**Signaling Through Cytoplasmic Calcium**

*) Extracellular concentrations (EM, blood) are 2 mM, and levels in cytoplasmic vesicles and the ER can reach up to 10 mM.

*) Baseline cytosolic Ca\(^{2+}\) concentration is around 100 nM in resting cells. Only 0.1% of the cellular Ca\(^{2+}\) is ionized (*Hodgkin & Keynes 1957*: radioactive Ca\(^{2+}\) shows different voltage-gradient mediated diffusion rates in solution than after microinjection into giant squid axon --> conclusion: ionized Ca\(^{2+}\) has to be less than 100 nM!!)

<table>
<thead>
<tr>
<th>Conc in mM</th>
<th>ECF</th>
<th>ICF</th>
</tr>
</thead>
<tbody>
<tr>
<td>K(^+)</td>
<td>4.5</td>
<td>160</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>144</td>
<td>7</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>114</td>
<td>7</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>2.2</td>
<td>0.0001</td>
</tr>
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</table>

*) High gradient makes this a very fast a sensitive signaling system since only slight changes in membrane permeability will result in dramatic changes in the concentration of [Ca\(^{2+}\)].

*) Low level of [Ca\(^{2+}\)]\(_i\) is also a necessity to facilitate the phosphate oriented cellular metabolism (high calcium and high phosphate concentrations are incompatible!!)

*) **Evolutionary challenge: Maintain calcium gradient** !!!

Evolution of proteins that bind Ca\(^{2+}\) with high affinity, but reject magnesium!

*) **Two classes of Ca-binding proteins:**

- membrane-integrated (unlimited capacity --> transporter systems: Ca-channels, calcium pumps)

- non-membranous (limited capacity --> not only buffering, but processing of signal through conformational changes that enable interaction with target proteins: Calmodulin, Troponin C ...)

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Extracellular compartment: (predominantly in nerve cells, cardiac cells; smooth muscle cells)

Two types of plasma-membrane localized calcium channels:

- Voltage operated calcium channels:
  Action potential depolarizes plasma membrane, which results in the opening of “voltage” dependent calcium channels (channels can be opened by increase in extracellular $K^+$).

Three types:

- **L-Type**: “slow channel”, most common; predominantly in heart and smooth muscle; produce currents of long duration; sensitive to dihydropyridines (nifedipine)
- **T-Type**: “rapid channel”, short duration currents
- **N-Type**: “neuronal channel” only in sensory neurons; opened by large depolarization by very negative transmembrane potential

<table>
<thead>
<tr>
<th>Property</th>
<th>Channel type</th>
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<tbody>
<tr>
<td></td>
<td>$T$</td>
</tr>
<tr>
<td>Activation voltage range</td>
<td>More positive than $-70$ mV</td>
</tr>
<tr>
<td>Inactivation voltage range</td>
<td>$-100$ to $-60$ mV</td>
</tr>
<tr>
<td>Single channel conductance</td>
<td>$8.10$ pS</td>
</tr>
<tr>
<td>Ca$^{2+}$ current kinetics</td>
<td>Long latency, brief current, moderate rate of current decrease</td>
</tr>
</tbody>
</table>

Blocked by dihydropyridines: No
Blocked by cadmium: Yes
Blocked by cobalt: Weakly

Opening of channel by depolarization

Where to get it from” (Ca-ON):
“Channels and stores”
Each channel protein has four homologous domains, each containing six membrane spanning $\alpha$-helices (the fourth one is thought to be the “voltage” sensor.

- **Ligand gated calcium channels:**
  Calcium channels opened after ligand binding to the receptor (e.g. glutamate/NMDA receptor; ATP receptor; also Nicotinic ACh receptors are coupled to ligand-gated cation channels (muscarinic ACh receptors signal through G-Proteins-->slower), prostaglandin receptors
- **Store operated calcium channels:**
  Activated by emptying of intracellular stores, exact mechanism unknown

*) **Intracellular stores:** (predominantly in muscle cells)
  Calcium stored in mM concentrations in endo/sarcoplasmatic reticulum bound to *Calsequestrin*. Previously mitochondria were thought to play an important role as Ca\(^{2+}\)-stores, but more recent evidence showed that the uptake rate is 10x lower than the ER/SR.

Calcium released from the ER/SR is regulated by two different calcium channels in the ER/SR membrane:

-** Ryanodine receptor controlled calcium channels (RYR):**
  Activity potentially regulated by cyclic ADP ribose as a second messenger (?).
  Ryanodine: plant alkaloid, irreversible inhibitor
  Caffeine: potent reversible activator of RYRs

-** IP3-sensitive calcium channels (IP3R):**
  Inositol-1,4,5-triphosphate is produced through the activity of receptor activated phospholipases-->diffuses through cytoplasm and binds IP3R on the ER/SR.
"How to make it go away" (Ca-OFF):
or
"Pumps, buffers and sensors"

* Ca\(^{2+}\) - pumps:
  Activity of these pumps is induced by increases in cytosolic calcium.

  - Plasma membrane Na\(^+\)/Ca\(^{2+}\) exchanger (mainly in excitable cells, e.g. cardiac cells)
    three Na\(^+\)-ions are exchanged for one Ca\(^{2+}\)-ion
    Digitalis alkaloids: Na\(^+\)/K\(^+\)-ATPase inhibitors => intracellular Na\(^+\) raises=> Na\(^+\)/Ca\(^{2+}\) exchange less efficient => Ca\(^{2+}\) intracellular increases=> stronger contractions

  - Plasma membrane Ca\(^{2+}\)-ATPase (PMCA)
    two Ca\(^{2+}\)- ions are transported per ATP molecule hydrolyzed
    become phosphorylated on aspartate during ion transport (causes the conformational change that propells the Ca\(^{2+}\)-ions to the ECF)
    regulated by CaM, PKA or PKC

  - SR/ER Ca\(^{2+}\)-ATPase (SERCA): 80% (!) of integral membrane protein of SR target of thapsigargin (=>Ca\(^{2+}\)-release from intracellular stores)

* Ca\(^{2+}\)-Buffers:
  Low affinity (!) but high capacity (50-100 Ca\(^{2+}\)-ions/molecule)
  - Calsequestrin (very acidic, 37% of aa are aspartic and glutamic acid), calreticulin, parvalbumin

* Ca\(^{2+}\)-Sensors:
  - Annexins (low affinity):
    Family of proteins w/ common feature that they interact w/ membranes in a Ca\(^{2+}\)-dependent manner.
    Low affinity for Ca\(^{2+}\)-ion s restricts action to membrane proximity (high local Ca\(^{2+}\) conc.!) ; implicated in the regulation of PLA2, cytoskeletal (re)organization and vesicle movement
EF-hand proteins (high affinity):
named after the shape created by the E and F $\alpha$-helices of the Ca$^{2+}$-binding domain

Calmodulin: ubiquitous expression; binds 4 Ca$^{2+}$-ions; acts through stimulation of either protein kinases (CaMKs) or protein phosphatases (Calcineurin). also activates cAMP phosphodiesterase

Troponin C: restricted expression, regulates contraction of skeletal and heart muscle
“What is it doing?”

*) Muscle contraction:
  -) Skeletal muscle:
  Contraction (=actin-myosin interaction) controlled by proteins on actin filaments (tropomyosin w/ troponin)
  *Troponin I inhibits formation of cross-bridges between actin and myosin => muscle relaxed.
  *Troponin C combines with Ca\(^{2+}\)-ions and blocks the action of Troponin I => muscle contracted

\[ \text{CONTRACTION} \]

\[
\begin{array}{c}
\text{Actin} \bullet \text{TM} \bullet \text{TM} \\
\text{Ca}^{2+} < 10^{-6} \text{M} \quad \text{Ca}^{2+} > 10^{-4} \text{M}
\end{array}
\]

\[ \text{RELAXATION} \]

-) Smooth muscle:
 Contraction controlled by proteins on either actin....
 NO Troponin=>regulation occurs through the CaM binding **Caldesmon**:
 Low Ca2+-conc.: Caldesmon forms complex with actin and tropomyosin=>
 access of myosin to actin is restricted=>muscle relaxed.

\[ \text{CONTRACTION} \]

\[
\begin{array}{c}
\text{Actin} \bullet \text{TM} \\
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\end{array}
\]

\[ \text{RELAXATION} \]

...OR myosin filaments:

phosphorylation of myosin light chain (MLC) by MLC kinase (MLCK):
phosphorylated myosin is able to interact w/ actin=>contraction
MLCK is regulated through interaction with calmodulin:

Additional modulation of smooth muscle response through inhibitory phosphorylation by PKC (activated by 1,2-diacylglycerol):
+ mediated through phosphorylation of MLC on inhibitory site
+ mediated through phosphorylation that inhibits the function of caldesmon
*) Neuronal excitibility and secretion:
Increase of Ca\(^{2+}\)-concentration induces fusion of the synaptic vesicles with the plasma membrane => this causes exocytosis of neuro-transmitters into the synaptic cleft.

*) Glycogen hydrolysis:
Glycogen hydrolysis is mediated by glycogen phosphorylase, which in turn is activated by glycogen phosphorylase kinase (GPK). GPK consists of multiple subunits, one of which (δ) is calmodulin. Phosphorylation of the α and β subunits by cAMP dependent kinase increases affinity of calmodulin for Ca\(^{2+}\)-ions.

*) Immune response:
TCR stimulation => Ca\(^{2+}\)-conc. Increases => activates Calcineurin => dephosphorylates NFATc on ser/thr => NFATc translocates to nucleus where it combines w/ NFATn and induces transcription of IL2 gene=> T cell proliferation

Cyclosporin A and FK506:
bind immunophillins : the drug/immunophilin complex binds then to calcineurin, blocking its catalytic activity => no IL2 => immunosuppression
“How to measure it”

*) $^{45}$Ca:
  Influx only!

*) Microelectrodes:
  Expensive, requires micromanipulators

*) Aequorin:
  21 kDa protein from *Aequorea forskalea* (jellyfish):
  protein emits light in an ATP dependent manner when bound to $\text{Ca}^{2+}$-ions.
  Very sensitive, but requires introduction of the protein into cells.

*) Fluorescence indicators:
  Derivatives of EGTA (=>$\text{Ca}^{2+}$-ions chelators)
  QUIN, INDO, FURA, FLUO...
  Compounds change there excitation/emission spectra when binding $\text{Ca}^{2+}$-ions.

Very hydrophilic=>$\text{Ca}^{2+}$-ion binding form.