Calcium:
General Principles, Modeling

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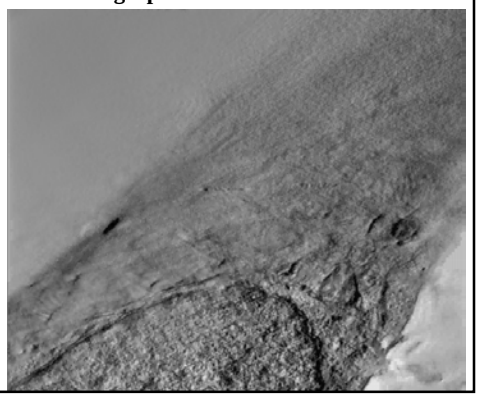
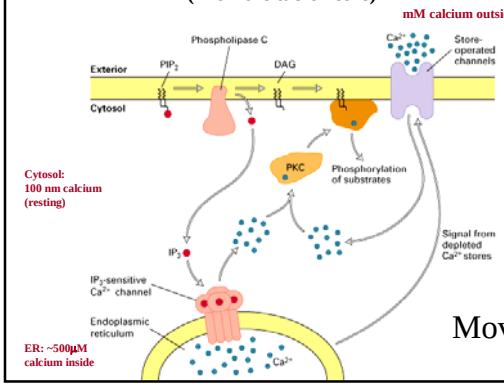
Can you pick out the cellular organelles in this tomographic tilt series?

- plasma membrane
- cytosol
- nucleus
- endoplasmic reticulum
- mitochondria



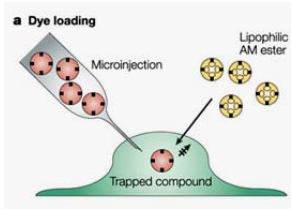
Basic signaling scheme to raise $[Ca^{2+}]_i$

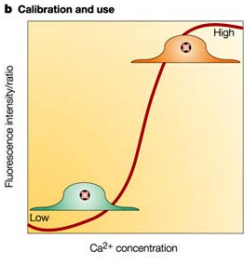
("non-excitable" cells)



Movie

How to measure [Ca2+]? ...typically use Fluorescent Probes





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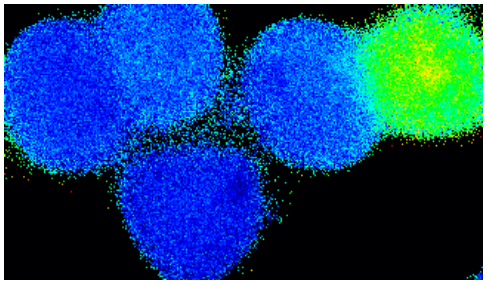
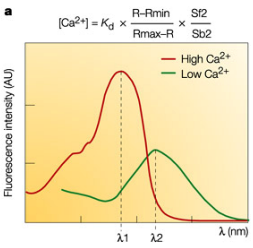
Movie
http://www.jcb.org/cgi/content/full/jcb.200206089/DC1/2

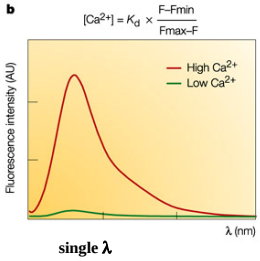
Imaging Live Cells



More on measurements...pick best dye for instrument you have access to.



Ratioing approach
(example: Fura)



single λ
(example: Fluo3)

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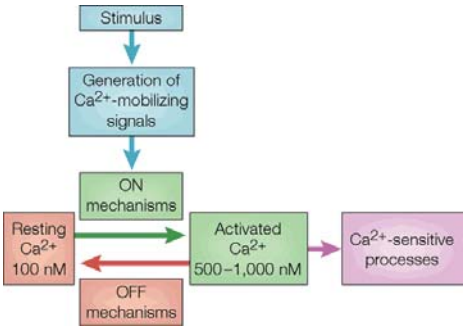
Typical Resting & Stimulated Levels of Cytoplasmic Calcium



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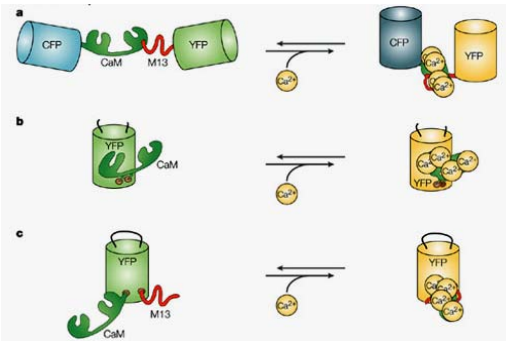
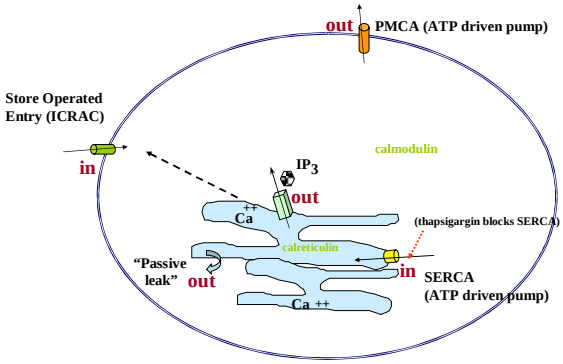
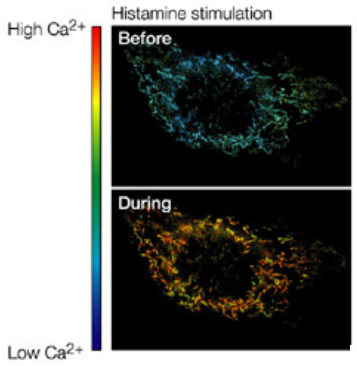
Also some newer GFP-based probes



In modeling, must consider flux both directions thru plasma membrane & ER membrane, as well as buffering proteins



....and Mitochondria, too!



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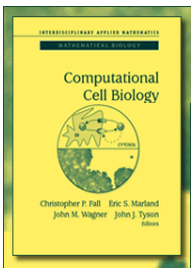
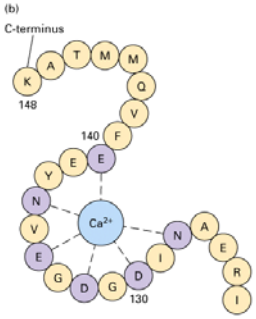
Most non-compartmental models treat mitochondria as an "immobile buffer" and represent with single ODE.

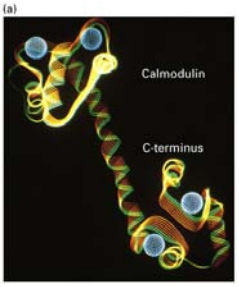
Recommended reading: Chapters in....



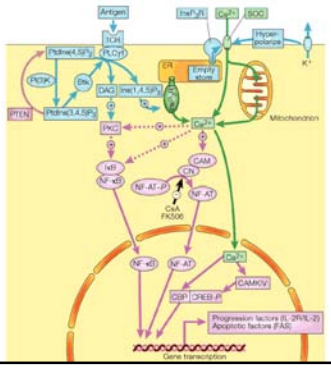
dedicated
to Joel E. Keizer
1942-1999

Why do we care? Calcium is a 2nd Messenger & binds directly to proteins such as calmodulin.





Calcium is important for signaling to the nucleus (such as PKC & Calmodulin/Calcineurin Pathways)



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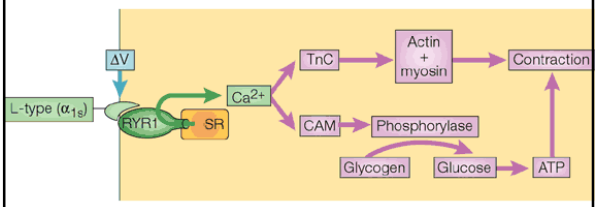
Outcomes of Elevated Calcium are <u>Cell-type Specific</u>

Example 1: Skeletal Muscle



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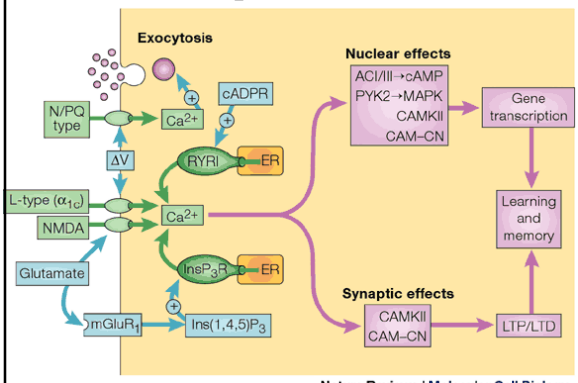
Example 2: NEURON



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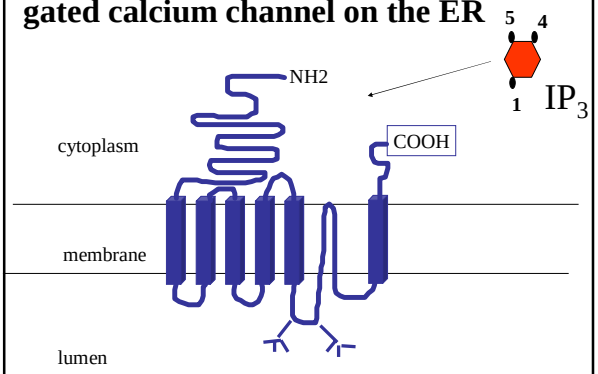
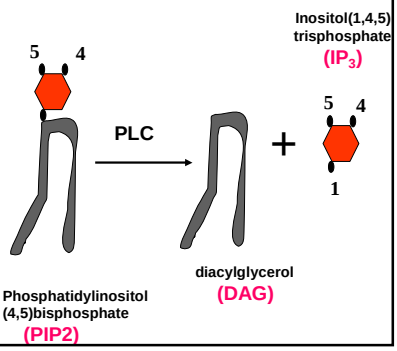
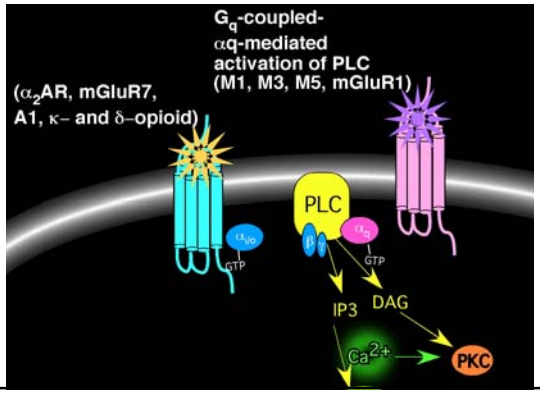
The IP_3 receptor is a ligand-gated calcium channel on the ER



The lipid PIP_2 is critical to this pathway

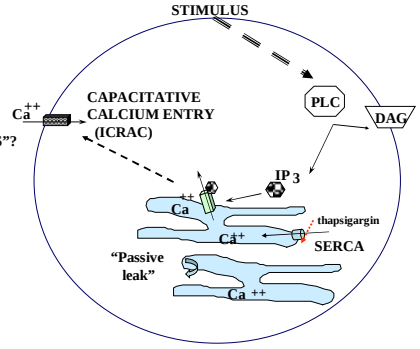


Example: Activation of phospholipase C by G_q- and G_i-coupled receptors



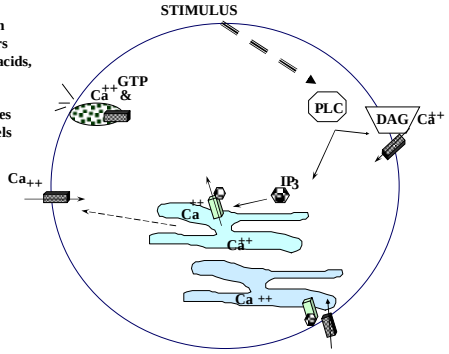
THE MYSTERY OF STORE-OPERATED" CALCIUM ENTRY

- Is there a sensor for "EMPTY STORES"?
- If so, where?
- How to transmit the message?

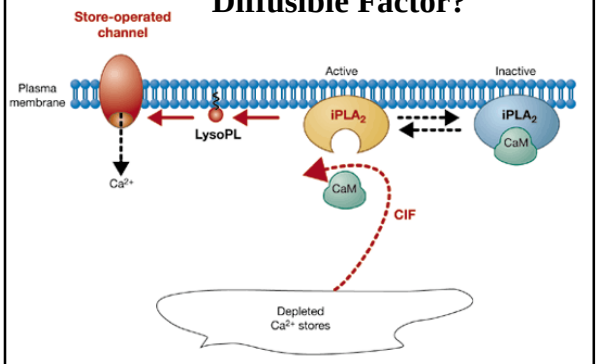


4 different hypotheses for regulation of Store-Operated Entry

1. Direct activation by 2nd messengers (ex: DAG, fatty acids, other?)
2. Fusion of Vesicles carrying channels
3. Diffusible Factor
4. Close Coupling (Conformational Coupling) model

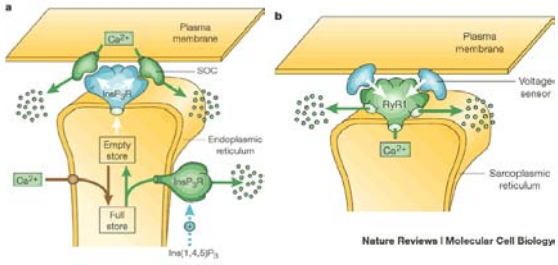


Diffusible Factor?



Tarik Smani, Sergey I. Zakharov, Peter Csutora, Endri Leno, Elena S. Trepakova & Victoria M. Bolotina *Nature Cell Biology* 6, 113 - 120 (2004)
A novel mechanism for the store-operated calcium influx pathway

Conformational Coupling or Close Coupling Model?



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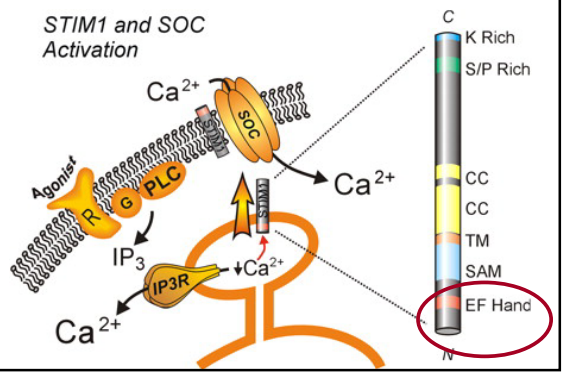
STIM: New Candidate for Sensor



Finding the identity of the store-operated channel has been "HOT" Topic


Science

CRACM1 Is a Plasma Membrane Protein Essential for Store-Operated Ca^{2+} Entry

M. Vig,[1*] C. Peinelt,[2] A. Beck,[2] D. L. Koomoa,[2] D. Rabah,[1] M. Koblan-Huberson,[1] S. Kraft,[1] H. Turner,[2] A. Fleig,[2] R. Penner,[2*] J.-P. Kinet[1*]

Store-operated Ca^{2+} entry is mediated by Ca^{2+} release–activated Ca^{2+} (CRAC) channels following Ca^{2+} release from intracellular stores. We performed a genome-wide RNA interference (RNAi) screen in *Drosophila* cells to identify proteins that inhibit store-operated Ca^{2+} influx. A secondary patch-clamp screen identified CRACM1 and CRACM2 (CRAC modulators 1 and 2) as modulators of *Drosophila* CRAC currents. We characterized the human ortholog of CRACM1, a plasma membrane–resident protein encoded by gene *FLJ14466*. Although overexpression of CRACM1 did not affect CRAC currents, RNAi-mediated knockdown disrupted its activation. CRACM1 could be the CRAC channel itself, a subunit of it, or a component of the CRAC signaling machinery.

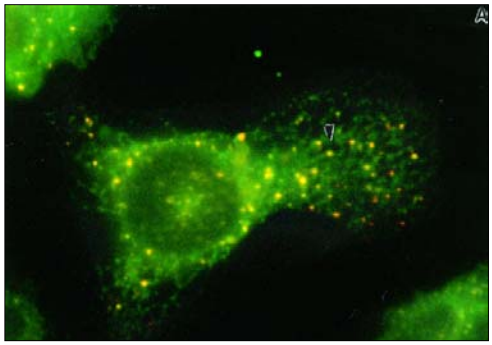
2006 VOL 312 SCIENCE www.sciencemag.org

To our knowledge, noone has modeled STIM or CRACM1 yet. More parameter data will be needed....

Our modeling strategies:

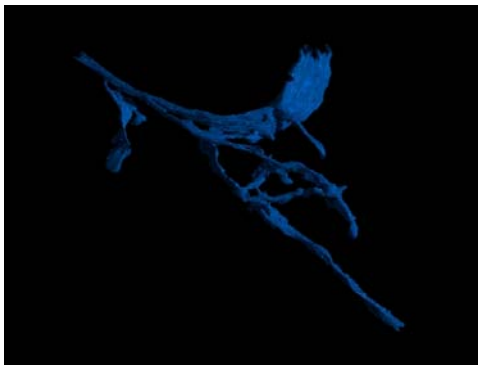
- **Build on existing literature**
- **Test interesting hypotheses**
- **Incorporate cell geometry**
- **Deterministic & stochastic approaches**
- **Support modeling with quantitative measurements; validate modeling results with more measurements**

<u>Starting premise:</u> our observation that IP_3 receptors cluster in ER after a rise in $[Ca^{2+}]_i$

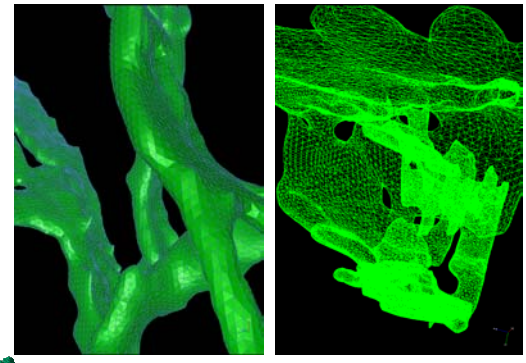


Wilson et al, Molecular Biol.Cell 1998

3D ER reconstruction



Tetrahedral mesh generation



using CUBIT (Sandia)

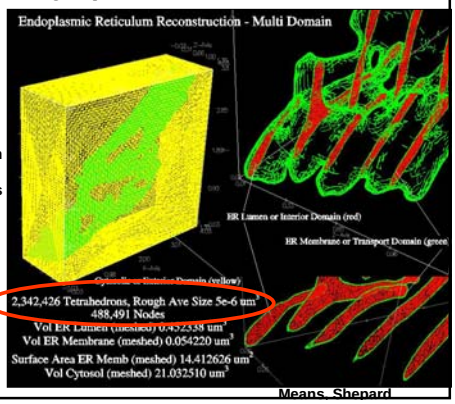
Smith, Shadid

ER & Cytoplasm MultiDomains

Simulations use Salsa

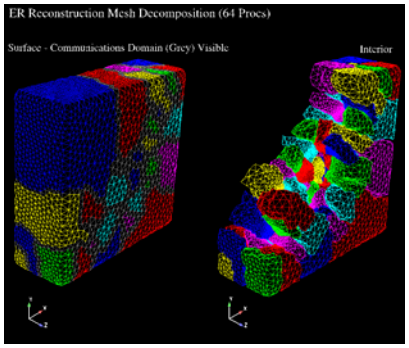
FEM Reacting Flows Solver, not originally designed for multiple domain problems. Code modifications allow for accurate representation of surface transport (Neumann Flux) with spatially-localized reactions (source term)

http://www.cs.sandia.gov/CRF/MPSalsa/

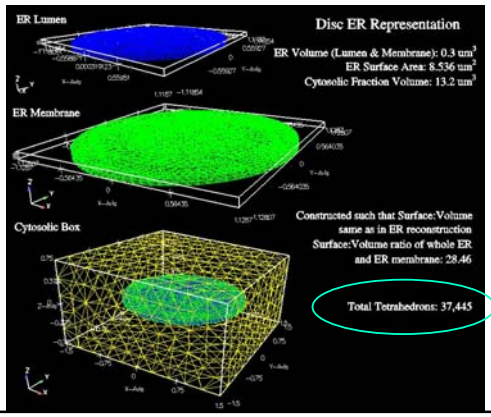


Means, Shepard

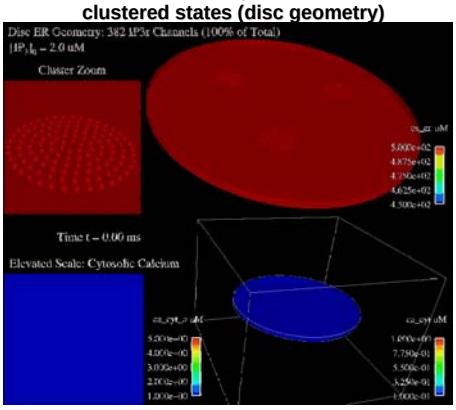
Decomposition for 64 Processors. communications domain colored in grey.



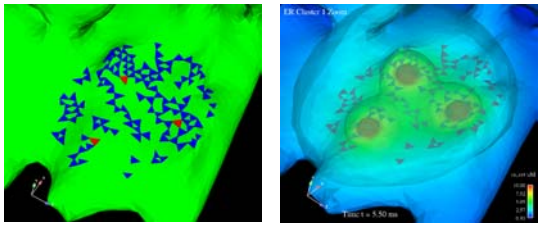
For quicker simulations, apply simpler geometries (discs & tubes).



Flux through IP_3 receptors in clustered states (disc geometry)

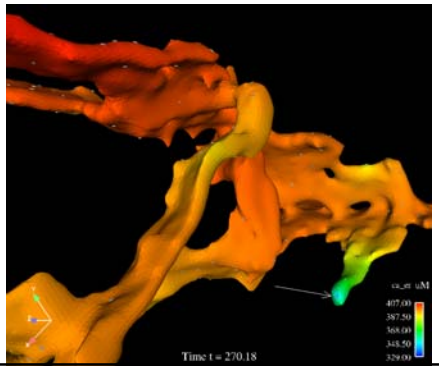


ER Geometry: Deterministic IP_3R Trial

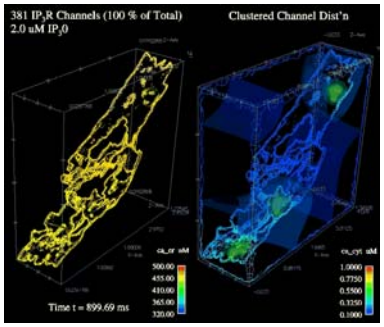


Three channels forced open and closed for 10 ms. Trio in clustered channels (left, red) and resulting calcium cloud shown.

ER Geometry: Small, Transient Concentration Gradients (Diffuse IP3R distribution)

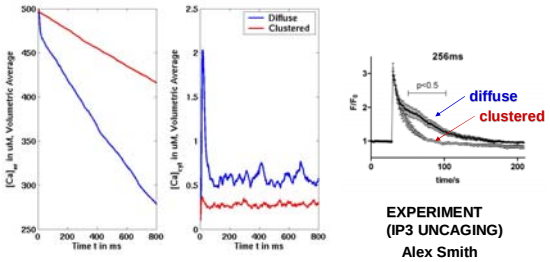


RESULTS IN THE FULL GEOMETRY



Exit for 2 movies..

THE ER EMPTIES SLOWER & CYTOSOLIC CALCIUM LEVELS ARE LOWER IN THE CLUSTERED IP3R STATE



EXPERIMENT (IP3 UNCAGING)
Alex Smith

SIMULATION