Intracellular Ca\(^{2+}\) Signaling

Why Calcium?

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

How cells keep [Ca\(^{2+}\)] 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], very high (dangerous) levels (>10\(^{-5}\) M)
  - Inner mitochondrial membrane
  - Uses the electrochemical gradient generated during electron-transfer of oxidative-phosphorylation

Calcium Concentrations

- [Ca\(^{2+}\)]\(_{\text{o}}\) / [Ca\(^{2+}\)]\(_{\text{i}}\) >10\(^4\)
  - [Ca\(^{2+}\)]\(_{\text{o}}\) ~10\(^{-3}\) M
  - [Ca\(^{2+}\)]\(_{\text{ER}}\) ~10\(^{-3}\) M
  - [Ca\(^{2+}\)]\(_{\text{i}}\) <10\(^{-7}\) M at rest

Ca\(^{2+}\) - a versatile signal

<table>
<thead>
<tr>
<th>Target Tissue</th>
<th>Signaling Molecule</th>
<th>Major Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Vasopressin</td>
<td>Glycogen breakdown</td>
</tr>
<tr>
<td>Pancreas</td>
<td>ACh</td>
<td>Amylase secretion</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>ACh</td>
<td>Contraction</td>
</tr>
<tr>
<td>Mast cells</td>
<td>Antigen</td>
<td>Histamine secretion</td>
</tr>
<tr>
<td>Blood platelets</td>
<td>Thrombin</td>
<td>aggregation</td>
</tr>
</tbody>
</table>

Ca\(^{2+}\) - a versatile signal

- Synaptic vesicle release (\(\mu\)s)
- 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 $\uparrow$ [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

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 Ca²⁺-binding protein
- Highly conserved in all eukaryotes
- Up to 1% of protein
- Binds 4 mol Ca²⁺/mol
- Binding affinity ~ 25-1x10⁶ M
  - Cooperativity with Hill constant ~ 2

Classical mode of CaM regulation
Relief of autoinhibition

CaMKII
Autoinhibition and Frequency Decoder

New modes of CaM interaction
New modes of CaM regulation

Intracellular Ca²⁺ Targets
- Protein kinase C
- Synaptotagmin
- Phospholipase A₂
C2 domains - PKC

Synaptotagmin and neurotransmitter release