging of growing vascular sprouts in response to stable molecular gradients. Cells labeled by fluorescent markers make it possible to study the organization and the dynamics of endothelial cells during active sprouting, to understand how organized collective cell migration of endothelial cells (in a sprout) is achieved.

LYMPH VESSEL FORMATION DURING EMBRYONIC DEVELOPMENT

Eduar Hejll

It is still debated how lymphatic vessels are formed during embryonic development. Two main hypotheses exist: Florence Sabin proposed already in the year 1902 that lymphatic

vessels arise by centrifugal sprouting from a few distinct hotspots located in the major embryonic veins. McLure and Huntington later suggested that lymphatics may be derived from non-vascular mesodermal precursor cells scattered in many different locations in the embryo. We study embryonic lymph vessel development inside several mouse organs at different stages of embryonic development. We have data indicating that lymph vessel formation in embryonic organs may occur both via invasive sprouting and by transdifferentiation of blood endothelial cells to lymphatic endothelial cells.

TUMOR VASCULAR BIOLOGY

Anna-Karin Olsson

Prolonged and excessive angiogenesis (formation of new capillary blood vessels) has been implicated in a number of pathological processes, for instance rheumatoid arthritis, retinopathy and tumor growth, and contribute to progression of the disease. To prevent or reduce angiogenesis in these situations is therefore of clinical interest. To date five anti-angiogenic drugs have been approved for clinical use. The aim of our group is to develop new and effective treatment strategies for cancer by targeting its vasculature. We are also interested in the genetic and molecular mechanisms responsible for deregulated blood vessel formation.

Members of the group during 2008

Anna-Karin Olsson, Associate Professor
Åsa Thulin, Ph.D student
Else Huijbers, Ph.D student
Maria Ringvall, post-doc
Julia Femel, ERASMUS student

Publications from 2004 to 2008

1. Olsson, A-K., Larsson, H., Dixelius, J., Johansson, I., Lee, C., Oellig, C., Björk, I. and Claesson-Welsh, L. A fragment of histidine-rich glycoprotein is a potent inhibitor of tumor vascularization. *Cancer Res. 64, 599-605, 2004.*
2. Olsson, A-K., Johansson, I., Larsson, H., Einarsson, B., Christofferson, R., Sasaki, T. and Claesson-Welsh, L. Endostatin's minimal active domain is a heparin-binding motif that mediates inhibition of tumor vascularization. *Cancer Res. 64, 9012-9017, 2004.*
3. Dixelius, J.§, Olsson, A-K.§, Thulin, Å., Lee, C., Johansson, I., and Claesson-Welsh, L. Minimal active domain and mechanism of action of the angiogenesis inhibitor histidine-rich glycoprotein. *Cancer Res. 66, 2089-2097, 2006.* §Equal contribution.
4. Vanwildemeersch, M.§, Olsson, A-K.§, Eva Gottfridsson, Claesson-Welsh, L., Lindahl, U. and Spillmann, D. Angiogenesis inhibition by histidine-rich glycoprotein involves Zn^{2+}-dependent binding of heparan sulfate to the His/Pro-rich domain. *J Biol Chem. 281, 10298-10304, 2006.* § Equal contribution.
5. Lee, C., Dixelius, J., Thulin, Å., Kawamura, H., Claesson-Welsh, L. and Olsson, A-K. Signal transduction in endothelial cells by the angiogenesis inhibitor HRGP targets focal adhesions. *Exp. Cell Res. 312, 2547-2556, 2006.*
6. Olsson, A-K, Dimberg, A., Kreuger, J. and Claesson-Welsh, L. VEGF receptor *signalling – in control of vascular function. Nat Rev Mol Cell Biol. 7, 359-371, 2006.*
7. Rydengård, V., Olsson A-K. Mörgelin, M. and Schmidtchen, A. Histidine-rich glycoprotein exerts antibacterial activities. *FEBS J. 274, 377-389, 2007.*
8. Dimberg A., Rylova, S., Dieterich LC, Olsson A-K., Schiller P., Wikner C., Bohman S., Botling, J., Lukinius A, Wawrousek EF. and Claesson-Welsh L. αB-crystallin promotes

tumor angiogenesis by increasing vascular survival during tube morphogenesis. *Blood. 111, 2015-2023, 2007.*
9. Rydengård, V., Shannon, O., Lundqvist, K., Kacprzyk, L., Olsson, A-K., Mörgelin, M., Jahnen-Dechent, W., Malmsten, M., and Schmidtchen, A. Histidine-rich glycoprotein protects from systemic *Candida* infection. *PloS Pathogens. 4(8):e1000116, 2008.*
10. Björklund, P., Svedlund, J., Olsson, A-K., Åkerström, G. and Westin, G. The internally truncated LRP5 receptor presents a therapeutic target in breast cancer. *PLoS ONE, in press.*

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HISTIDINE-RICH GLYCOPROTEIN IN PHYSIOLOGICAL AND PATHOLOGICAL ANGIOGENESIS

Maria Ringvall, Åsa Thulin

Histidine-rich glycoprotein (HRGP; alternatively, HRG/HPRG) has been identified as an angiogenesis inhibitor *in vitro* and *in vivo* by us and others. We have demonstrated that HRGP reduces tumor growth and vascularization in mice. The anti-angiogenic effect of HRGP is mediated via its histidine-proline (His/Pro)-rich domain, which needs to be released from the mother protein to exert its effects. We are presently addressing the role of HRGP in physiological and pathological angiogenesis using HRGP-deficient mice. One model that we use for this purpose is the transgenic Rip1-Tag2 mouse, which develops orthotopic insulinoma through a multistep process. One of the stages is characterized by induction of angiogenesis, the so called “angiogenic switch”, which renders the Rip1-Tag2 mouse an excellent model for studies of tumor angiogenesis. Because of the local expression of the transgene in pancreas, these mice can also be used to address the role of HRGP in developmental and physiological angiogenesis, for instance in the retina of newborn mice.

THE ROLE OF PLATELETS IN TUMOR VASCULARIZATION

Åsa Thulin, Maria Ringvall

Platelets are anuclear cellular fragments, which play a crucial role in regulating blood hemostasis as well as non-hemostatic processes such as immunity, tumor metastasis and angiogenesis. A number of both pro-angiogenic and anti-angiogenic factors are stored in platelets. Cancer patients commonly have elevated numbers of activated platelets in their circulation and complications related to increased blood clotting, a condition that may

promote angiogenesis. The angiogenesis inhibitor HRGP is a platelet protein and mice lacking HRGP display enhanced coagulation. Our research is focused on the mechanisms by which platelets affect tumor vascularization, with a special focus on the role of HRGP in this process.

THERAPEUTIC VACCINATION AS A NOVEL TUMOR THERAPY

Else Huijbers, Maria Ringvall, Julia Femel

A small number of molecules have been identified as specifically expressed by tumor blood vessels, either by the endothelial cells themselves or by the adjacent stroma. These molecules are interesting targets for therapeutic vaccination against cancer, since they are absent in normal tissue and not expressed by the actual tumor cells, which often escapes the immune system. Therapeutic vaccination should be used as a therapy, not as a preventive strategy. Moreover, therapeutic vaccination does not induce a life-long immunity, but is instead reversible. We are developing therapeutic vaccines using tumor vascular antigens, aimed at reducing tumor development and growth. So far we have found one very promising vaccine target, the extra domain B (ED-B) of fibronectin, expressed by the tumor stroma. We have managed to break self-tolerance and induce antibody production in vaccinated mice against this self-antigen. Moreover, tumor growth is significantly reduced in the immunized mice. Electron microscope analysis of the tumor vasculature in ED-B immunized mice reveals significant changes, indicative of impaired function. The unique aspect of this vaccination project is the combination of an effective therapeutic vaccination technology with antigens specific for tumor vessels.

LOOSE CONNECTIVE TISSUES - TARGETS FOR NOVEL THERAPIES IN CANCER AND INFECTIOUS DISEASES

PI Kristofer Rubin
PI Cecilia Rydén

Loose connective tissues embed peripheral blood vessels and underlie epithelial sheets. Inflammatory processes, cancer invasion and infections occur in and are dependent on this compartment. Loose connective tissue structures are intrinsic to all organs outside the central nervous system. They are important not only as structural organ compartments but also actively maintain fluid homeostasis and filtration, as well as fulfilling important functions in innate immunity. The cellular and molecular mechanisms that control function and regeneration of loose connective tissue are less well understood. Our research aims at increasing the understanding of such mechanisms during homeostasis and pathologic conditions such as cancer growth, infection and chronic inflammation.

TUMOR STROMA AS A TARGET FOR NOVEL CANCER THERAPY

PI Kristofer Rubin

We have established a mechanistic model for the control of capillary-to-interstitium transport of fluid. Interstitial fluid pressure (IFP) is one of the Starling forces that determine fluid transport in the connective tissues, which embed all peripheral blood capillaries. Acute inflammation in normal tissues lowers IFP and leads to edema formation. Our proposed model holds that connective tissue cells apply tensile forces on ECM-fibers that in turn restrain the under-hydrated ground substance from taking up fluid and swell. A decrease in cellular tension on the ECM fibers allows the ground substance to swell, *i.e.* form edema. During this process negative IFP values can be recorded if refilling of the tissue with fluid is inhibited. The tensile forces are mediated by integrins. Furthermore, these forces depend on cytoskeletal dynamics and can be pharmacologically modulated. Our data also show that fibroblast mediated collagen gel contraction *in vitro* serves as a model for cellular control of IFP *in vivo*. We propose that modulation of IFP is part of the innate immune response and is relevant for edema formation. Edema formation is one of the classic signs of inflammation induced by *e.g.* invading bacteria. The pathophysiologic relevance of edema formation is most likely to increase drainage from the tissue, as well as to facilitate for phagocytes and soluble anti-microbial proteins to reach the infectious foci.

The stroma of both experimental and clinical carcinomas displays several pathologic features distinguishing it from a normal connective tissue but resembling chronically inflamed tissues. Characteristics include infiltrating myeloid cells, distorted blood vessels, hypoxia, low pH, pathologically high IFP and activated connective tissue cells that commonly produce a fibrotic ECM. These properties result in a disturbed physiology and *e.g.* poor uptake of anti-cancer drugs into the carcinoma. Our interest in tumor stroma physiology is fueled by the need for improvements of existing chemotherapy.

We have shown that lowering of IFP in experimental carinoma increases uptake and efficacy of chemotherapeutic agents. Notably, using microdialysis, or magnetic resonance imaging, we have pro forma demonstrated that lowering of carcinoma IFP increased capillary-to-interstitium transport of low-molecular weight compounds including 5-fluorouracil (5FU) in experimental carcinoma. In addition, our data suggest that inflammatory processes and IFP are related functionally. We are engaged in elucidating the mechanisms by which IFP can be lowered in experimental carcinoma. Furthermore, our research aims at devising clinically relevant methods to improve efficacy of existing chemotherapy for the treatment of carcinoma.

CONNECTIVE TISSUE INFECTIONS

PI Cecilia Rydén

The normal human bacterial flora on skin and mucous membranes is regarded as being beneficial to the host as long as the balance is well kept, and the barrier is intact. Staphylococci and certain streptococci are part of the normal flora of these body barriers. Small numbers of bacteria get access to deeper tissue when the barriers are damaged, something that happens almost constantly. Sometimes this process leads to infection, but most often the human defense system keeps the balance and prevents infection. Bacterial virulence mechanisms include toxin and enzyme production as well as cell wall associated molecules including adhesins with potential importance for pathogenicity. Some of the staphylococcal cell wall associated proteins belong to the family of Sdr proteins, containing stretches of SD repeats (serine and aspartic acid) in the carboxyterminal end of the protein. We have characterized, cloned and sequenced the gene for one of these proteins namely a bone sialoprotein binding protein, bbp, of S. aureus strain O24. A bbp-negative mutant of S. aureus strain O24 was made using transposon mutagenesis with a plasmid containing an ermB-casette introduced into the A-region of bbp. The bbp+ and bbp- strains have been studied in an experimental model of sepsis, work that is currently undertaken. The clinical diagnosis of osteomyelitis by radiological methods is often delayed, leading to difficulties in optimizing antibiotic treatment. The potential of recombinant bbp as an antigen for serologic diagnosis of osteomyelitis in adults has been investigated.

In many bacterial infections edema contributes to the morbidity of the patient, and is likely to decrease the possibility of antibiotics as well as of immune cells to reach the focus of infection. Edema formation may also contribute to the possibility for toxins to spread while the water content of the tissue is high. We are studying if and how bacterial products influence IFP and if such an influence constitutes a virulence mechanism.

Group members during 2008

Annelie Barrueta, Medical student (summer 2008)
Maria Blomquist, Master degree (exam) student
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Publications from 2004 to 2008

1. Heldin, C.-H., Rubin, K., Pietras, K. and Östman, A. (2004) High interstitial fluid pressure - an obstacle in cancer therapy. *Nature Rev Cancer* 4:806-813
2. Nowinski, D., Gardner, H., Rubin, K., Gerdin, B. and Ivarsson, M. (2004) Analysis of gene expression in fibroblasts in response to keratinocyte-derived factors *in vitro* – potential implications for the wound healing process. *J Invest Dermatol 122:216-221*
3. Åhlén, K., Ring, P., Tomasini-Johansson, B., Holmquist, K., Magnusson, K.-E. and Rubin, K. (2004) Platelet-derived growth factor-BB modulates focal adhesion assemblies and membrane motility of β1 integrins. *Biochem Biophys Res Commun 314:89-96*
4. Rubin, K., Salnikov, A.V. and Sundberg, C. (2006) Knockout mouse models have provided invaluable insights into vascular development In: Encyclolpedia of the Microvasculature (David Shepro (Editor), Elsevier Academic, San Diego, (NYP) Chapter 162 pp:1109-1114
5. Torell E., Molin D., Tano E., Ehrenborg C. and Rydén C. (2005) Community aquired pneumonia and bacteremia in a healthy young woman caused by methicillin-resistant Staphylococcus aureus (MRSA) carrying the genes encoding Panton-Valentine leukocidin. *Scand. J. Infect. Dis. 37:902-904.*
6. Salnikov, A.V., Roswall, P., Sundberg, C., Gardner, H., Heldin, N.-E., and Rubin, K. (2005) Inhibition of TGF-β modulates macrophages and vessel maturation in parallel to a lowering of interstitial fluid pressure in experimental carcinoma. *Lab Invest 85:512-521.*
7. Roswall, P., Bu, S., Rubin, K., Landström, M. and Heldin, N.-E. (2006) 2-Methoxyestradiol induces apoptosis in human anaplastic thyroid carcinoma cells. *Thyroid 16:143-150*
8. Hultgård-Ekwall, A.-K., Mayerl C., Rubin, K., Wick, G. and Rask-Andersen, H. (2006) An interstitial network of podoplanin-expressing cells in the human endolymphatic duct. *J Assoc Res Otolaryngol 7:38-47*
9. Lidén, Å., Karlström, Å., Lannergård, J., Kalamajski, S., Guss, B., Rubin, K. and Rydén, C. A fibronectin-binding protein from Streptococcus equi binds collagen and modulates cell-mediated collagen gel contraction. (2006) *Biochem Biophys Res Commun 340:604-610*

10. Lidén. Å, Berg, A., Nedrebø, T., Reed, R. and Rubin, K. (2006) PDGF BB-mediated normalization of dermal interstitial fluid pressure after mast cell degranulation depends on β3- but not on β1-integrins. *Circ Res 98:635-641*
11. Sthur, L.E.B., Salnikov, A.V., Ivresen, I.V., Salvesen, G., Rubin, K. and Reed, R.K. (2006) High-dose, short-term, anti-inflammatory treatment with dexamethasone reduces growth and augments the effects of 5-fluorouracil on dimethyl-a-benzanthracene-induced mammary tumors in rats. *Scand J Clin Lab Invest 66:477-486*
12. Salnikov, A.S., Heldin, N.-H., Stuhr, L.B., Wiig, H., Gerber, H., Reed, R.K. and Rubin, K. (2006) Inhibition of carcinoma cell-derived VEGF reduces inflammatory characteristics in xenograft carcinoma. *Int J Cancer 119:295-302*
13. Fortin, M.-A., Salnikov, A.V., Nestor, M., Heldin, N.-E., Rubin, K. and Lundqvist H. (2007) Immuno-PET of undifferentiated thyroid carcinoma with radioiodine-labeled antibody cMAb U36: application to antibody tumour uptake studies *Eur J Nuclear Med Mol Imag 4:1376-87*
14. Oldberg, Å, Kalamajski, S., Salnikov, A.V., Stuhr, L., Mörgelin, M., Reed, R.K., Heldin. N.-E. and Rubin, K. (2007) The collagen-binding proteoglycan fibromodulin can determine stroma matrix structure and fluid balance in experimental carcinoma. *Proc Natl Acad Sci (USA) 104:13966-13971*
15. Rubin, K., Oldberg, Å and Heldin, N.-E. (2007) Dämpad inflammation gör kemoterapi effektivare. Onkologi i Sverige 6:35-41 [in Swedish]
16. Lidén, Å., van Wieringen, T., Lannergård, J., Kassner, A., Heinegård, D., Reed, R.K., Guss, B. and Rubin, K. (2008) A secreted collagen and fibronectin-binding streptococcal protein modulates fibroblast-mediated collagen gel contraction and interstitial fluid pressure *J Biol Chem 283:1234-1242*
17. Stuhr, L.E.B., Moen, I., Nedrebø, T., Salnikov, A.V., Wiig, H., Rubin, K. and Reed, R.K. (2008) TNFα-administration influences tumor stroma structure and physiology independently of growth in DMBA-induced mammary tumors in rats. *Scand J Clin Lab Invest 9:1-10*
18. Svendsen, Ø.S., Lidén, Å., Nedrebø, T., Rubin, K. and Reed, R.K. (2008) Integrin αVβ3 acts downstream of insulin in normalization of interstitial fluid pressure in sepsis and in cell-mediated collagen gel contraction *Am J Physiol 295:H555-560*.
19. Rodriguez, A., Karen, J., Gardner, H., Gerdin, B. and Rubin, K. and Sundberg, C. (2008) Integrin α1β1 dictates development of myofibroblasts and their function in adult reactive tissues. *J Cell Mol Med. In Press*
20. Rubin, K., Lidén, Å., van Wieringen, T. and Reed, R.K. (2008) Control of interstitial fluid homeostasis - roles of growth factors and integrins In: Vascular Complications in Human Disease: Mechanisms and Consequences (Eds: Handler C, Dashwood M, Coghlan JG & Abraham D) Springer, London UK. pp 105-115

Publications by Lars Rask from 2004 to 2008:

1. Irobi, E., Aguda, A.H., Larsson, M., Guerin, C., Yin, N.L., Burtnick, L.D., Blanchoin, L. and Robinson, R.C. (2004) Structural basis of actin sequestration by thymosin-beta4: implications for WH2 proteins. EMBO J. 23, 3599-3608.

2. Sakwe AM, Larsson M, Rask L. (2004) Involvement of protein kinase C-alpha and epsilon in extracellular Ca^{2+} signaling mediated by the calcium sensing receptor. Exp. Cell Res. 297, 560-573.
3 Pontoppidan, B., Hopkins, R., Rask, L. and Meijer, J. (2005) Differential wound induction of the myrosinase system in oilseed rape (Brassica napus): contrasting insect damage with mechanical damage. Plant Sci. 168, 715-722.
4. Sakwe, A.M., Rask, L. and Gylfe, E. (2005) Protein kinase C modulates agonist-sensitive release of Ca^{2+} from internal stores in HEK293 cells overexpressing the Calcium sensing receptor. J. Biol. Chem. 280**,** 4436-4441.
5. Wycliffe, P., Sitbon, F., Ellerström, M., Wernersson, J., Ezcurra, I. and Rask L. (2005) Continuous expression in tobacco leaves of the *Brassica* PEND homologue blocks plastid differentiation and palisade cell development Plant J. 44, 1-15.
6. Wicher, G., Larsson, M., Rask, L. and Aldskogius, H. (2005) Low density lipoprotein receptor-protein (LRP)-2/megalin is transiently expressed in a subpopulation of neural rogenitors in the embryonic mouse spinal cord. J. Comp. Neurol. 492, 122-131.
7. Ellerström, M., Reidt, W., Ivanov, R., Tiedemann, J., Melzer, M., Tewes, A., Moritz, T., Mock, H.-P., Sitbon, F., Rask, L. and Bäumlein, H. (2005) Effector of Transcription (ET): A novel plant protein family is involved in the repression of gibberlin mediated processes. Plant Mol. Biol. 59, 663-681.
8. Gianni, S., Engström, Å, Larsson, M., Calosci, N., Malatesta, F., Eklund, L., Ngang, C.C., Travaglini-Allocatelli, C. and Jemth, P. (2005) The kinetics of PDZ domain-ligand interactions and implications for the binding mechanism. J. Biol. Chem. 280, 34805-34812.
9. Wicher G., Larsson, M., Fex Svenningsen, Å., Gyllencreutz, E., Rask, L. and Aldskogius, H. (2006) Low density lipoprotein receptor-related protein-2/megalin is expressed in oligodendrocytes in the mouse spinal cord white matter. J. Neurosci. Res.83, 864-873.
10. Aguda, A.H., Sakwe, A.M., Rask, L. and Robinson, R.C. (2007) Expression, crystallisation and preliminary crystallographic data analysis of filamin A repeats 14-16. Acta crystallogr. Sect. F Struct. Biol. Crysst. Commun. 63:291-293.

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1. TUMOR INTERSTITIAL FLUID PRESSURE, EXTRACELLULAR MATRIX COMPOSITION AND STROMAL CELL ACTIVITY

Sebastian Kalamajski, Renata Gustafsson

We have shown that stroma production of fibromodulin, a collagen-binding proteoglycan rich in leucine repeats (SLRP) is one key regulator of extracellular matrix structure as well as of fluid balance in carcinoma. Using gene ablation or down-regulation by specific inhibition of TGF-β1 and -β3, or inhibitors of inflammation we have established that fibromodulin

modulates the formation of the collagen scaffold structure in tumor stroma. Furthermore, we discovered a clear link between the structure of this scaffold, interstitial fluid pressure (IFP) and extracellular volume in experimental carcinoma. These results are now extended. We will study to what extent various carcinoma cells are able to stimulate production of collagen-binding SLRPs, interstitial collagens and enzymes involved in collagen maturation and cross-linking. Similar analysis will be performed using differentiated and activated macrophages. We are establishing experimental in vitro systems in which we combine carcinoma cells, monocytes/macrophages with fibroblasts. We take advantage of inhibitors of TGF-β, PDGF, VEGF and IL-1, as well as dexamethasone that are known to lower IFP in carcinoma, dampen inflammation and for some of the inhibitors also to normalize the tumor vasculature.

2. IMPORTANCE OF INTEGRIN β3 FOR INTERSTIRIAL FLUID PRESSURE IN EXPERIMENTAL CARCINOMAS

Tomas Friman, Renata Gustafsson, Sebastian Kalamajski

Integrins containing the β3 subunit are expressed and functional in various reactive tissue conditions, *e.g.* inflammation. Carcinomas are to various degrees in a constant state of inflammation and the integrin β3 is also expressed in several types of carcinomas. We have previously shown that β3 integrins are active downstream of PDGFR-β in the control of interstitial fluid pressure (IFP) in normal dermis. Expression of PDGFR-β is up-regulated in the stroma of many types of carcinoma. Upon stimulation of PDGFR-β, the αVβ3 integrin mediates contraction of interstitial cells and a subsequent increase in the IFP. Increased IFP is a characteristic feature of carcinomas that constitute or reflect a barrier for capillary-to-interstitium transport of therapeutic substances into the carcinoma tissue. Earlier experiments have shown that inhibition of PDGFR-β signaling decreases the interstitial fluid pressure and increases the efficacy of drug treatment. In this project, the aim is to investigate the IFP in syngeneic carcinomas implanted into integrin β3-negative mice. We also investigate other important parameters of the carcinomas, such as vascularity, inflammatory cell content, oxygenation, flow and tumor cell viability as well as the extra cellular matrix composition and structure. Initially, we have found differences in the IFP between tumors implanted into integrin β3 deficient and wild type mice. This difference could be explained by corresponding differences in extra cellular matrix architecture.

3. EXPRESSION OF SMALL COLLAGEN-BINDING PROTOGLYCANS (SLRP) IN HUMAN CARCINOMA

Renata Gustafsson, Tomas Friman

Our results suggest that expression of SLRPs affect the structure and properties of the collagen scaffold in carcinoma. We are investigating the expression of SLRP in biopsies from human

carcinoma in a defined selection of samples from patients. Clinical outcome and treatment results are known. There is also a need to define the spatial relation of areas that express SLRPs to vascular organization and infiltrating inflammatory cells. Finally we will extend these studies to also include defined clinical materials from head and neck, thyroid and cervix carcinomas. These studies will be carried out in close collaborations with oncologists and surgeons at the University hospital in Lund.

4. TGF-b AND PATHOPHYSIOLOGY OF TUMOR STROMA

Kristofer Rubin, Sebastian Kalamajski

TGF-β has several functions in proliferation and differentiation of cells, and can depending on context act as a tumor suppressor or promoter. We have shown that specific inhibition of TGF-β1/-β3 lowers interstitial fluid pressure in experimental carcinoma and leads to a downregulation of several macrophage-associated genes and of the small leucine rich repeat proteoglycan fibromodulin. Data from our group show that a well-characterized anti-αVβ6 antibody affects physiological parameters in KAT-4 experimental tumors. The αVβ6 integrin binds and activate "latent" inactive TGF-β. Currently, we investigate KAT-4 xenograft tumors treated with this antibody with regard to macrophage infiltration and expression of SLRPs.

5. PDGF β-RECEPTOR SIGNALING AND CYTOSKELETON DYNAMICS DURING COLLAGEN GEL CONTRACTION

Tijs van Wieringen, Vahid Reyhani

We study how PDGF-BB controls cell-mediated collagen gel contraction *in vitro*. Cell-mediated collagen gel contraction is widely used as an *in vitro* model of wound contraction and tissue repair. We have shown that collagen gel contraction can serve as an *in vitro* model of *in vivo* control of interstitial fluid pressure (IFP). Collagen-binding integrins and growth factors, *e.g.* PDGF-BB, collaborate to control collagen gel contraction. A lowering of IFP in normal rat skin, resulting from anaphylactic reactions could be restored by PDGF-BB. We use PAE cells transfected with PDGF β-receptors bearing different mutations in sites required for signaling molecule activation. This approach allowed identification of two major signaling molecules that are required for PDGF-BB affected contraction. Furthermore, in these specific mutants the PDGF-BB-induced activation of the actin-binding protein cofilin is disturbed. Cofilin is a key molecule in actin-turn over, a process required for contraction. We are investigating further downstream events from the PDGF β-receptor with a focus on cofilin using several specific inhibitors, activators and siRNA.

6. THE EFFECT OF PROTEIN CNE FROM *STREPTOCOCCUS EQUI* ON COLLAGEN GEL CONTRACTION *IN VITRO*

Tijs van Wieringen, Sebastian Kalamajski

The collagen receptor-devoid cell line C2C12 only contracts collagen gels in presence of PDGF-BB and this effect is mediated by αVβ3 integrins. αVβ3 cannot directly bind collagen. Preliminary data shows that a recombinant protein from *Streptococcus equi* (CNE) abrogates the PDGF-BB-stimulated C2C12 cell-mediated collagen gel contraction. In contrast, C2C12-α2 (transfected with α2 integrin subunit) mediated contraction is only marginally inhibited. We aim to identify the biochemical nature of this CNE-effect. Electron microscope scanning demonstrated that presence of CNE generates smaller collagen fibers and leads to a lower melting point for the triple helical conformation of collagen. Furthermore, cell death was apparent in C2C12-seeded gels while addition of PDGF-BB led to cell survival. We hypothesize the presence of a collagen-binding protein that simultaneously binds αVβ3 but is only expressed after PDGF-BB stimulation. CNE would compete with this protein for a binding site on the collagen triple helix and therefore reduce the adhesion and consequent collagen gel contraction.

7. IDENTIFYING FIBRONECTIN- AND/OR COLLAGEN-BINDING PROTEINS THAT STIMULATE CELL-MEDIATED COLLAGEN GEL CONTRACTION

Tijs van Wieringen, Vahid Reyhani

PDGF-BB induces contraction of C2C12-seeded collagen lattices and normalizes a lowered interstitial fluid pressure (IFP) in rat skin. The streptococcal protein FNE bridges collagen to fibronectin, which in turn is bound by integrin αVβ3. We have shown that FNE stimulates C2C12-mediated collagen gel contraction and normalizes anaphylaxis-induced lowering of IFP *in vivo*. These effects depend on the multi-specific αVβ3 integrin, which does not recognize native collagen. We hypothesize the existence of a protein secreted by C2C12 cells that bridges the integrin to collagen in a manner similar to FNE. PDGF-BB stimulates the production of fibronectin in C2C12 cells, a protein that is crucial for the FNE-mediated contraction. Using Western ligand blotting, protein purification and mass spectrometry we plan to identify proteins from C2C12 cell conditioned medium and cell monolayers that bind both collagen and fibronectin, and that are upregulated by PDGF-BB. Purified proteins will be used in solid phase assays to demonstrate the bridging function between collagen and fibronectin or directly between cells and collagen. Apart from fibronectin, other extracellular proteins that bind to the αVβ3 integrin, and that therefore could function as a linker between integrin and the collagen fiber network, are of interest and will be tested in similar assays. Furthermore, we plan to use immunofluorescence stainings on inflamed- and tumor tissues for proteins that tested positive in the before mentioned assays to see whether they are expressed and upregulated. The hypothesis is that in an inflamed tissue this/these protein(s) will be

produced after PDGF-BB release and contribute to controlling edema formation and/or giving rise to an elevated tumor IFP, a hindrance for anti-cancer therapy.

8. INTERPLAY BETWEEN α2β1 AND αVβ3 INTEGRINS DURING COLLAGEN GEL CONTRACTION

Tijs van Wieringen

Collagen binding integrins are important for regulating the tissue interstitial fluid pressure (IFP) by supplying a physical link between extracellular matrix (ECM) and the cytoskeleton in cells. In this project two integrins are of specific interest: the collagen binding α2β1 and the RGD sequence recognizing multi-specific αVβ3. It is believed that the signaling pathways of these two integrins are interconnected. C2C12 cells do not express collagen-binding β1 integrins and transfection of C2C12 with integrin α2 allows comparison between cells that express no or only one collagen binding integrin. Results indicate that PDGF-BB stimulates contraction in both cell lines and that clustering of β1-integrins modulates this effect. This study aims at distinguishing between signaling pathways that are involved in collagen gel contraction mediated by α2β1 (via integrin clustering) or by αVβ3 (with or without PDGF-BB stimulation). Of specific interest is the MAP kinase ERK1/2 as well as the small, cytoskeleton associated GTPase molecules Rho and Rac. A pull-down assay has been designed to detect active RhoA and Rac1. This assay is being used to investigate the effects of (PDGF-stimulated) integrin-mediated cell signaling leading to cytoskeletal rearrangements during cell contraction.

9. EFFECTS OF INFLAMMATORY MEDIATORS AND PDGF-BB ON ADHESION RECEPTORS, CYTOSKELETON, CELLULAR MOTORS AND INTRACELLULAR PROTEINS OF HUMAN FIBROBLASTS *IN VITRO*

Tijs van Wieringen

A lowering of interstitial fluid pressure (IFP) leads to edema formation *in vivo*. Inflammatory mediators such as prostaglandin E_1 and Interleukin-1 lower the IFP *in vivo* in rat dermis. These factors also inhibit fibroblast-mediated collagen gel contraction *in vitro*. PDGF-BB, on the contrary, promotes this contraction by an integrin dependent process and leads to normalization of lowered IFP *in vivo*. The aim of this project is to elucidate the mechanisms behind these processes and especially the effects on cytoskeletal and actin-binding proteins. Fibroblasts are stimulated with different factors and their effects on cytoskeletal rearrangements are studied by immuno-fluorescence. We also examine the expression and localization of actin microfilaments, β1 and β3 integrins. We have identified several changes in expression and phosphorylation of actin-binding proteins using 2D gel electrophoresis and Mass Spectrometry (MALDI-TOF).

10. THE EFFECT OF PROTEINS FROM *STREPTOCOCCUS EQUI*, SUBSPECIES *EQUI*, ON COLLAGEN GEL CONTRACTION *IN VITRO* AND NORMALIZATION OF INTERSTITIAL FLUID PRESSURE *IN VIVO*

Lena Persson, Maria Blomquist, Tijs van Wieringen, Annelie Barrueta

We postulate that secreted ECM-binding proteins could participate in a novel virulence mechanism in which the bacterial proteins modulate fluid balance, edema formation and shock. *Streptococcus equi*, subspecies *equi*, is a pathogen in horses that causes strangles. Recently we published a report in which we demonstrated that a secreted protein (FNE) from this species induced C2C12 cell-mediated collagen gel contraction and normalized a lowered interstitial fluid pressure in rat skin. A mouse model of skin abscess formation was set up in collaboration with Elisabeth Josefsson and the late Andrej Tarkowski at the Sahlgrenska Akademin in in Gothenburg to study virulence of different streptococcal and staphylococcal strains having different repertoires of ECM-binding proteins. Clinical differences were seen in mice injected intradermally with streptococci expressing FNE and strains that do not express FNE. Read outs include changes in loose connective tissue structure and physiological properties during localized infections. In an experimental model of skin abscess formation the possibility of FNE playing a role in the tissue pressure and the development of disease will be studied. In this model treatment possibilities could be studied with regard to antibiotic penetration into the lesion as a function of interstitial fluid pressure.

11. SEROLOGICAL TEST FOR OSTEOMYELITIS BASED ON STAPHYLOCOCCAL BONE SIALOPROTEIN-BINDING PROTEIN

Lena Persson

We have earlier shown that the presence of the BSP-binding protein, Bbp, is clinically associated with infection of bone tissue, and now we have found also that Bbp is immunogenic both in children and adults. Serological detection of IgG antibodies against certain staphylococcal proteins may aid in the differential diagnosis between bone destructive disease due to disorders with neuropathic and angiopathic changes as in diabetes mellitus, Charcot foot and staphylococcal infection. Such discrimination is very important in avoiding unnessecary antibiotic use with risks for side effects as well as increased antibiotic resistance in bacteria. A bbp-negative mutant of S.aureus O24 has been shown to be more virulent in a sepsis model of staphylococcal infection than the bbp wildtype, whereas the impact on osteitis or arthritis is more complex. These bacteria will also be studied in the mouse model for skin abscess formation that is currently being set up.

MECHANISMS OF OPTIMAL TISSUE REGENERATION VERSUS FIBROSIS AND THE ROLE OF THE MICROVASCULATURE

PI: Christian Sundberg

The main focus of this group is to understand the biology of blood vessels and their role in tumor formation and fibrosis. Fibrosis is a common denominator in a wide variety of diseases characterized by chronic inflammation including stroma formation in solid tumors, rheumatoid arthritis and inflammatory bowel disease, connective tissue diseases, atherosclerosis, heart failure, transplant rejection and wound healing to name a few. The progression of fibrosis in these diseases leads to the derangement of tissue architecture and subsequent failure of the organ. In many of these diseases current therapeutic approaches have only marginally contributed to cure and must be seen as approaches that delay the progression of the disease. However, in certain circumstances in the adult, diseased organs (for instance the kidney in glomeruloid nephritis, the liver after hepatitis, and the heart during ventricular hypertrophia) are capable of healing themselves with minimal damage to the tissue and its function. Tissue regeneration following damage to an organ during embryogenesis and infancy is also an example of tissue repair with minimal functional sequel. Thus, the body has mechanisms by which to adequately repair damaged organs. Why the body does not always achieve this, and what causes progression in one instance, and healing in another, is largely unknown and is one of the main subjects of study in the laboratory.

Group members 2008

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PUBLICATIONS from 2005 to 2008

1. Salnikov AV, Roswall P, Sundberg C, Gardner H, Heldin NE and Rubin K. Inhibition of TGF-b moduates macrophages and vessel maturation in parallel to a lowering of interstitial fluid pressure in experimental carcinoma. Lab Invest. 2005; 85:512-521.
2. Jackson SW, Hoshi T, Enjyoji K, Cszimadia E, Robson SC, Sundberg C. Disordered purinergic signaing inhibits angiogenesis and tumor growth in cd39/Entpd1-null mouse, Am J Pathol.2007; 171:1395-1404.
3. Rodriguez A, Karen J, Gardner H, Gerdin B, Rubin K, and Sundberg C. Integrin a_1b_1 dictates development of activated connective tissue cell phenotypes and their function in adult reactive tissues. In Press, J Cellular and Molecular Medicine.
4. Sundberg C, Friman T, Hecht LE, Kuhl C and Solomon KR. PDGF-BB producing cells dictate biological endpoints in receptor bearing cells through targeting specific cohorts of PDGFb-receptors. Under Revision. Am J Pathol.

5. Rubin K, Salnikov A and Sundberg C. Knockout mouse modes have provided invaluable insights into microvascular development and functions. 2005. Microvascular Research edited by David Shepro. Chapter 162: 1113-1118.

Patent: Methods of reducing angiogenesis ID #632. International patent.
Inventors: Sundberg C, Robson S, Sevigney J, Hoshi T. 2005.

1. DEFINING THE PERICYTE-FIBROBLAST LINEAGE AND THEIR COMMON STEM CELL

Jakob Karén and Christian Sundberg

We have published the novel concept that microvascular pericytes have the ability to differentiate into collagen type I producing fibroblasts, thereby coupling the process of angiogenesis and fibrosis in a previously unrecognized way. Our research involves the isolation and study of stem cells that define the pericyte-fibroblast lineage, and to identify different stages of this differentiation process. Preliminary results show that five different stages are involved in this process. We will study differences in gene expression and gene products during this differentiation process using cDNA microarray techniques as well as proteomics (2-D gel electrophoresis). By defining this new lineage novel insights into the process of fibrosis and potential modulation will be identified.

2. GENE THERAPEUTIC APPROACH FOR STUDYING FIBROSIS AND BLOOD VESSEL FORMATION IN THE BODY

Alejandro Rodriguez, Tomas Friman and Christian Sundberg

We have previously published a novel animal model by introducing VPF/VEGF into normal tissues by adenoviral vectors. These studies have led to the discovery of three previously unknown modes of angiogenesis. We will further study cell progression and events that occur during blood vessel formation and fibrosis in the body. To this effect gene therapy techniques using adenoviral vectors will be used in order to induce genes for growth factors that are believed to modulate the development of the tumor stroma. Growth factors will be introduced into normal and diseased tissues, both individually and in combination. Effects of these growth factors will be studied using advanced morphological and physiological techniques which are being developed. This approach might be used for treatment of heart disease as well as diseases in other arteries in the body resulting from arteriosclerosis and diabetes.

3. APPLYING GENE THERAPY IN ORDER TO STUDY AND THERAPEUTICALLY MODULATE ANGIOGENESIS

Alejandro Rodriguez, Tomas Friman and Christian Sundberg

Angiogenesis and desmoplasia are two major processes that contribute to tumor stroma formation. We have recently published a novel animal model, which allows for a detailed

temporal analysis of the in vivo effects of growth factors, by introducing VPF/VEGF into normal tissues using adenoviral vectors. We intend to use adenoviral vectors that are engineered to express other growth factors that are believed to modulate the development of the tumor stroma. This approach will result in useful models, which will allow for a detailed analysis of the cellular and molecular mechanisms underlying the formation of tumor stroma, angiogenesis and fibrosis. Knowledge from these animal models will then be used as a basis for a rational approach for studying the stroma in tumor models, which we have already extensively characterized in our laboratories.

4. DIFFERENCES IN BIOLOGICAL ENDPOINTS BASED ON SUBCELLULAR CONFINEMENT OF SIGNALING COMPLEXES

Tomas Friman and Christian Sundberg

A long standing scientific question has been why a growth factor can evoke different biological endpoint responses for instance migration versus proliferation. We are working on the hypothesis that mode of growth factor delivery stimulates different subpopulations of receptors localized in different cell membrane compartments. This issue is of importance considering the growing number of compounds undergoing clinical trials that are directed towards specific down stream signaling events following growth factor/ligand interactions.

5. STUDYING EARLY EVENTS DURING THE PROCESS OF ANGIOGENESIS IN THE LIVING ANIMAL

Tomas Friman and Christian Sundberg

We are studying the earliest structural and biochemical events during the initial stages of angiogenesis evoked by growth factors or in reponse to wound healing. Methods have been developed allowing for us to visualise these events in the living animal using different fluorescent tracers. The aim of the project is to identify precursor structurers that form different componente of the vascular tree and follow them over time.

Much effort has been directed towards isolating stemcells, propagating them *in vitro* and then reimplanting them inorder to achieve organ regeneration. We are studying a novel structure which we have named “stem structure” which is a conglomerate of cells and matrix which we are trying to activate *in situ* inorder to achieve organ regeneration based on an *in vivo* model which we are currently developing.

THE BIOLOGICAL ROLE OF MAST CELLS DURING INFLAMMATION AND PATHOGENIC DISORDERS

Magnus Åbrink

Mast cells (MCs) are most well known for their harmful effects in allergy and asthma. In addition they also seem to act negatively in other inflammatory disorders, for example in rheumatoid arthritis. In contrast, they have been shown to be essential for host defense against parasites and bacteria, and in the destruction of snake and bee venoms. MCs are considered to be "guardian" cells as they are situated in the mucosa and the skin, waiting to take action upon the encounter of an intruder. In response to an assault, MCs release their granules filled with potent mediators, and synthesize cytokines to attract other immuno -regulatory and -defensive cells, such as lymphocytes and granulocytes, that will kill the pathogen. The de-granulation releases an enormous amount of proteases that likely will affect the surrounding tissue in multiple ways. To address the function of the proteases in inflammatory disorders, we have developed two different knockout mice strains. The mMCP-4, one of the connective tissue type MC chymases, have been shown to be the major protein with chymotryptic activity in skin and peritoneal MCs. The serglycin, a proteoglycan expressed in many hematopoietic cell types, is believed to be involved in intracellular storage. The serglycin-deficient strain show defects in both connective tissue and mucosal type MCs, cytotoxic cells, macrophages, neutrophils and platelets. Current work focuses on the role of chymase and serglycin-dependent mechanisms in chronic inflammation, e.g. rheumatoid arthritis, as well as in host defense against parasitic infection, (infection with *Toxoplasma gondii* and *Trichinella spiralis).* The *in vivo* models are supplemented with *in vitro* cell culture methods, using bone marrow or peritoneal-derived MCs. In addition, we have successfully established ES-derived MC-cultures. *In vitro* we analyze the production and release of mast cell mediators, including newly synthesized cytokines.

Members of the group during 2008

Ananya Roy, PhD student
Osama Sawesi, PhD student
Magnus Åbrink, Associate professor, Group leader

Publications from 2004 to 2008

1. Magnusson S, Pejler G, Kleinau S, Åbrink M. Mast cell chymase contributes to the antibody response and the severity of autoimmune arthritis. FASEB J In press
2. Braga T, Ringvall M, Tveit H, Åbrink M, Pejler G. Reduction with dithiothreitol causes serglycin-specific defects in secretory granule integrity of bone marrow derived mast cells. Molecular Immunology In press
3. Bengtsson H, Epifantseva I, Åbrink M, Kylberg A, Kullander K, Ebendal T, Usoskin D. Generation and characterization of a Gdf1 conditional null allele. Genesis 46(7): 368-72 2008

4. Grujic M, Christensen JP, Sörensen MR, Åbrink M, Pejler G, Thomsen AR. Delayed contraction of the CD8+ T cell response toward lymphocytic choriomeningitis virus infection in mice lacking serglycin. J Immunology 181(2): 1043-51 2008
5. Piliponsky AM, Chen CC, Nishimura T, Metz M, Rios EJ, Dobner PR, Wada E, Wada K, Zacharias S, Mohanasundaram UM, Faix JD, Åbrink M, Pejler G, Pearl RG, Tsai M, Galli SJ. Neurotensin increases mortality and mast cells reduce neurotensin levels in a mouse model of sepsis. Nat Med. 14(4): 392-8 2008
6. Ringvall M, Rönnberg E, Wernersson S, Duelli A, Henningsson F, Åbrink M, Garcia-Faroldi G, Fajardo I, Pejler G. Serotonin and histamine in mast cell secretory granules is dependent on serglycin proteoglycan. J Allergy Clin Immunol. 121(4): 1020-6 2008
7. Woulfe DS, Lilliendahl JK, August S, Rauova L, Kowalska MA, Åbrink M, Pejler G, White JG, Schick BP. Serglycin proteoglycan deletion induces defects in platelet aggregation and thrombus formation in mice. Blood 111(7): 3458-67 2008
8. Pejler G, Åbrink M, Ringvall M, Wernersson S. Mast cell proteases. Advances in Immunology, 95: 167-255 2007
9. Mizuno K, Tolmachova T, Ushakov DS, Romao M, Åbrink M, Ferenczi MA, Raposo G, Seabra MC. Rab27b regulates mast cell granule dynamics and secretion. Traffic 8(7): 883-92 2007
10. Tolmachova T, Åbrink M, Futter CE, Authi KS, Seabra MC. Rab27b regulates number and secretion of platelet dense granules. Proc Natl Acad Sci U S A, 104(14): 5872-7 2007
11. Moller C, Karlberg M, Åbrink M, Nakayama KI, Motoyama N, Nilsson G. Bcl-2 and Bcl-XL are indispensable for the late phase of mast cell development from mouse embryonic stem cells. Exp Hematol. 35(3): 385-93 2007
12. Niemann CU, Åbrink M, Pejler G, Fischer RL, Christensen EI, Knight SD, Borregaard N. Neutrophil elastase depends on serglycin proteoglycan for localization in granules. Blood 109(10): 4478-86 2007
13. Braga T, Grujic M, Lukinius A, Hellman L, Åbrink M, Pejler G. Serglycin proteoglycan is required for secretory granule integrity in mucosal mast cells. Biochem J. 403(1): 49-57 2007
14. Lundequist A, Åbrink M, Pejler G. Mast cell-dependent activation of pro matrix metalloprotease 2: A role for serglycin proteoglycan-dependent mast cell proteases. Biol Chem. 387(10-11): 1513-9 2006
15. Henningsson F, Hergeth S, Cortelius R, Åbrink M, Pejler G. A role for serglycin proteoglycan in granular retention and processing of mast cell secretory granule components. FEBS J. 273(21): 4901-12 2006
16. Metz M, Piliponsky AM, Chen CC, Lammel V, Åbrink M, Pejler G, Tsai M, Galli SJ. Mast cells can enhance resistance to snake and honeybee venoms. Science 313(5786): 526-30 2006
17. Zernichow L, Åbrink M, Hallgren J, Grujic M, Pejler G, Kolset SO. Serglycin is the major secreted proteoglycan in macrophages and has a role in the regulation of macrophage tumor necrosis factor-alpha secretion in response to lipopolysaccharide. J Biol Chem. 281(37): 26792-801 2006
18. Tolmachova T, Anders R, Åbrink M, Bugeon L, Dallman MJ, Futter CE, Ramalho JS, Tonagel F, Tanimoto N, Seeliger MW, Huxley C, Seabra MC. Independent degeneration of photoreceptors and retinal pigment epithelium in conditional knockout mouse models of choroideremia. J Clin Invest. 116 (2): 386-94 2006

19. Grujic M, Braga T, Lukinius A, Eloranta ML, Knight SD, Pejler G, Åbrink M. Serglycin-deficient cytotoxic T lymphocytes display defective secretory granule maturation and granzyme B storage. J Biol Chem. 280(39): 33411-8 2005
20. Tchougounova E, Lundequist A, Fajardo I, Winberg JO, Åbrink M, Pejler G. A key role for mast cell chymase in the activation of pro-matrix metalloprotease-9 and pro-matrix metalloprotease-2. J Biol Chem. 280(10): 9291-6 2005
21. Barnes BR, Marklund S, Steiler TL, Walter M, Hjalm G, Amarger V, Mahlapuu M, Leng Y, Johansson C, Galuska D, Lindgren K, Åbrink M, Stapleton D, Zierath JR, Andersson L. The 5'-AMP-activated protein kinase gamma3 isoform has a key role in carbohydrate and lipid metabolism in glycolytic skeletal muscle. J Biol Chem. 279(37): 38441-7 2004
22. Åbrink M, Grujic M, Pejler G. Serglycin is essential for maturation of mast cell secretory granule. J Biol Chem. 279(39): 40897-905 2004
23. Lundequist A, Tchougounova E, Åbrink M, Pejler G. Cooperation between mast cell carboxypeptidase A and the chymase mouse mast cell protease 4 in the formation and degradation of angiotensin II. J Biol Chem. 279(31): 32339-44 2004
24. Looman C, Hellman L, Åbrink M. A novel Kruppel-Associated Box identified in a panel of mammalian zinc finger proteins. Mamm Genome 15(1): 35-40 2004

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DEVELOPMENT OF NEW MODELS FOR FUNCTIONAL STUDIES OF MAST CELLS

Ananya Roy

The usage of a MC deficient mouse strain (W^v) has lead to many cutting edge findings on the role of MCs in disease. This mouse strain completely lacks MCs because it carries a mutation in the c-KIT gene. c-KIT is a cell surface receptor for the stem cell factor (SCF), which is an important factor for growth and differentiation of MCs. The MC deficient mice have been used to study many different pathological conditions and by simply reconstituting W^v mice with in vitro derived MCs, these studies provided convincing evidence for a major role of MCs in disease progression. However, these studies do not specify what factor(s) from the MCs that are responsible for either the protective or the harmful effect observed. Therefore, we are in the process of developing two novel MC specific mouse strains.

MAST CELL DEFENSE DURING TOXOPLASMA AND TRICHINELLA INFECTION

Osama Sawesi, Ananya Roy

Mast cells (MCs) are involved in the first line of defense in innate and acquired immunity and play a major role during inflammation, e.g. in allergic reactions and in bacterial and

parasitic infections. Upon infection MCs are activated and release into the tissue a wide array of preformed inflammatory mediators, including histamine, many cytokines, prostaglandins, leukotrienes, heparin and MC-proteases. The primary sites of infection are often mucosal membranes but many parasites also invade other tissues of the host. The protozoan *Toxoplasma gondii* is an intracellular parasite capable of invading all cell types. It causes severe problems in livestock with stillbirths and abortions. *Trichinella spiralis* is an intestinal nematode which infectious larval stage is encysted in muscle cells of the host. It can infect a wide range of domestic and wild animals. Both *T. gondii* and *T. spiralis* are zoonotic parasites and will infect humans if we eat undercooked meat from infected animals. Recently, MCs have been shown to have a protective role during parasitic infections. But, what MC specific factor(s) that are involved in resolving the complication is not known. These projects aims at identifying new MC mediated mechanisms in parasite driven host responses. The use of two different knockout (KO) mouse strains, i.e. one with a defect in a wide range of cells (the intracellular proteoglycan serglycin) and one MC specific (the chymase, mMCP-4), give us the possibly to address these issues in a very broad manner. Prelimary results indicate that both chymase and serglycin deficient mice show an altered neutrophil recruitment and cytokine response to Toxoplasma infection.

AUTOIMMUNE ARTHRITIS AND THE ROLE OF SERGLYCIN

Ananya Roy

In inflammatory processes many different cell types play important and diverse roles. MCs are usually considered to be an immediate early cell-type in the inflammatory cascade releasing many potent mediators, i.e. histamine, tissue remodeling proteases, prostaglandins, leukotrienes and several cytokines. The release of these mediators activates macrophages, attracts neutrophils and/or eosinophils (depending on site), and recruit T- and B-cells to drive the response beyond the early phase. In rheumatoid arthritis MCs have been shown to be crucial for the onset of disease. In collaboration with Sandra Kleinau and Sofia Magnusson at ICM, Uppsala University, we will make use of the serglycin-deficient mouse strain to study the role of serglycin in two models of experimental arthritis. First we will investigate a passive model by administration of antibodies against collagen. This model bypasses the antibody response to antigen, i.e collagen, and develops experimental arthritis within days after the immunization. Secondly, we will immunize with collagen directly in an active model of collagen-induced arthritis (CIA). This model develops slower and involves both the innate and the adaptive immune system to evolve. We aim at identifying the potential role of the only committed intracellular proteoglycan, serglycin, in this inflammatory autoimmune disease. Preliminary results indicate that, in the passive model, serglycin contributes with approximately 50% of the disease severity since the knockout mice have a 50% reduction in the clinical score as compared to wild type animals.

SCIENTIFIC PAPERS PUBLISHED 2008

Akusjärvi, G. (2008). Temporal regulation of adenovirus alternative RNA splicing.
Front Biosci., 13, 5006-5013.

Andersson DI. (2008) Shrinking bacterial genomes.
Microbe. March.

Andersson, L. (2008). Domestic animals - a treasure trove for comparative genomics. In: *Comparative Genomics*: Basic and Applied Research (ed. Brown, J.R.) Taylor and Francis Group, Boca Raton, Fl., pp. 341-362.

Andersson, L. (2008). Genome-wide association analysis in domestic animals – a powerful approach for genetic dissection of trait loci.
Genetica Aug. 15.

Annerén C. (2008). Tyrosine kinase signaling in embryonic stem cells.
Clin Sci. 115:43-55.

Bach, A., Chi, C. N., Olsen, T. B., Pedersen, S. W., Clausen, R. P., Jemth, P., and Strømgaard, K.* (2008). Peptidomimetic inhibitors of the PSD-95/NMDA receptor interaction.
J. Med. Chem. 51:6450-6459.

Barkefors, I., Le Jan, S., Jakobsson, L., Hejll, E., Carlson, G., Johansson, H., Jarvius, J., Park, J.W., Jeon, N.L., and Kreuger, J. (2008). Endothelial cell migration in stable gradients of VEGFA and FGF2: Effects on chemotaxis and chemokinesis
J. Biol. Chem, May 16;283:13905-12.

Barkefors, I., Thorslund, S., Nikolajeff, F., and Kreuger, J., (2008). "A fluidic device to study directional angiogenesis in complex tissue and organ culture" models.
Lab. Chip, DOI:10.1039/B814691H

Bengtsson H, Epifantseva I, Åbrink M, Kylberg A, Kullander K, Ebendal T, Usoskin D. (2008). Generation and characterization of a Gdf1 conditional null allele.
Genesis 46:368-72

Bergström-Lind, S., Molin, M., Savitski, M.M., Emilsson, L., Åström, J., Hedberg, L., Adams, C., Nielsen, M.L., Engström, Å., Elfineh, L., Andersson, E., Zubarev, R.A. and Pettersson, U. (2008). Immunoaffinity enrichments followed by mass spectrometric detection for studying global protein tyrosine phosphorylation.
J. Proteome Res. 7:2897-910.

Bode, L., Salvestrini, C., Park, P.W., Li, J.-P., Esko, J.D., Yamaguchi, Y., Murch, S. and Freeze, H.H. (2008) Heparan sulfate and syndecan-1 are essential in maintaining Intestinal Epithelial barrier Function,
J. Clin. Invest. 118: 229-238

Brännvall, K., Corell, M., Forsberg-Nilsson, K. and Fex Svenningsen, Å., (2008) Environmental cues from CNS, PNS, and ENS cells regulate CNS progenitor differentiation,
Neuroreport 19, 1283-1289

Calosci, N., Chi, C. N., Richter, B., Camilloni, C. Engström, Å, Eklund, L., Travaglini-Allocatelli, C., Gianni, S.*, Vendruscolo, M.* and Jemth, P.* (2008) Comparison of successive transition states for folding reveals alternative early folding pathways of two homologous proteins..
Proc. Natl Acad. Sci. USA. 105 (49), 19241 - 6.

Carlsson, K., Linder, C. (2008), Introduktion till mikrobiologi – med inriktning mot naturvetare och farmaceuter,
Studentlitteratur, ISBN: 9789144048468

Carlsson*, P., Presto*, J., Spillmann, D., Lindahl, U., Kjellén, L. (2008). Heparin/heparan sulfate biosynthesis: processive formation of N-sulfated domains.
J. Biol. Chem. 283:20008-20014. * shared first author

Cébe-Suarez*, S., Grünewald*, F. S., Jaussi, R. Li, X., Claesson-Welsh, L., Spillmann, D., Mercer, A. A., Prota, A. E. , Ballmer-Hofer, K. (2008). Orf virus VEGF-E NZ2 promotes paracellular NRP-1/VEGFR-2 coreceptor assembly via the peptide RPPR.
FASEB J 22:3078-3086.

Chi, C. N., Elfström, L., Shi, Y., Snäll, T., Engström, Å, and Jemth, P.* (2008) Reassessing a sparse energetic network within a single protein domain.
Proc. Natl Acad. Sci. USA. 105, 4679-4684.

Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, Sougnez C, Greulich H, Muzny DM, Morgan MB, Fulton L, Fulton RS, Zhang Q, Wendl MC, Lawrence MS, Larson DE, Chen K, Dooling DJ, Sabo A, Hawes AC, Shen H, Jhangiani SN, Lewis LR, Hall O, Zhu Y, Mathew T, Ren Y, Yao J, Scherer SE, Clerc K, Metcalf GA, Ng B, Milosavljevic A, Gonzalez-Garay ML, Osborne JR, Meyer R, Shi X, Tang Y, Koboldt DC, Lin L, Abbott R, Miner TL, Pohl C, Fewell G, Haipek C, Schmidt H, Dunford-Shore BH, Kraja A, Crosby SD, Sawyer CS, Vickery T, Sander S, Robinson J, Winckler W, Baldwin J, Chirieac LR, Dutt A, Fennell T, Hanna M, Johnson BE, Onofrio RC, Thomas RK, Tonon G, Weir BA, Zhao X, Ziaugra L, Zody MC, Giordano T, Orringer MB, Roth JA, Spitz MR, Wistuba II, Ozenberger B, Good PJ, Chang AC, Beer DG, Watson MA, Ladanyi M, Broderick S, Yoshizawa A, Travis WD, Pao W, Province MA, Weinstock GM, Varmus HE, Gabriel SB, Lander ES, Gibbs RA, Meyerson M, Wilson RK. (2008). Somatic mutations affect key pathways in lung adenocarcinoma.
Nature 455:1069-75.

Drogemüller C, Karlsson EK, Hytönen HK, Perloski M, Dolf G, Sainio K, Lohi H, Lindblad-Toh K & Leeb T. (2008) Identification of the canine hairless mutation reveals an essential role for *FOXI3* in ectodermal development
Science 321:1462.

Dutt A, Salvesen HB, Chen TH, Ramos AH, Onofrio RC, Hatton C, Nicoletti R, Winckler W, Grewal R, Hanna M, Wyhs N, Ziaugra L, Richter DJ, Trovik J, Engelsen IB, Stefansson IM, Fennell T, Cibulskis K, Zody MC, Akslen LA, Gabriel S, Wong KK, Sellers WR, Meyerson M, Greulich H. (2008). Drug-sensitive FGFR2 mutations in endometrial carcinoma.
Proc Natl Acad Sci U S A. 105:8713-7.

Dyachok, O., Idevall-Hagren, O., Sågetorp, J., Tian, G., Wuttke, A., Arrieumerlou, C., Akusjärvi,G., Gylfe, E. and Tengholm, A. (2008). Glucose-idnuced cyclic AMP oscillations regulate pulsatile insulin secreation.
Cell Metab. 8, 26-37

Eriksen J, Mwankusye S, Mduma S, Veiga MI, Kitua A, Tomson G, Petzold MG, Swedberg G, Gustafsson LL, Warsame, M. (2008). Antimalarial resistance and DHFR/DHPS genotypes of *Plasmodium falciparum* three years after introduction of sulfadoxine-pyrimethamine and amodiaquine in rural Tanzania.
Trans R Soc Trop Med Hyg 102: 137-42

Eriksson, J., Larson, G., Gunnarsson, U., Bed'hom, B., Tixier-Boichard, M., Strömstedt, L., Wright, D., Jungerius, A., Vereijken, A., Randi, E., Jensen, P. and Andersson, L. (2008). Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken.
PLoS Genetics 4: e1000010.

Forsberg, M., Forsberg-Nilsson K and Ebendal, T,, (2008). Genetiskt modifierade försöksdjur (Genetically modified mice),
Book chapter in *Försöksdjurskunskap (Animal Experimentation),* Studentlitteratur eds., in press

Geli P, Andersson M, Svensson Å and Andersson DI. A (2008). Multi-Type Branching Model with Varying Environment for Bacterial Dynamics with Postantibiotic Effect.
J Theoretical Biology. Oct 9 Epub.

Götte*, M, Spillmann*, D., Yip*, G. W., Versteeg, E., Echtermeyer, F. G., van Kuppevelt T. H., Kiesel, L. (2008): Changes in heparan sulfate are associated with delayed wound repair, altered cell migration, adhesion and contractility in the galactosyltransferase I (bGaltT-7) deficient form of Ehlers-Danlos syndrome.
Hum. Mol. Genet. 17:996-1009.

Grujic M, Christensen JP, Sörensen MR, Åbrink M, Pejler G, Thomsen AR. (2008). Delayed contraction of the CD8+ T cell response toward lymphocytic choriomeningitis virus infection in mice lacking serglycin.
J Immunology 181:1043-51

Gustavsson, M. and Ronne, H. (2008) Evidence that tRNA modifying enzymes are important in vivo targets for 5-Fluorouracil in yeast.
RNA 14, 666-674.

Gustavsson, M., Barmark, G., Larsson, J., Murén, E. and Ronne, H. (2008) Functional genomics of monensin sensitivity in yeast: implications for post-Golgi transport and vacuolar H^+-ATPase function.
Mol. Genet. Genomics 280, 233-248.

Hjelm, F., Karlsson, M,C.I., and Heyman, B. (2008). A novel B-cell mediated transport of IgE immune complexes to the follicle of the spleen.
J. Immunol. 180: 6604-6610.

Jemth, P., Johnson, C. M., Gianni, S., and Fersht, A. R.* (2008) Demonstration by burst phase analysis of a robust folding intermediate in the FF domain.
Prot. Eng. Des. Sel. 21, 207-214

Kawamura, H., Li, X., Goishi, K., van Meeteren, L.A., Jakobsson, L., Cébe-Suarez, S., Shimizu, A., Edholm, D., Ballmer-Hofer, K., Kjellén, L., Klagsbrun, M. and Claesson-Welsh, L. (2008) "Neuropilin-1 in regulation of VEGF-induced activation of p38MAPK and endothelial cell organization"
Blood 112, 3638-3649

Larsson J., Forsberg M., Brännvall K., Zhang X-Q., Enarsson M., Hedborg F. and Forsberg-Nilsson K., (2008) Nuclear Receptor Binding Protein 2 is induced during neural progenitor differentiation and affects cell survival.
Molecular and Cellular Neuroscience 39, 32-39

Li, J.-P., (2008) Heparin, heparan sulfate and heparanase in cancer: remedy for metastasis?
Anticancer Agents Med Chem. Jan;8:64-76; Review

Lidén*, Å., van Wieringen*, T., Lannergård, J., Kassner, A., Heinegård, D., Reed, R.K., Guss, B. and Rubin, K. (2008) A secreted collagen and fibronectin-binding streptococcal protein modulates fibroblast-mediated collagen gel contraction and interstitial fluid pressure
J Biol Chem 283:1234-1242 * shared first author.

Lind PA and Andersson DI. (2008). Whole-genome mutational biases in bacteria.
Proc Natl Acad Sci U S A. 105, 17878-17883.

Lindahl, U. (2008): Artificial polysaccharide in contaminated heparin. Acute hypersensitivity reactions explained — but how could it happen?
Läkartidningen 105:24-30.

Lindås, A.-C., Eriksson, S., Jozsa, E. & Tomkinson, B. (2008): Investigation of a role for Glu-305 and Glu-331 in substrate binding of tripeptidyl-peptidase II
Biochim. Biophys. Acta 1784, 1899-1907

Löfmark S, Jernberg C, Billström H, Andersson DI and Edlund C. (2008). Restored fitness leads to long-term persistence of resistant Bacteroides strains in the human intestine.
Anaerobe 14:157-160.

Lövkvist, L., Sjölinder, H., Plant, L., Eriksson, J., Aro, H., Jones, A., Norrby-Teglund, A., and Jonsson, A-B. (2008) The ScpC protease of Streptococcus pyogenes affects the outcome of sepsis.
Infect. Immun. 76:3959-3966.

Lövkvist, L., Sjölinder, H., Wehelie, R., Aro, H., Norrby-Teglund, A., Plant, L., and Jonsson, A-B. (2008) CD46 contributes to the severity of group A streptococcal infection.
Infect. Immun. 76: 3951-3958.

Magnusson S, Pejler G, Kleinau S, Åbrink M. Mast cell chymase contributes to the antibody response and the severity of autoimmune arthritis.
FASEB J 208 Nov 14

Markljung,E., Braunschweig,M.H., Karlskov-Mortensen,P, Bruun,C.S., Sawera,M., Cho,I.C., Hedebro-Velander,I, Josell,A, Lundstrom,K, von Seth,G, Jorgensen,C.B., Fredholm,M,, and Andersson,L. (2008). Genome-wide identification of quantitative trait loci in a cross between Hampshire and Landrace II: meat quality traits.
BMC Genetics 9:22.

McCarroll SA, Huett A, Kuballa P, Chilewski SD, Landry A, Goyette P, Zody MC, Hall JL, Brant SR, Cho JH, Duerr RH, Silverberg MS, Taylor KD, Rioux JD, Altshuler D, Daly MJ, Xavier RJ. (2008). Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease.
Nat Genet. 40:1107-12.

Miller W, Drautz D, Ratan A, Pusey B, Lesk A, Tomsho L, Packard M, Gilbert T, Sher A, Raney B, Lindblad-Toh K, Lander E, Knight J, Irzyk GP, Perbost CS, Fredrikson KM, Harkins TT, Sheridan S, Pringle T & Schuster SC. (2008) Sequencing the nuclar genome of the extinct wolly mammoth.
Nature 456:387-390

Nigrovic PA, Gray DH, Jones T, Hallgren J, Kuo FC, Chaletzky B, Gurish M, Mathis D, Benoist C, Lee DM. (2008). Genetic inversion in mast cell-deficient Wsh mice interrupts corin and manifests as hematopoietic and cardiac aberrancy.
Am J Pathol. 173:1693-1701.

O' Callaghan, P., Sandwall, E., Li, J.-P., Yu, H., Ravid, R., Guan, Z. van Kuppevelt, TH., Nilsson, L., Ingelsson, M., Hyman B.T., Kalimo, H., Lindahl, U., Lannfelt, L. and Zhang, X. (2008) Heparan Sulfate Accumulation with Aβ Deposits in Alzheimer's Disease and Tg2576 Mice is Contributed by Glial Cells;
Brain Pathology 18: 548-561

Orzechowski Westholm, J., Xu, F., Ronne, H. and Komorowski, J. (2008) Genome-scale study of the importance of binding site context for transcription factor binding and gene regulation.
BMC Bioinformatics 9:484

Orzechowski Westholm, J., Nordberg, N., Murén, E., Ameur, A., Komorowski, J. and Ronne, H. (2008) Combinatorial control of gene expression by the three yeast repressors Mig1, Mig2 and Mig3.
BMC Genomics 9, 601.

Pettersson M, Sun S, Andersson DI and Berg OG. Evolution of new gene functions: simulation and analysis of the amplification model.
Genetica. June 22 Epub.

Piliponsky AM, Chen CC, Nishimura T, Metz M, Rios EJ, Dobner PR, Wada E, Wada K, Zacharias S, Mohanasundaram UM, Faix JD, Åbrink M, Pejler G, Pearl RG, Tsai M, Galli SJ. (2008). Neurotensin increases mortality and mast cells reduce neurotensin levels in a mouse model of sepsis.
Nat Med. 14: 392-8 2008

Pränting M, Negrea A, Rhen M, and Andersson DI. (2008). Mechanism and fitness costs of PR-39 resistance in Salmonella enterica serovar Typhimurium LT2.
Antimicrob Agents Chemother. 52:2734-2741.

Presto, J., Thuvesson, M., Carlsson, P., Busse, M., Wilén, M., Eriksson, I., Kusche-Gullberg, M. & Kjellén, L. (2008) "Heparan sulfate biosynthesis enzymes EXT1 and EXT2 affect NDST1 expression and heparan sulfate sulfation"
Proc. Natl. Acad. Sci. USA 105, 4751-4756

Richard, B., Swanson, R, Schedin-Weiss, S., Ramirez, B, Izaguirre, G., Gettins, P.G.W. and Olson, S.T. (2008) Characterization of the conformational alterations, reduced anticoagulant activity, and enhanced antiangiogenic activity of prelatent antithrombin.
J. Biol. Chem., 283:14417–14429.

Ringvall M, Rönnberg E, Wernersson S, Duelli A, Henningsson F, Åbrink M, Garcia-Faroldi G, Fajardo I, Pejler G. (2008). Serotonin and histamine in mast cell secretory granules is dependent on serglycin proteoglycan.
J Allergy Clin Immunol. 121:1020-6

Rosengren Pielberg,G., Golovko,A., Sundström,E., Curik,I., Lennartsson,J., Seltenhammer,M.H., Druml,T., Binns,M., Fitzsimmons,C., Lindgren,G., Sandberg,K., Baumung,R., Vetterlein,M., Strömberg,S., Grabherr,M., Wade,C., Lindblad-Toh,K., Pontén,F., Heldin,C.-H., Sölkner,J. and Andersson,L. (2008). A cis-acting regulatory mutation causes premature hair greying and susceptibility to melanoma in the horse.
Nature Genetics 40: 1004 - 1009.

Rubin, K., Lidén, Å., van Wieringen, T. and Reed, R.K. (2008) Control of interstitial fluid homeostasis - roles of growth factors and integrins
In: *Vascular Complications in Human Disease*: Mechanisms and Consequences (Eds: Handler C, Dashwood M, Coghlan JG & Abraham D) Springer, London UK. pp 105-115

Rydengård, V., Shannon, O., Lundqvist, K., Kacprzyk, L., Olsson, A-K., Mörgelin, M., Jahnen-Dechent, W., Malmsten, M., and Schmidtchen, A. (2008). Histidine-rich glycoprotein protects from systemic *Candida* infection.
PloS Pathogens. 4:1000116.

Sandegren L, Lindqvist A, Kahlmeter G and Andersson DI. (2008). Nitrofurantoin resistance mechanism and fitness cost in Escherichia coli.
J Antimicrob Chemother 62:495-503.

Sandwall, E., Bodevin, S., Nasser, NJ., Nevo, E., Avivi, A., Vlodavsky, I. and Li, J.-P. (2008) "Molecular Structure Of Heparan Sulfate From *Spalax:* Implications Of Heparanase And Hypoxia"
J. Biol. Chem. Online pub Dec 10.

Schedin-Weiss, S., Richard, B., Hjelm, R. and Olson, S.T. (2008) Antiangiogenic forms of antithrombin specifically bind to the anticoagulant heparin sequence.
Biochemistry, 47:13610-9.

Schwartz, S. (2008). HPV-16 RNA processing.
In *"Protein-RNA interactions in viral RNA processing"*, Ed: T. Zheng, Frontiers in Bioscience, 13:5880-5891.

Sjölinder, H., Eriksson, J., Maudsdotter, L., Aro, H., and Jonsson, A-B. (2008) Meningococcal outer membrane protein NhhA is essential for colonization and disease by preventing phagocytosis and complement attack.
Infect. Immun. 76:5412-5420.

Sjölinder, H., Lövkvist, L., Plant, L., Eriksson, J., Aro, H., Jones, A., and Jonsson, A-B. (2008) The ScpC Protease of Streptococcus pyogenes Affects the Outcome of Sepsis in a Murine Model.
Infect. Immun. 76:3959-3966

Sjölinder, H., Mogensen, T. H., Kilian, M., Jonsson, A-B*., and Paludan, S. P.* (2008) Important role for TLR-like receptor 9 in host-defense against meningococcal sepsis.
Infect. Immun. 76: 5421-5428. *contributed equally

Snir, O., Widhe, M., von Spee, C., Lindberg, J., Padyukov, L., Lundberg, K., Engström, Å., Venables, P.J., Lundeberg, J., Holmdahl, R., Klareskog, L. and Malmström, V. (2008). Multiple antibody reactivities to citrullinated antigens in sera from rheumatoid arthritis patients - association with HLA-DRB1 alleles.
Ann Rheum Dis. Jul 17.

Sol, E., Sargsyan, E., Akusjärvi, G. and Bergsten, P. (2008). Glucolipotoxicity in INS-1E cells is counreracted by carnitine palmitoyltransferase 1 over-expression.
BBRC 375, 517-521.

Somberg, M., Zhao, X., Fröhlich, M., Evander, M., Schwartz, S. (2008). PTB induces HPV-16 late gene expression by interfering with splicing inhibitory elements at the major late 5'-splice site SD3632.
J. Virol., 82:3665-3678.

Ståhl, U., Stålberg, K., Stymne, S. and Ronne, H. (2008) A family of eukaryotic lysophospholipid acyltransferases with broad specificity.
FEBS Lett. 582, 305-309.

Stålberg, K., Neal, A., Ronne, H. and Ståhl, U. (2008) Identification of a novel glycerophosphocholine acyltransferase activity and a new pathway for phosphatidylcholine biosynthesis in *Saccharomyces cerevisiae*.
J. Lipid Res. 49, 1794-1806.

Stuhr, L.E.B., Moen, I., Nedrebø, T., Salnikov, A.V., Wiig, H., Rubin, K. and Reed, R.K. (2008) TNFα-administration influences tumor stroma structure and physiology independently of growth in DMBA-induced mammary tumors in rats.
Scand J Clin Lab Invest 9:1-10

Sun, W., Eriksson, A.S. and Schedin-Weiss, S. (2008) Heparin enhances the inhibition of factor Xa by protein C inhibitor in the presence but not in the absence of Ca^{2+}.
Biochemistry, in press.

Sun, W., Parry, S., Panico, M., Morris, H.R., Kjellberg, M., Engström, Å., Dell, A. and Schedin-Weiss, S. (2008) N-glycans and the N-terminus of protein C inhibitor affect the cofactor-enhanced rates of thrombin inhibition.
J. Biol. Chem., 283: 18601-18611.

Sundström,E. and Andersson,L. (2008). Den vita hästens gåta löst.
Forskning och Framsteg 8:40-43.

Svendsen, Ø.S., Lidén, Å., Nedrebø, T., Rubin, K. and Reed, R.K. (2008) Integrin αVβ3 acts downstream of insulin in normalization of interstitial fluid pressure in sepsis and in cell-mediated collagen gel contraction
Am J Physiol 295:H555-560.

Thelander, M., Nilsson, A. and Ronne, H. (2008) Carbon and energy allocation.
In *The Moss Physcomitrella patens*, Eds. Perroud P.-F., Knight, C. and Cove, D., Blackwell Publishing, Annual Plant Reviews Series (in press)

Thomas R, Duke SE, Karlsson EK, Evans A, Ellis P, Lindblad-Toh K, Langford CF, Breen M. (2008) A genome assembly-integrated dog 1 Mb BAC microarray: a cytogenetic resource for canine cancer studies and comparative genomic analysis.
Cytogenet Genome Res. 122:110-21

van den Berg L, Vos-Loohuis M, Schilder MB, van Oost BA, Hazewinkel HA, Wade CM, Karlsson EK, Lindblad-Toh K, Liinamo AE, Leegwater PA. (2008) Evaluation of the Serotonergic Genes htr1A, htr1B, htr2A, and slc6A4 in Aggressive Behavior of Golden Retriever Dogs.
Behav Genet. 38:55-66

van den Hoven, MJ., Wijnhoven, TJ., Li, J.-P., Zcharia, E., Dijkman, HB., Wismans, RG., Rops, AL., Lensen, JF., van den Heuvel, LP., van Kuppevelt, TH., Vlodavsky, I., Berden, JH.and van der Vlag, J. (2008) Reduction of anionic sites in the glomerular basement membrane by heparanase does not lead to proteinuria,
Kidney Int. 73: 278-287

Velling T, Stefansson A, Johansson S. (2008). "EGFR and β1-Integrins utilise different signalling pathways to activate Akt".
Exp Cell Res., 314, 309-316.

Vielfort, K., Sjölinder, H., Roos, S., Jonsson, H., and Aro, H. (2008) Adherence of clinically isolated lactobacilli to human cervical cells in competition with *Neisseria gonorrhoeae*.
Microbes and Infection, doi: 10.1016/j.micinf.2008.07.032

Wiik AC, Wade C, Biagi T, Ropstad E-O, Bjerkås E, Lindblad-Toh K[2], and Lingaas F. (2008) A mutation for recessive cone-rod dystrophy in standard wire haired dachshund.
Genome Research 18: 1415 – 1421.

Woulfe DS, Lilliendahl JK, August S, Rauova L, Kowalska MA, Åbrink M, Pejler G, White JG, Schick BP. (2008). Serglycin proteoglycan deletion induces defects in platelet aggregation and thrombus formation in mice.
Blood 111: 3458-67

Wright, D., Kerje, S., Brändström, H., Schütz, K., Kindmark, A., Andersson, L., Jensen, P. and Pizzari, T. (2008). The genetic architecture of a female sexual ornament.
Evolution 62:86-98.

Zhao, X. and Schwartz, S. (2008). Inhibition of HPV-16 L1 expression from L1 cDNAs correlates with the presence of hnRNP A1 binding sites in the L1 coding region.
Virus Genes, 36: 45-53.

Zody MC, Jiang Z, Fung HC, Antonacci F, Hillier LW, Cardone MF, Graves TA, Kidd JM, Cheng Z, Abouelleil A, Chen L, Wallis J, Glasscock J, Wilson RK, Reily AD, Duckworth J, Ventura M, Hardy J, Warren WC, Eichler EE. (2008). Evolutionary toggling of the MAPT 17q21.31 inversion region.
Nat Genet. 40:1076-83.

DISSERTATIONS 2008

Carlsson, Pernilla, Cellular design of heparan sulfate. The NDST enzymes and their regulation. September 27, 2008

Jastrebova, Nadja, Role of heparan sulfate structure in FGF-receptor interactions and signaling, May 22, 2008

Martin, Nathalie, Studies on the regulation of the Napin napA promoter by ABI3, bZIP and bHLH transcription factors, May 20, 2008

Valsecchi, Isabel, AtZDP, a plant 3'DNA phosphatase, involved in DNA repair, June 4, 2008

Xu, Ning, Adenoviral control of RNAi/miRNA pathways in human cells, December 10, 2008

Friman, Tomas, Growth factor activation of mural cells and their role in vascular remodeling, May 21, 2008, Licentiate thesis.

Petersson, Ludvig, Mechanisms of vascularization; the endothelial cell and the (myo) fibroblast , June 4, 2008, Licentiate thesis.

Somberg, Monika, Cellular and viral factors that control human papillomavirus late gene expression, November 20, 2008. Licentiate thesis.

Tobin, Christina, Evolution of a minimal ribosome, February 28, 2008, Licentiate thesis.

Hejll, Eduar, Mechanisms of lymph and blood vessel formation in the mouse embryonic kidney, December 17, 2008, Licentiate thesis

ECONOMY
(kSEK)

	2007	**2008**
Undergraduate Education Grant	17.881	19.024
Faculty Grant	37.044	39.255
External Grants	47.205	59.477
Others	0	0
Total	**102.130**	**117.756**

UNDERGRADUATE TEACHING AT IMBIM

IMBIM has about 18 full professors and associate professors, all of whom participate in undergraduate teaching. In addition there are about 6 assisstant professors and research fellows who also contribute to the teaching. Finally, there are some 40 PhD students who act as teaching assistants in the practical course work.

IMBIM participates in four different study programmes: medicine, pharmacy, dispensing pharmacy, biomedicine and biomedical laboratory science. In all of these, laboratory work is an important part and IMBIM has about 600 m^2 dedicated to this purpose; some 20 different practicals on various subjects are given each year, some of which are common to two or three of the programmes.

Medicine

A reform of the medical programme was recently initiated. Now each course focuses on a specific medical topic - rather than the subject of a department - and the teachers come from different departments. Thus, teachers from IMBIM take part in courses covering topics like ”Energy and food stuff balance”, ”Homeostasis and endocrine regulation” and ”Attack and defense”. Teaching is done through regular lectures, study groups and practicals. The overall objective of these courses is to provide basic knowledge of the biological function of the human body and to create a basis for later clinical studies and future work in the medical profession. The major part of the Department´s contribution is in the field of metabolism and microbiology. Some 90 students are enrolled in this programme every semester.

Pharmacy

This 5-year programme leads to a Master of sciences in Pharmacy and is designed to prepare the students for work in retail and hospital pharmacies, pharmaceutical industry, government agencies and academic institutions. IMBIM is responsible for the teaching of molecular genetics and microbiology. In this programme 90 students are enrolled every semester.

Dispensing pharmacy

This 3-year programme leads to a Dispensing Pharmacist Degree which prepares the students for work in retail and hospital pharmacies. IMBIM is responsible for the teaching of gene technology, molecular biology and infection biology. Some 40 students are enrolled every semester.

Biomedicine

This 3-year programme aims to give students a sound understanding of the physiological and pathological processes occurring in humans. It contains different courses describing these processes from a molecular, cellular, genetical and medical perspective. Through practical

sessions throughout the programme the students obtain experience in techniques used in current biomedical research. The programme aims at providing training for future activity in research, development and information. About 40 students are enrolled each year and the staff of IMBIM takes part in the teaching of biochemistry, cell biology and microbiology.

Biomedical Laboratory Sciences

This 3-year programme leads to a Bachelor of Medical Science (Major in Biomedical Laboratory Science) which prepares the students for work as biomedical scientists in diagnostic and research laboratories. Placements at external laboratories constitute a substantial part of the curriculum allowing the students to specilize within the programme. The major part of the Department's contribution to this programme is in the field of biochemistry. Some 35 students are enrolled in this programme every year.

MEDIGLY

Swedish network in medical glycobiology

The Swedish Research Council/Scientific Council for Medicine finances the network which will promote and stimulate Swedish glycoscience between groups with different goals and methodological expertise, but with a common interest in the role of carbohydrates in medicine and biology. The network, which builds on the previous SSF-finaced GLIBS program, spans from organic synthesis and characterization of protein-carbohydrate interactions to transplantation and cancer treatment. The 38 groups in the network are found in Uppsala, Umeå, Gothenburg, Linköping, Stockholm and Lund. The network is coordinated from Uppsala with contact persons at the participating universities. The activities include annual meetings and advanced graduate courses.

Contact:
Lena Kjellén, scientific coordinator
Barbro Lowisin adm. coordinator

Homepage: http://www.imbim.uu.se/medigly

UPPSALA GRADUATE SCHOOL IN BIOMEDICAL RESEARCH, UGSBR

Uppsala Graduate School in Biomedical Research (UGSBR) started in January 1997 as one of originally six local biomedical preparatory research schools initiated through support by the Foundation for Strategic Research (SSF). The preparatory research school in Uppsala is based on collaboration between the Faculties of Medicine, Pharmacy and Natural Sciences at Uppsala University and the Faculties of Veterinary Medicine and Animal Science and Natural Resources and Agricultural Sciences at the Swedish University of Agricultural Sciences. The school has as its prime objective to give university students, heading for a research education, a deeper knowledge about research and development, increasing possibilities to make active choices among the multitude of biomedical research fields and to establish productive national and international contacts.

As of Aug 2008, approximately 230 students have been accepted to UGSBR since the start in Jan 1997. Almost all UGSBR students have continued with PhD studies and over 90 students have until now received their Doctoral degree.

Management:
Catharina Svensson, program director
Barbro Lowisin, program administrator

Program board:
Leif Kirsebom, Cell and Molecular Biology, chairman
Prof Lennart Dencker, Pharmaceutical Biosciences
Prof Caroline Fossum, Biomedical Sciences and Veterinary Public Health, SLU
Prof Lena Kjellén, Medical Biochemistry and Microbiology
Magnus Malmqvist, Bioventia Capital AB

Deputy members
Prof. Stellan Sandler, Medical Cell Biology
Researcher Maria Norlin, Pharmaceutical Biochemistry
Prof Inger Andersson, Molecular Biology, SLU
Per Lindström, Meadowland Business Partners AB

Student representatives
Jonas Fagerberg, UGSBR 2007
Jessica Nordlund, UGSBR 2007
Xiang Gao, UGSBR 2008
Anne-Li Lind, UGSBR 2008

PROTEOMICS RESOURCE CENTER

Åke Engström and Eva Andersson

This laboratory gives the scientific community an opportunity to at a low cost make use of recent developments in techniques and instrumentations for the analysis and identification of proteins. The facility for Expression Proteomics is equipped with 2-D electrophoresis systems, systems for post or pre-gel labeling of proteins, visible and UV light scanners, software for image analysis, spot picking systems, semi automated spot processing, and MALDI-TOF/TOF and MS/MS instrumentation. The facility has expertise for 2-D analysis, mass, spectrometry, image analysis, data base searches and general protein chemistry.

Our area of work is analysis and comparison of proteomes, identification of proteins in protein spots/bands by mass spectrometry, analysis of expressed proteins for quality control and analysis of proteins for post-translational modifications. The facility is open for all scales of problem solving or analysis, although the capacity for 2D gels might be a limiting factor for very large undertakings.

The service is primarily intended for identification of proteins from species with large numbers of genes or proteins characterized. The facility has in addition a limited capacity for de novo sequencing of proteins from any species. The service includes straightforward methods for characterization of expressed recombinant proteins. Considering the low cost for analysis this is highly recommended to avoid the potential risk of doing experiments with the "wrong" or modified protein. An MS analysis of intact expressed protein and a peptide mapping with MS give much better confidence than a simple SDS-gel analysis. If suitable for our techniques and knowledge we provide analysis of any type of sample

Organization

The expression proteomics facility is organized for running samples for researchers or for the researcher to use the equipment after approval. The latter is recommended for longer series of experiments.

Location

Equipment for handling and performing 2-D gel electrophoresis experiments and MALDI-Tof/Tof instrumentation are located at the Dept. of Medical Biochemistry and Microbiology, Biomedical Center (building D9 floor 3), Uppsala.

Contact

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eva.andersson@imbim.uu.se

UPPSALA UNIVERSITY TRANSGENIC FACILITY (UUTF)

Maud Forsberg, PhD, Acting Facility Head
Anne-Mari Olofsson, Technician
Xiao-Qun Zhang, PhD
Karin Forsberg Nilsson, associate professor, Facility Head on leave

Generation of transgenic and knockout mice

Uppsala University Transgenic Facility (UUTF) is a core facility that offers services to research groups at Uppsala University as well as other universities in Sweden and abroad. UUTF is located in a Specific Pathogen Free (SPF) barrier facility in the animal house at the Biomedical Center. Our object is to assist scientists to develop new transgenic and knockout mouse models. We perform gene targeting in embryonic stem (ES) cells, blastocyst injection of genetically modified ES cells and pronuclear injection of DNA constructs. We also assist with advice on construct design, genotyping and initial breeding of novel transgenic lines. In addition, we perform embryo transfers to clean infected mouse strains from pathogens.

Derivation of new ES cell lines and in vitro differentiation

With support from the Wallenberg Consortium North we have developed, and are now offering as a service, the technique of in vitro differentiation of embryonic stem (ES) cells. Use of ES cells opens several experimental approaches in the field of developmental biology. ES cells can be differentiated to many cell lineages in vitro under defined conditions and can function as a tool for dissection of cell fate decision in the early embryo. Using ES cell in vitro differentiation, cell lineage commitment can be studied and cell types can be generated that are not easily accessible from early time points in vivo. Wild type ES cells can be in vitro differentiated for studies of specific cell type development, toxicity tests and other specialized applications. UUTF also offers the service of derivation of new ES cell lines from transgenic and knockout mice. These cell lines can be used for studying the consequences of genetic alterations on in vitro differentiation to various cell lineages.

Publications from 2004 to 2008

1. Andrae J, Afink G, Zhang X-Q, Wurst W, Nistér M. (2004) Forced expression of platelet-derived growth factor B in the mouse cerebellar primordium changes cell migration during midline fusion and causes cerebellar ectopia. Mol. Cell. Neurosci. Jun;26(2):308-321
2. Zhang X-Q, Afink GB, Hu X, Forsberg-Nilsson K, Nistér M. (2005) Gli1 is not required for Pdgfrα expression during mouse embryonic development. Differentiation 73:109-119
3. Lord A, Kalimo,H, Eckmang C, Zhang X-Q, Lannfelt L, Nilsson L. (2005) The Arctic Alzheimer mutation facilitates early intraneuronal A aggregation and senile plaque formation in transgenic mice. Neurobil Aging 27(1): 67-77
4. Nistér M, Tang M, Zhang X-Q, Yin C, Beeche M, Hu X, Enblad G, van Dyke T, Wahl GM. (2005) P53 must be competent for transcriptional regulation to suppress tumor formation. Oncogene 19;24 (22):3563-73

5. Li Q, Zhang X-Q, Nie L, Chen G-S, Li H, Zhang F, Zhang L-Y, Hong L, Wang S-F, Wang H. (2007) Expression of interferon-gamma in human adrenal gland and kidney tumors. British Journal of Cancer 97:420-425
6. Kriz V, Mares J, Wentzel P, Funa NS, Calounova G, Zhang XQ, Forsberg-Nilsson K, Forsberg M, Welsh M. (2007) Shb null allele is inherited with a transmission ratio distortion and causes reduced viability in utero. Dev Dyn. Sep;236(9):2485-92.
7. Larsson J, Forsberg M, Brännvall K, Zhang XQ, Enarsson M, Hedborg F, Forsberg-Nilsson K. (2008) Nuclear receptor binding protein 2 is induced during neural progenitor differentiation and affects cell survival. Mol Cell Neurosci. Sep;39(1):32-9.

The services of UUTF have contributed to at least 28 additional publications during 2004-2008.

Financial support for UUTF

The Faculties of Medicine and Pharmacy
The Wallenberg Foundation (Wallenberg Consortium North)

Homepage

http://www.imbim.uu.se/resource/transgenic.html

FACILITY FOR RECOMBINANT ADENOVIRUS CONSTRUCTION

Anette Carlsson

Viral vectors have become popular as vehicles to introduce genes for basic studies of protein function, vaccination, treatment of cancer and correction of genetic disorders. Viruses are in some aspects "ideal" for this purpose since they have evolved efficient mechanisms to deliver nucleic acid to specific cell types while avoiding immunosurveillance by an infected host.

A core facility for recombinant adenovirus construction was established in 2001 on an initiative from the Swedish Research Council, the Cancer Society, the Swedish Foundation for Strategic Research, and the Wallenberg Foundation who also supported it financially up to the summer of 2004. The facility is physically located at the renovated virus cell culture facility at IMBIM (B9 corridor). At the facility recombinant viruses are reconstructed based on the so-called AdEasy system (for further information see http://www.qbiogene.com), although alternative viral vector backbones can be used. Customers are asked to transfer their gene of interest to a suitable shuttle vector (provided by the facility). This will then be reconstructed to a virus at the facility. In a typical order the investigator will receive a small-scale virus batch that for many purposes has to be amplified. Larger bathces of purified virus can be purchased from the facility. It is important to note that the facility does not produce clinical grade viruses.

http://www.imbim.uu.se/resource/adenovirus.html