Intracellular Ca^{2+} Signaling

2004

Why Calcium?

- Na^+ and Cl^- are sea water
  - Excluded to maintain low osmotic pressure
  - $[K^+]_i$ kept high for electrical neutrality
- $[Ca^{2+}]_i$ maintained very low
  - Prevents precipitation of organic anions
- Mg^{2+} helps solubilize organic anions

How cells keep $[Ca]_i$ low

- All eukaryotic cells have PM Ca^{2+}-ATPase
  - Excitable cells also have Na^+/Ca^{2+} exchanger (NCX)
- ER Ca^{2+}-ATPase (against a high grad)
- Mitochondrial high capacity (low affinity) pump
  - When $[Ca]_i$ very high (dangerous) levels (>10^{-5} M)
  - Inner mitochondrial membrane
  - Uses the electrochemical gradient generated during electron-transfer of oxidative-phosphorylation

Calcium Concentrations

- $[Ca^{2+}]_o$ / $[Ca^{2+}]_i$ >10^4
  - $[Ca^{2+}]_o$ ~10^{-3} M
  - $[Ca^{2+}]_{ER}$ ~10^{-3} M
  - $[Ca^{2+}]_i$ <10^{-7} M at rest

Ca^{2+} - a versatile signal

Target Tissue	Signaling Molecule	Major Responses
Liver	Vasopressin	Glycogen breakdown
Pancreas	ACh	Amylase secretion
Smooth muscle	ACh	Contraction
Mast cells	Antigen	Histamine secretion
Blood platelets	Thrombin	aggregation

Ca^{2+} - a versatile signal

- Synaptic vesicle release (μs)
- Excitation-contraction coupling (ms)
- Smooth muscle relaxation (ms-sec)
- Excitation-transcription coupling (min-h)
- Gene transcription (h)
- Fertilization (h)

How Ca^{2+} is measured

- Ratiometric dyes
- Cameleons

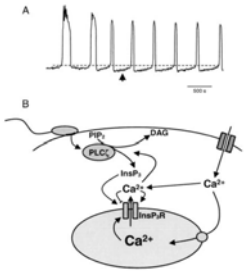
How cells ↑ $[Ca]_i$

- Voltage-gated Ca^{2+} Channels
  - Membrane potential drives Ca^{2+} down its chemical gradient
  - Different channels in different cells
    - Different properties for different purposes

Ca^{2+} shut-off pathways

- Voltage-gated Ca^{2+} channels inactivate
- IP_3 rapidly dephosphorylated
- Ca^{2+} rapidly pumped out

Ca^{2+} oscillations



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Intracellular Ca^{2+} Targets

- EF hand-containing proteins
  - Calmodulin
- C2 domain proteins
  - PKC
  - Synaptogamin

Intracellular Ca^{2+} Targets EF hand proteins

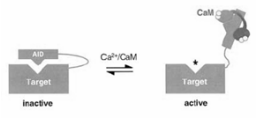
- Troponin C
- Calmodulin
- Calcineurin
- Calpain
- Recoverin
- S100

Calmodulin (CaM)

- 16.8 kDa Ca^{2+}-binding protein
- Highly conserved in all eukaryotes
- Up to 1% of protein
- Binds 4 mol Ca^{2+}/mol
- Binding affinity ~ .25-1x10^6 M
  - Cooperativity with Hill constant ~ 2

Classical mode of CaM regulation

Relief of autoinhibition



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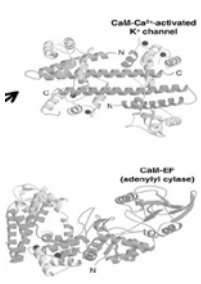
CaMKII

Autoinhibition *and*
Frequency Decoder

New modes of CaM interaction



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New modes of CaM interaction

New modes of CaM regulation

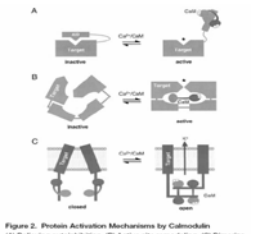

Figure 2. Protein Activation Mechanisms by Calmodulin
(A) Relieving autoinhibition. (B) Active site remodeling. (C) Dimerization. Calcium ions are depicted in yellow, and active sites by asterisks.

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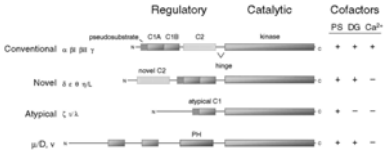
Intracellular Ca^{2+} Targets
C2 Domains

- Protein kinase C
- Synaptotagmin
- Phospholipase A_2

C2 domains - PKC



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Synaptotagmin and neurotransmitter release