Regulation | Metabolite Channeling in Energy Metabolism

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This is an update of U. Schlattner, M. Tokarska-Schlattner, T. Wallimann, Metabolite Channeling: Creatine Kinase Microcompartments, in Encyclopedia of Biological Chemistry (Second Edition), Edited by: William J. Lennarz, M. Daniel Lane, Elsevier Inc., 2013, https://doi.org/10.1016/B978-0-12-378630-2.00283-8.

Glossary

Metabolite channeling Local transfer of metabolic intermediates between sequential enzyme or transport reactions without equilibration with bulk solution.
Metabolic compartmentation Segregation of intermediates and enzymes of a metabolic pathway by membranes, through basic principles of liquid-liquid phase separation (membraneless), by binding to a specific surface, or via direct interaction in protein complexes allowing metabolite channeling.
Microcompartment Structural unit allowing metabolic compartmentation, also called "metabolon."
Mitochondrial contact sites Close adhesions of inner and outer mitochondrial membrane that can be observed by electron microscopy and can be isolated as a separate microcompartment. Contact sites consist of multi-lipid/protein complexes with variable composition. They are involved in energy transduction (e.g., containing ANT/mtCK/VDAC), protein import or shaping mitochondrial structure (e.g. MICOS).
Mitochondrial permeability transition pore A multiprotein complex of the inner mitochondrial membrane that is crucially involved in early events that trigger apoptosis like release of cytochrome *c* and other apoptosis-inducing factors into the cytosol.

Definition of Metabolite Channeling

Subcellular microcompartments, consisting of multienzyme complexes that are embedded within the cellular, highly viscous matrix, associated with the cytoskeleton, or situated along membranes, operate according to exclusion principles and favor preferred pathways of intermediates. This process, called metabolite or substrate channeling, is defined as transfer of intermediates between sequential enzymes without equilibration of these metabolites with the surrounding bulk solution. Such associations between two or more sequential enzyme- or transport reactions in a microcompartment, forming a distinct functional pool of intermediates, are also known as functionally coupled reactions or metabolons. They can be considered as a general mechanism to increase the efficiency of sequential reactions in a metabolic pathway (Holthuis and Ungermann, 2013; Ovadi, 1995; Ovadi and Srere, 2000). Since metabolite channeling leads to segregation of a metabolic pathway from other cellular reactions, it represents a specific kind of metabolic compartmentation similar to that operating within membrane-separated organelles or by being restricted two-dimensional diffusion at surface boundary layers. Here, metabolite channeling is described with examples from energy metabolism, where local fueling of ATPases and GTPases is maintained by isoenzymes of creatine kinase (CK), nucleoside diphosphate kinase (NDPK) or glycolytic enzymes.

Subcellular Microcompartments and Mechanisms of Metabolite Channeling

Life possibly originated autotrophically *de novo* in metabolic complexes organized on FeS_2 (pyrite) mineral surfaces, the earliest form of microcompartments. Regarding this situation, a cell cannot be represented by a well-mixed bag of enzymes, behaving in complete equilibrium according to solution kinetics. Because of the intricate structural and functional organization of living cells, enzymes and metabolites do not behave as if they were freely diffusible in solution. Instead, they may form structurally, functionally, and temporally defined subcellular microcompartments, either via strong static, or via fickle, dynamic interactions with other enzymes, proteins, or subcellular structures. Such a structural organization of pathway components is a general prerequisite for metabolite channeling. It may involve (1) huge covalently linked enzyme-complexes (or multifunctional enzymes) such as fatty acid synthase (FAS), (2) kinetically stable multienzyme complexes like pyruvate dehydrogenase complex (PDHC) or bacterial and plant tryptophan synthase (TS), (3) more dynamic, reversibly associating enzymes such as glycolytic complexes containing glyceraldehyde phosphate dehydrogenase (GAPDH) or glycerol phosphate dehydrogenase (GPDH), or (4) colocalization on subcellular particles or biological membranes. These associations allow the transfer of intermediates between the channeling components by different mechanisms: (1) physical hindrance or electrostatic effects prevent mixing with bulk solution and drive a directed diffusion (e.g., TS, FAS), (2) sequential covalent binding to very close active sites in the reaction sequence (e.g., PDHC),

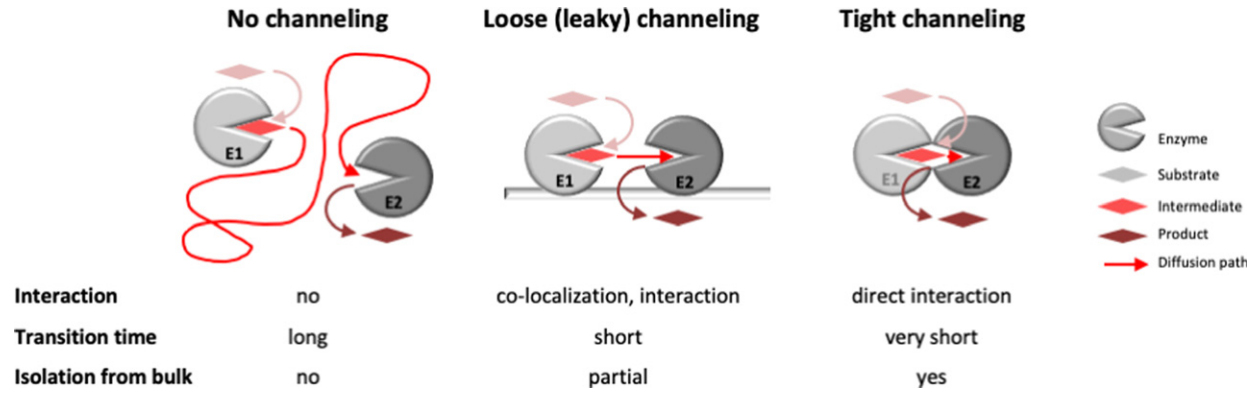

Fig. 1 Free diffusion (no channeling) versus loose (leaky) or tight metabolite channeling. Compartmentation of a reaction sequence without (full) equilibration with bulk solution leads to shorter transition times and further advantages (see text).

(3) transfer of noncovalently bound intermediates between active sites (e.g., NADH dehydrogenase), (4) transfer in dynamic multi-enzyme complexes (GAPDH, GPDH) or in an unstirred membrane surface layer (mtCK). These mechanisms can be fulfilled in both, static and dynamic enzyme associations. However, while static associations often allow for an almost perfect or "tight" channeling of metabolites, dynamic channeling is often only partial or "leaky" (**Fig. 1**).

Advantages of Metabolite Channeling

Sequestering of intermediates in a microcompartment through metabolite channeling provides kinetic and regulatory advantages for not only the reaction sequence itself (**Fig. 1**), but also for cellular metabolism (Ovadi, 1995; Zala *et al.*, 2017). In general: (1) enzyme reaction rates and equilibria are controlled by local and enzyme bound substrates, rather than bulk phase substrate concentrations, (2) for a readily reversible reaction, local supply of substrate and removal of product may drive the reaction in the desired direction, (3) sequestered intermediates are present at high local concentrations and an apparently low K_m for these intermediates can be observed with the channeling complex compared to the non-channeling situation measured with isolated components, (4) metabolites are isolated from competing reactions, e.g., between anabolic and catabolic pathways, (5) the lifetime of the intermediate in the solvent phase is shortened relative to free diffusion, which may be essential in case of unstable intermediates, (6) in certain cases, the unfavorable energetics of desolvating the substrate that precedes binding to the enzyme is avoided, (7) channeling components can be regulated by modulators that affect enzyme associations, and (8) a larger degree of metabolic control of the over-all-flux of the reactions can be achieved, e.g., via feed-back regulatory mechanisms such as substrate activation, product inhibition, and cooperativity.

Creatine Kinase: Channeling of High-Energy Phosphates for Efficient ATP Supply

The Creatine Kinase/Phosphocreatine Circuit or Shuttle

One fundamental requirement of life is energy supply. Cellular energy demand and supply are balanced, and tightly regulated for economy and efficiency of energy use. In eukaryotic cells, CK is a major enzyme that helps to cope with high and fluctuating energy demands to maintain cellular energy homeostasis in general and to guarantee stable, locally buffered ATP/ADP ratios in particular (Bessman and Carpenter, 1985).

The enzyme catalyzes the reversible phosphoryl transfer from ATP to creatine (Cr) to generate ADP and phosphocreatine (PCr). Thus, CK is able to conserve energy in the form of metabolically inert PCr and vice versa, to use PCr to replenish global as well as local cellular ATP pools. Since PCr can accumulate to much higher cellular concentrations than ATP, the CK/PCr-system constitutes an efficient and immediately available cellular "energy buffer". In addition, tissue-specific CK isoenzymes are located in the cytosol (dimeric muscle-type MM-CK and brain-type BB-CK) and within the mitochondrial intermembrane space (sarcomeric smtCK and ubiquitous umtCK, both forming octamers and dimers). CK isoenzymes are often associated with sites of ATP supply, where they generate PCr, or with sites of ATP consumption, where they regenerate ATP by using PCr. Thus, together with the faster diffusion rate of PCr as compared to ATP, the CK/PCr system also supports an intrinsic energy transfer system (CK/PCr-circuit or -shuttle), coupling sites of energy generation (oxidative phosphorylation or glycolysis) with sites of energy consumption (Wallimann *et al.*, 1992) (**Fig. 2(A)**). This circuit is particularly important in cells with high or fluctuating energy demands like cardiac and skeletal muscle or brain cells (Guzun *et al.*, 2015), as well as in large and/or polar cells, such as spermatozoa. In the latter, diffusional limitations of adenine nucleotides, especially ADP, along the sperm tail become especially apparent (Wallimann *et al.*, 2011).

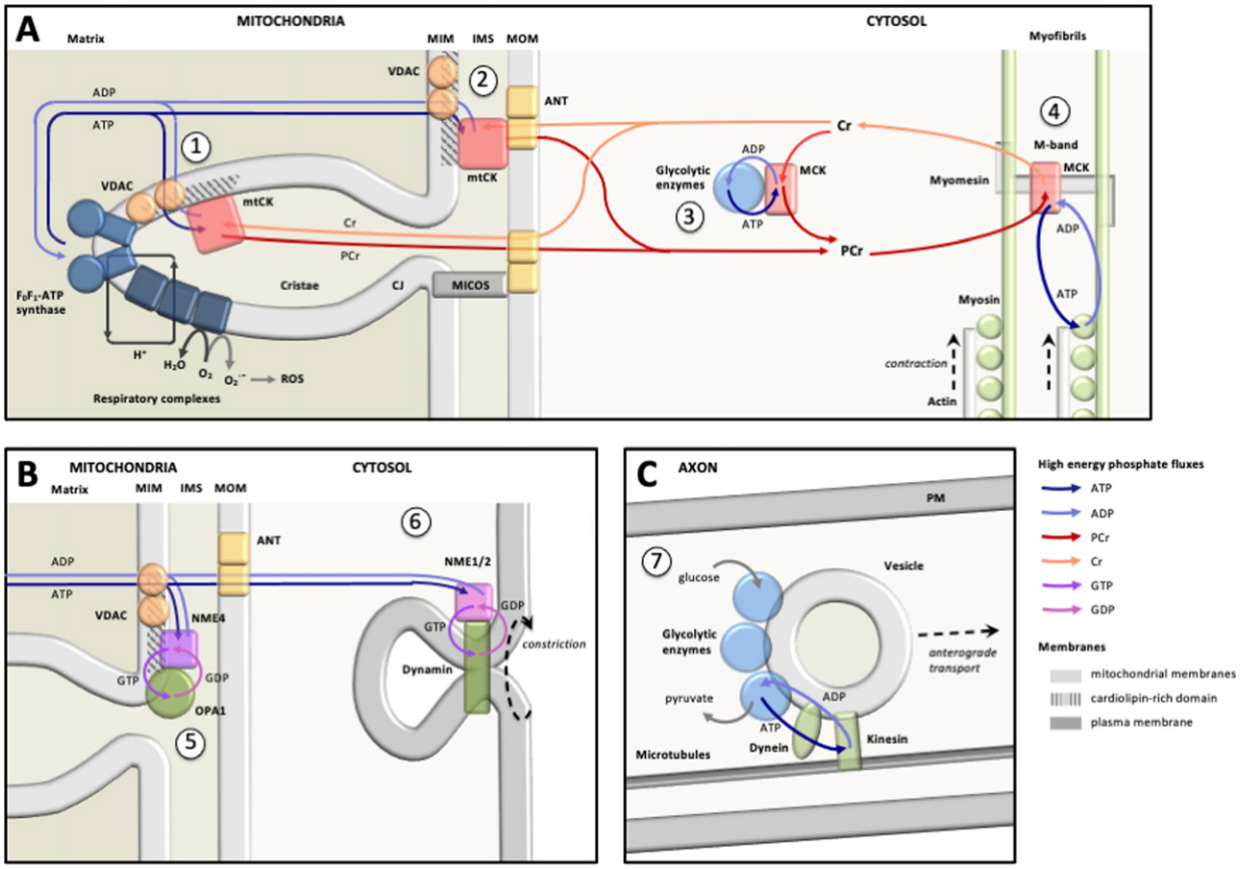

Fig. 2 High energy phosphate channeling in specific cellular microcompartments. Colored symbols depict proteins that transport energy phosphates (yellow, orange) or convert high energy phosphates (ATP generation, blue; ATP/PCr conversion, red; ATP/GTP conversion, magenta; ATP or GTP consumption, green). Metabolite fluxes are depicted as arrows. (A) Creatine kinase (CK) isoenzymes, together with easily diffusible, highly concentrated phosphocreatine (PCr; up to 30 mM), maintain a unique energy buffer and shuttle system between ATP-providing and -consuming processes, in particular in cells that are polarized and/or have very high or localized ATP consumption. Isoenzymes of CK are found in mitochondria (octameric mtCK ① ②) and the cytosol (dimeric cytCK ③ ④), either soluble (not shown) or structurally associated with ATP-delivering or -consuming sites. Metabolite channeling occurs where CK is associated with ATP-providing or -consuming transporters, pumps or metabolic enzymes. ① ② Mitochondrially generated ATP is exported from the matrix through the mitochondrial inner membrane (MIM) via adenine nucleotide translocase (ANT) and then channeled to mtCK, both proteins being in close proximity via their preferential interaction with the mitochondrial phospholipid cardiolipin. ① In cristae, ANT and mtCK can be part of supercomplexes that also include F0F1-ATPase and respiratory chain complexes. This maintains a local, efficient ADP/ATP circuit favoring correct respiratory function and reducing reactive oxygen species (ROS). Generated PCr then diffuses along cristae and cristae junctions (CJ) to cross the mitochondrial outer membrane (MOM) via large channels (VDAC) into the cytosol. ② In the intermembrane space, mtCK associates simultaneously with the mitochondrial inner membrane (MIM) and VDAC, forming so-called mitochondrial contact sites between MIM and MOM for direct, vectorial channeling of PCr into the cytosol. ③ In the cytosol, cytCK can associate with glycolytic ATP-generating enzymes or enzyme assemblies for PCr synthesis. While in oxidative tissues like heart, PCr is mainly produced by mtCK using ATP from oxidative phosphorylation, PCr in fast-twitch glycolytic muscle mainly comes from cytCK and glycolytic ATP. PCr is then used by cytCK to buffer global cellular ATP/ADP ratios (not shown). ④ More specifically, cytCK associated with or close to cytosolic ATPases uses PCr to keep local ATP/ADP ratios high for maximal ΔG° of ATP hydrolysis. In muscle, for example, the cytosolic MCK isoenzyme is bound to myomesin in the myofibrillar M-band to regenerate ATP close to the myosin head ATPases for contraction. In addition, a significant fraction of cytosolic MCK is also specifically bound to the sarcoplasmic reticulum (SR)-Ca^{2+} ATPase to support energy demanding Ca^{2+} pumping (not shown here). (B) Isoenzymes of nucleoside diphosphate kinase (NDPK, also called NME or NM23) use ATP to maintain the cellular levels of different NTPs. Part of these NDPKs associate with GTPases or G-proteins for direct channeling of the required GTP. ⑤ In mitochondria, NDPK-D (NME4) directly interacts with the dynamin-related GTPase OPA1 to fuel GTP for MIM dynamics. ⑥ At the plasma membrane, cytosolic NDPK isoenzymes, NDPK-A (NME1) and NDPK-2 (NME2) associate with dynamin to fuel GTP for membrane constriction and dynamin-mediated endocytosis. (C) The ten enzymes of the glycolytic pathway (including glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, pyruvate kinase) associate with vesicles that travel along axons. This fast-axonal transport (FAT) is realized by the vesicle-bound molecular motors kinesin and dynein that attach to microtubule filaments and propel vesicles in either anterograde or retrograde direction, respectively. ⑦ The ATP for efficient FAT by kinesin or dynein ATPases is mainly channeled from on-board vesicular glycolysis, but is not generated by mitochondria or in bulk cytosol.

Channeling With Cytosolic CK

Cytosolic CK is only partially soluble. A significant fraction is structurally and functionally associated or co-localized with different, structurally bound ATPases or ATP-regulated processes (Schlattner *et al.*, 2016). These include different ion pumps or the ATP-gated K^+-channel at the plasma membrane, the sarcomeric M-band of the myofibrils in muscle (**Fig. 2(A)**, #4), or the calcium pump of the muscular sarcoplasmic reticulum. Cytosolic CK is also structurally associated with the key regulatory enzyme of glycolysis, phosphofructokinase (PFK), which itself is regulated by ATP. In all these cases, PCr is used for the local regeneration of ATP, which is directly channeled from CK to the ATP-consuming ATPase or ATP-regulated enzyme without major dilution by the surrounding bulk solution.

Only in some cases, the physical basis for the metabolite channeling is known. For example, MM-CK uses a "charge clamp" consisting of four lysine residues to specifically bind to partner molecules, myomesin and M-protein in the M-band (Hornemann *et al.*, 2000). This allows for an isoenzyme-specific association of MM-CK with the sarcomeric M-band of the myofibrillar apparatus, where CK is ideally positioned to regenerate in situ during muscle contraction the ATP hydrolyzed in the acto-myosin overlap zones that are situated symmetrically on both sides of the M-band.

A tight functional coupling of CK to ATPases, e.g., acto-myosin ATPase and ion pumps, such as the K^+/Na^+-ATPase or the Ca^{2+}-ATPase, has the advantage (1) that product inhibition of the ATPase by ADP and H^+ is avoided, since the latter are both substrates of the CK reaction ($PCr + ADP + H^+ \leftrightarrows Cr + ATP$) and (2) that the high free energy of ATP hydrolysis (ΔG_{ATP}) at sites of ATP hydrolysis is preserved by keeping locally very high ATP/ADP ratios due to coupling of CK with those ATPases in situ and thus preventing energy dissipation that would otherwise be caused by transport of ATP and mixing it with the bulk surrounding (Wallimann *et al.*, 1992). Transgenic mice no longer expressing cytosolic MM-CK and mitochondrial mtCK in muscle have significant difficulties with intracellular calcium handling and muscle relaxation. This emphasizes the physiological importance of the CK system for the energetics of intracellular calcium homeostasis in general and the delivery of ATP to the energetically demanding Ca^{2+}-ATPase, in particular.

Channeling in Energy Transducing Mitochondrial Microcompartments

The mtCK forms mainly large, cuboidal octamers that are present (1) between the mitochondrial outer (MOM) and inner membrane (MIM), the so-called "intermembrane space" of mitochondria, preferentially localizing in "mitochondrial contact sites" between these two membranes (**Fig. 2(A)**, #2), as well as (2) in the cristae space (**Fig. 2(A)**, #1) (Schlattner *et al.*, 2006; Schlattner *et al.*, 2018). This CK isoenzyme catalyzes the direct transphosphorylation of intramitochondrial produced ATP and Cr from the cytosol into ADP and PCr. ADP then enters the matrix space to stimulate oxidative phosphorylation, giving rise to mitochondrial recycling of a specific pool of ATP and ADP, while PCr is the primary "high energy" phosphoryl compound that leaves mitochondria entering into the cytosol. The molecular basis for such directed metabolite flux is channeling between the large, cuboidal mtCK octamer and two transmembrane proteins, adenylate translocator (ANT) and mitochondrial porin or voltage-dependent anion channel (VDAC). ANT is an obligatory antiporter for ATP/ADP exchange across MIM, while VDAC is a nonspecific, potential-dependent pore in the MOM. The mtCK-linked metabolite channeling is based on (1) colocalization, (2) direct interactions, and (3) diffusion barriers. mtCK tightly binds to cardiolipin, an acidic phospholipid that is specific for the MIM. Since ANT molecules are situated in cardiolipin patches, this leads to colocalization and metabolite channeling between both proteins, mtCK and ANT, in the cristae as well as in the intermembrane space. Octameric mtCK in the intermembrane space further interacts with outer membrane phospholipids and VDAC, thus virtually cross-linking inner and outer membrane and contributing to the "mitochondrial contact sites." Increasing the external calcium concentrations strengthens the interaction of mtCK with VDAC, which may improve channeling under cytosolic calcium overload as occurs at low cellular energy state. Finally, the limited and potentially regulated permeability of VDAC and thus of the entire outer membrane creates a dynamic microcompartmentation of metabolites in the intermembrane space that contributes to mtCK-linked channeling and separate mitochondrial ATP- and ADP pools. Similar to mtCK, hexokinase is able to use intramitochondrially produced ATP by binding to VDAC from the cytosolic mitochondrial surface at contact sites containing only ANT and VDAC. The direct functional coupling of mtCK to oxidative phosphorylation can be demonstrated with oxygraph respirometry on skinned muscle fibers from normal and transgenic mice lacking mtCK. While in normal muscle fibers Cr stimulates mitochondrial respiration, this phenomenon is missing in fibers of mtCK knock-out mice.

By favoring ATP/ADP exchange through ANT in MIM, cytosolic Cr and the mtCK reaction not only stimulate the rate of mitochondrial respiration, but also improve coupling between respiration and ATP synthesis and thus enhance mitochondrial energy channeling, which finally reduces formation of potentially deleterious reactive oxygen species (ROS; **Fig. 2(A)**, #1 lower part). Both effects of mtCK-related metabolite channeling, stimulation of mitochondrial respiration and lowering of ROS production, may contribute to the remarkable cell- and specifically neuro-protective action of Cr supplementation in neurodegenerative disorders observed in vitro and in vivo. This is an instructive example of how efficient, multiple metabolite channeling events can be beneficial for human health.

Cellular low-energy stress induces expression of mtCK, be it caused by chronic endurance training, fasting, Cr depletion, or pathologies in ATP generation like mitochondrial dysfunction seen in patients with mitochondrial cytopathies. In the latter, highly up-regulated mtCK can even crystallize into sheets to form characteristic "railway-track inclusions" within enlarged mitochondria as a hallmark of the pathology. Similar mtCK inclusions were observed with chronic Cr depletion in rodents. On the other hand,

cellular stress that generates reactive oxygen and nitrogen species like cardiac infarction or anthracycline chemotherapy leads to molecular damage to mtCK. This then triggers inactivation and dimerization of mtCK, as well as its dissociation from MIM. All of these impair an efficient channeling of high-energy phosphates by mtCK and thus contribute to cardiac energy failure or specific anthracycline cardiotoxicity. Finally, mtCK together with its substrate Cr participates in regulating the mitochondrial permeability transition pore that is crucially involved in triggering apoptosis. Likely because of metabolite channeling in the mtCK/ANT microcompartment, a high ADP concentration is maintained in the matrix space that is inhibitory for permeability transition. Thus, the channeling CK-system may exert additional effects that are not necessarily directly related to improved cell energetics. Such pleiotropic effects of creatine may explain some of the cell- and neuro-protective effects seen with Cr supplementation (Wallimann *et al.*, 2011).

Nucleoside Diphosphate Kinase: Channeling GTP

Many cellular processes require GTP instead of ATP as an energy source, including biosynthesis and membrane translocation of proteins, cellular membrane dynamics or cell signaling. The involved GTPases have either rather low turnover, like signaling heterotrimeric G proteins and small GTPases, or high turnover like the motor proteins of the dynamin family. Cellular GTP is largely supplied by members of the NME/NDPK/NM23 protein family that have nucleoside diphosphate kinase (NDPK) activity, namely isoenzymes NME1 to NME4 (Boissan *et al.*, 2018). These hexameric proteins use mainly cellular ATP to regenerate other nucleoside triphosphates (NTPs), including GTP, in the reaction ATP + NDP⇆ADP + NTP. However, while ATP- and GTP-binding proteins have often similar affinities for their respective nucleotide, cellular concentrations of guanine nucleotides are largely below those of adenine nucleotides. To overcome this mismatch, NMEs localizing in close vicinity of GTP-dependent processes locally increase GTP concentrations, or even directly interact with and channel GTP to the guanylate binding site of specific GTPases (**Fig. 2(B)**).

The cytosol harbors three NME isoenzymes, NME1-NME3, that can form homo- and heterohexamers. These are partially soluble, but partially also associate with membranes, in particular NME3 that has an N-terminal hydrophobic membrane anchor. At the plasma membrane, NME2/NME3 heterohexamers recruit heterotrimeric G proteins to locally provide the GTP necessary for G protein activation via GTP/GDP exchange (Hippe *et al.*, 2009). In addition, NME2 can directly transfer "high energy phosphates" from its active site histidine to a G protein histidine close to the guanylate binding site, from where it is used to regenerate GTP from GDP directly on-site (Abu-Taha *et al.*, 2018). This histidine phospho-relay mechanism allows G protein activation independent of the classical receptor-induced activation of heterotrimeric G proteins. Another example of GTP channeling is the membrane-remodeling GTPase dynamin which drives membrane fission during clathrin-mediated endocytosis. Here, NME1/2 localize to clathrin-coated pits at the plasma membrane where they directly interact with the proline-rich domain of dynamin to channel the GTP necessary for dynamin-mediated membrane constriction and efficient endocytosis (Boissan *et al.*, 2014) (**Fig. 2(B)**, #6).

Two NME isoenzymes localize to mitochondria. NME4 is imported into the intermembrane and matrix spaces of mitochondria, where it binds to MIM via interaction with the phospholipid cardiolipin (Schlattner *et al.*, 2013). NME3 anchors at the outer surface of the MOM via its N-terminal hydrophobic stretch. In their respective membranes, both NMEs directly interact with and channel GTP to specific dynamin-related GTPases that drive membrane remodeling and mitochondrial fusion (**Fig. 2(B)**, #5). While NME4 fuels OPA1 at MIM (Boissan *et al.*, 2014), NME3 fuels MFN1/2 at MOM (Chen *et al.*, 2019). Although all the mentioned GTPases of the dynamin family can also work in the absence of NMEs, their full efficiency is only achieved in association with NMEs that provide high and constant local GTP levels.

Glycolytic Multienzyme Complexes: Roles in Muscle, Sperm Flagella and Neurons

In muscle, glycolytic enzymes are targeted to the actin-containing thin filaments at the sarcomeric I-band region where they form highly complex metabolons. The I-band in *Drosophila* flight muscle contains a multienzyme complex consisting of GDPH-1, aldolase, and GAPDH. By elegant experiments with transgenic *Drosophila* expressing GDPH-3 instead of GDPH-1, it could be shown that all three glycolytic enzymes no longer colocalize in the I-band to form a microcompartment. Even though the full complement of active glycolytic enzymes was still present, their failure to colocalize in the sarcomer resulted in the inability to fly (Wojtas *et al.*, 1997). Thus, correct targeting and formation of multienzyme complexes that lead to functionally coupled microcompartments and substrate/product channeling seem to be a prerequisite for proper function of glycolysis and ultimately for correct muscle function. In mammalian cells, CK also participates in the glycolytic metabolon.

Since glycolysis is able to produce ATP at lower yield but higher rate as compared to oxidative phosphorylation that produces ATP with a higher yield but lower rate, glycolysis is often used for activities requiring sudden high levels of ATP. An example illustrating such high energy demand is the motility of many mammalian sperm flagella that are powered by glycolysis. Notably, multiple glycolytic enzymes are localized within the flagellum and more precisely arrayed on the fibrous sheath, the cytoskeletal structure separating the head from the sperm tail. The sperm glycolytic enzymes differ from their somatic counterparts by containing an anchoring domain to the fibrous sheath of the flagellum. This property of the sperm glycolytic enzyme was used to tether ten glycolytic enzymes, from hexokinase (HK) to pyruvate kinase (PK) and lactate dehydrogenase (LDH), to nanoparticles thus reconstituting in vitro the conversion of glucose to lactate. While the enzymatic efficiency of each enzyme alone was higher when in solution, the conversion of glucose to lactate was significantly higher when the ten enzymes were tethered to

nanoparticles as compared to their soluble counterparts (Mukai *et al.*, 2017). These findings illustrate how substrate/product channeling is improved by the ordering of sequential reaction in functionally coupled microcompartments.

In the brain, energy demands are primarily met by glucose oxidation through glycolysis and oxidative phosphorylation. These ATP-generating pathways have to respond in a rapid and localized manner to large changes in energy demand. This high demand in energy is particularly evident at synapses since vesicles containing neurotransmitters and factors such as the brain-derived neurotrophic factor (BDNF), are released to allow neurotransmission upon neuronal activity. Mitochondria have been shown to be essential for sustaining synapse function by maintaining energy metabolism at the presynapse as well as by clearing calcium. However, glycolysis by producing ATP at high rate likely allows synapses to adapt to the sudden needs in energy. This was demonstrated for *Caenorhabditis elegans*. Under conditions of energy stress, glycolytic enzymes dynamically relocalize at synapses to form a glycolytic metabolon thus maintaining high local levels of ATP at the synapse, the proper functioning of the synaptic vesicle cycle and subsequent locomotion of the worms (Jang *et al.*, 2016). In addition to this dynamic redistribution of the glycolytic metabolon upon high energy demand (e.g., neuronal activity) or energy depletion, the glycolytic enzymes GAPDH and phosphoglycerate kinase (PGK) have been shown to associate to synaptic vesicles and the glycolytic machinery is required to activate the vesicular proton pump required for vesicle glutamate reuptake at the synapse further illustrating the compartmentalization of glycolytic pathway in neurons.

While such compartmentalization of the glycolytic metabolon appears to fulfill energy demand at specific subcellular localizations, one long-standing question has been how motile organelles such as vesicles or mitochondria move within neurons and what is the source of energy for this transport. Indeed, neurons are highly polarized cells with axons that can measure up to one meter in humans. Neurons require efficient intracellular transport referred to as fast axonal transport (FAT) to ensure exchange of material, mainly organelles, between the cell body and the synapses. Dysregulated FAT plays crucial roles in different neurodegenerative disorders (Hinckelmann *et al.*, 2013). FAT relies on ATP-dependent motor proteins, namely kinesin and dynein for anterograde and retrograde transport, respectively, moving cargo like vesicles or mitochondria along microtubules. Numerous adapter or regulatory proteins are associated with the FAT machinery thereby modulating transport efficacy, selectivity for the cargo and directionality within axons and dendrites. The observation of the specific association and function of the glycolytic enzymes on vesicles led to the demonstration that these enzymes provide energy for the long distance transport of vesicles within axons (Hinckelmann *et al.*, 2016; Zala *et al.*, 2013). While mitochondria generate their own energy for their locomotion, small vesicles contain the whole glycolytic metabolon. Vesicles are able to produce energy from glucose and to self-propel on microtubules independently of mitochondrial or bulk ATP in the cell (**Fig. 2(C)**, #7). Thus, these vesicles have their energy supply machinery "on board".

Together, these different examples illustrate the importance of the glycolytic metabolon to sustain a wide range of energy-consuming activities within the cell. The extraordinary high rate activity of the glycolytic machinery makes this metabolon essential for the cells to adapt to sudden changes in the environment.

Conclusions

Creatine kinases, nucleoside diphosphate kinases, and glycolytic enzymes provide instructive examples of how metabolite channeling within microcompartments or metabolons can provide the ATP or GTP necessary for fueling local motor proteins or other energy-requiring reactions. Many more cases of metabolite channeling are becoming evident in energy metabolism (a good example being the Krebs cycle (Zhang *et al.*, 2017)), and many other metabolic pathways in pro- and eukaryotes. However, it remains technically challenging to access metabolite channeling experimentally, and there are continuing efforts to develop appropriate methodologies (Abernathy *et al.*, 2019; Zhang *et al.*, 2017). Finally, there is rising interest to exploit the biological principle of metabolite channeling for applications in metabolic engineering and synthetic biology to increase efficiency and yield (Abernathy *et al.*, 2017; Castellana *et al.*, 2014; Obata, 2020).

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