11 th Calcium signaling course
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Capacitative $Ca^{2+}$ entry

Store-operated $Ca^{2+}$ entry

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$Ca^{2+}$ readdition protocol

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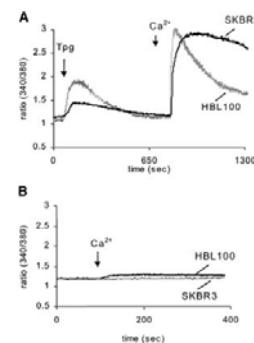


Baldi C *J Cell Biochem.* 88:1265, 2003

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$Mn^{2+}$ Quench technique

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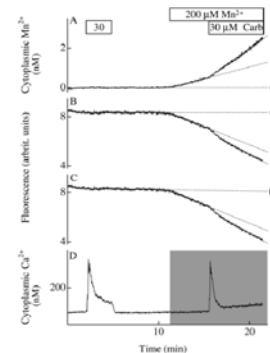
## Blockers of capacitative Ca$^{2+}$ entry

- **Gd$^{3+}$, La$^{3+}$**
- **SK&F 96365**
- **2-aminoethoxydiphenyl borate**
- **MRS 1845 (Sigma)**

J L Harper *Biochem.Pharmacol.* 65:329, 2003.
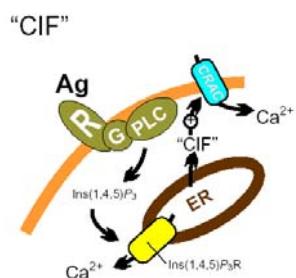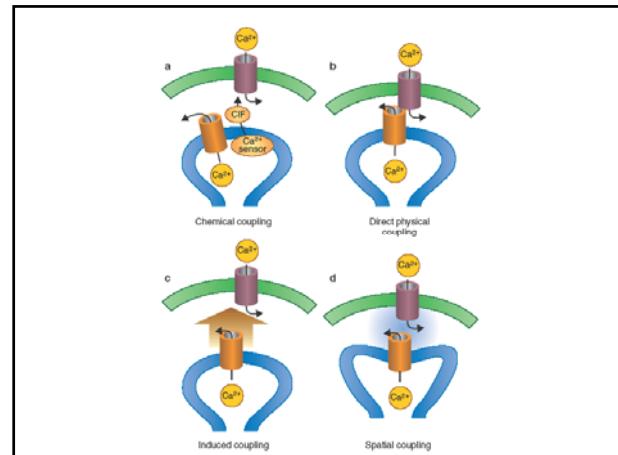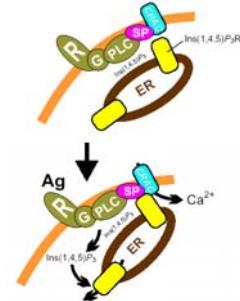






## Store-operated calcium entry

- Capacitative Ca2+ entry
- Receptor-operated Ca2+ entry
- Finite amount of ER Ca2+
- Thapsigargin and SOCE
- SOCE refills ER and prolongs Ca2+ increase

## Two types of SOCs

- Ca2+ release-activated Ca2+-selective (CRAC) channels
- Other SOC(s)
  – Non-selective current

## CRAC channel

- Highly Ca$^{2+}$-selective
- Formed by four Orai1 monomers that forms a tetrameric structure, which is associated to two STIM1 molecules
- Orai1:STIM1 ratio determine the biophysical properties of the channel
- SCID is due to loss of $I_{crac}$ due to mutation of Orai1

## Orai1 ("keepers of the gates of heaven")

- Prwevious name Ca2+ release-activated Ca2+ channel protein 1 (CRACM1)
- Orai1, Orai2, Orai3
- 301 aa; Four transmembrane domains
- Forms multimeric ion channels in the PM
- Low concentrations of 2-APB activate and high conc inhibit Orai1
- Orai3 is stimuleted by 2-APB
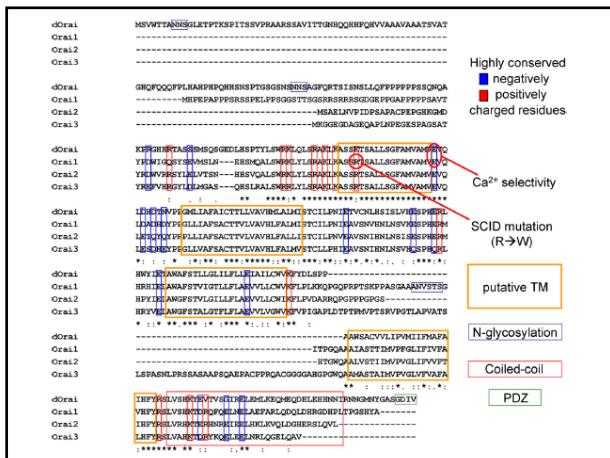


**Orai domains**

| Region | Location | Function |
|---|---|---|
| Arginine/proline-rich region | aa 3-8, aa 28-33, aa 39-47 | Orai1 assembly |
| Arginine/lysin-rich region | aa 77-88 | Orai1 assembly |
| Transmembrane regions: TM1 TM2 TM3 TM4 | aa 88-105, aa 118-140, aa 175-197, aa 236-258 | Four transmembrane segments |
| Selectivity filter | aa 106-114, E190 | Pore-forming domain |
| Coiled-coil region | aa 265-294 | Involved in protein-protein interactions (STIM1-Orai1 interaction) |





Putney,J.W.,Jr. et al J. Cell Biol. 2005, 169:381:2
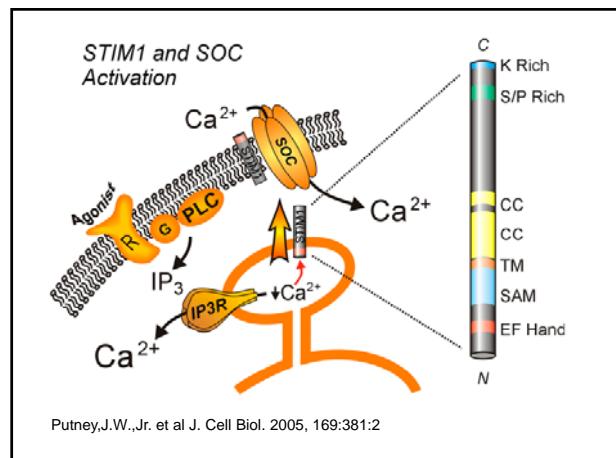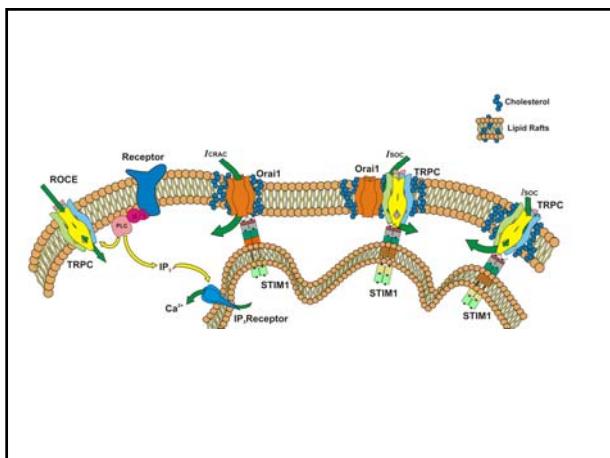
# STIM1

- Stromal Interaction Molecule 1
- 685 amino acids, single transmembrane spanning domain
- A $Ca^{2+}$ sensor Located on the ER
- Mutation of EF hand domain: constitutive SOCE
- Communicates filling state of ER to PM
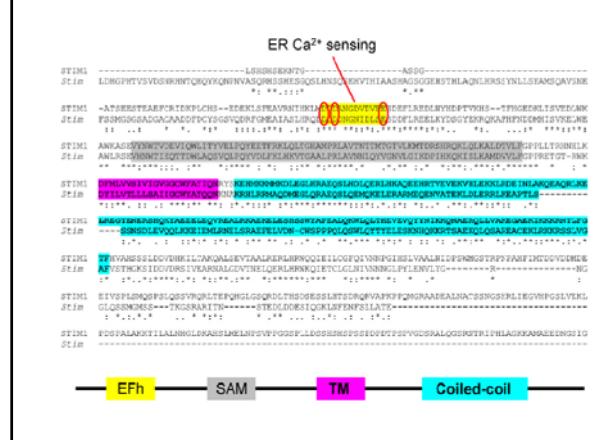


| STIM1 domains | Region | Location | Function |
|---|---|---|---|
| | EF-hand domain | aa 67-95 | $Ca^{2+}$ binding domain that senses ER $Ca^{2+}$ concentration |
| | SAM motif | aa 132-200 | sterile-α motif involved in protein-protein interaction |
| | Transmembrane region | aa 215-234 | A single transmembrane segment |
| | Coiled-coil regions | aa 238-343 aa 363-389 | Include the regions involved in Orai1/CRAC channel activating domains and overlap with the ERM-like domain |
| | ERM-like domain | aa 251-535 | Ezrin-radixin-moesin (ERM)-like domain. Includes de Orai1/CRAC channel activating domain |
| | CAD SOAR OASF CCb9 | aa 342-448 aa 344-442 aa 233-450/474 aa 339-444 | Orai1/CRAC activating domain. |
| | Homomerization domain | aa 400-474 | Clusters STIM1 into regions close to the plasma membrane |
| | STIM1 inhibitory domain | aa 445-475 | Inhibits the Orai1/CRAC activating domain at rest |
| | CMD | aa 474-485 | CRAC modulatory domain that induces Orai1/CRAC cannel closure |
| | Serine/proline-rich region | aa 600-629 | Localization of STIM1 into ER–PM junctions |
| | Polybasic region | aa 672-685 | Involved in puncta formation |

# STIM1 movement

- In resting cell moves in tubulovesicular shape on the ER
- On store depletion rapidly redistributes into discrete puncta underneath the PM
- SAM motif is necessary for puncta formation
- CC, SAM and Ser/Thr-rich domain are essential for SOC activation

**TTIM1 puncta**
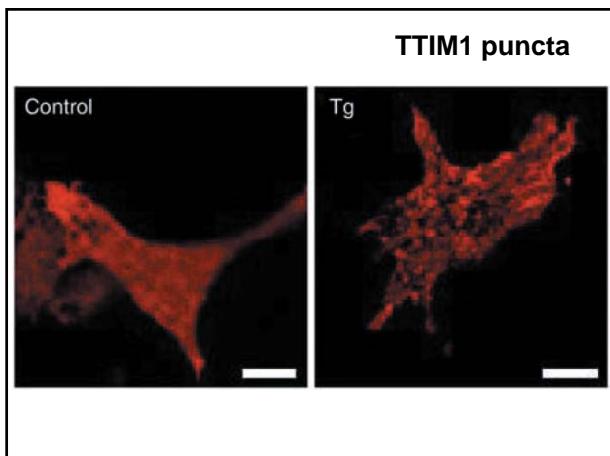


# TRPCs as SOCs

- TRPC1, TRPC2, TRPC3, TRPC4, TRPC5, TRPC6, TRPC7
- SOCE depends on expression level of TRPCs
- Low level of expression promotes SOCE
- High level of expression inhibits SOCE

## Transient receptor potential (*trp*) in drosophila

- Mutant flies have **transient** rather than sustained response to light

## TRP superfamily

- TRPC
  - TRP-Canonical, (Short TRPs, STRPs)
- TRPV
  - Vanilloid Receptor 1, (OTRPC)
- TRPM
  - Melastatin (Long TRPs, LTRPs)
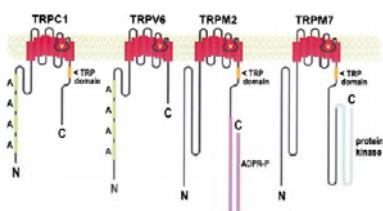


Montell C et al 2002 *Cell* 108:595
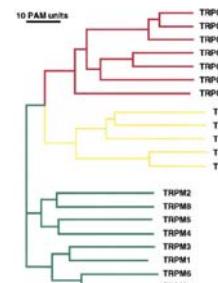


Phylogenic tree of TRP superfamily

## STIM1 and TRP

- STIM1 is a ER Ca2+ sensor
- Functional association between STIM1 and TRPC1 in many cell types
- STIM1 gates TRPC1 and TRPC3 by intermolecular electrostatic interaction
- Cytosolic C terminus is sufficient to activate TRPC1 and SOCE

## Orai-TRPCs-STIM interactions

- SOC channels are formed by combination of Orai1 and TRPC1 heteromers
- Filling state of ER is communicated to the SOC channel by STIM1 and Orai1
- Orai-TRPC complexes recruited to lipid rafts mediate SOCE