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Calcium ions Function as a Ubiquitous Intracellular Messenger

Objectives:

To acquaint the students about:

- i) Difference in intracellular and extracellular levels of Ca^{2+} ions and its maintenance
- ii) Ca^{2+} ions as signaling molecules
- iii) Ca^{2+} /Calmodulin-dependent Protein Kinases (CaMKinases)

- Many extracellular signals induce an increase in cytosolic Ca^{2+} level.
 - e.g. In egg cells, sudden rise in cytosolic Ca^{2+} concentration upon fertilization by a sperm triggers a Ca^{2+} wave that is responsible for the onset of embryonic development.
 - In muscle cells, Ca^{2+} triggers contraction, and in many secretory cells, including nerve cells, it triggers secretion.

- **Ca^{2+} ions can be used as a signal because -**

- its concentration in the cytosol is normally kept very low ($\sim 10^{-7}$ M), whereas
 - its concentration in the extracellular fluid ($\sim 10^{-3}$ M) and in ER lumen is high.
- Thus, a large gradient tends to drive Ca^{2+} into the cytosol across both PM and ER membrane.

- When a signal transiently opens Ca^{2+} channels in either of these membranes, Ca^{2+} rushes into the cytosol, increasing the local Ca^{2+} concentration by 10-20 fold and triggering Ca^{2+} -responsive proteins in the cell.

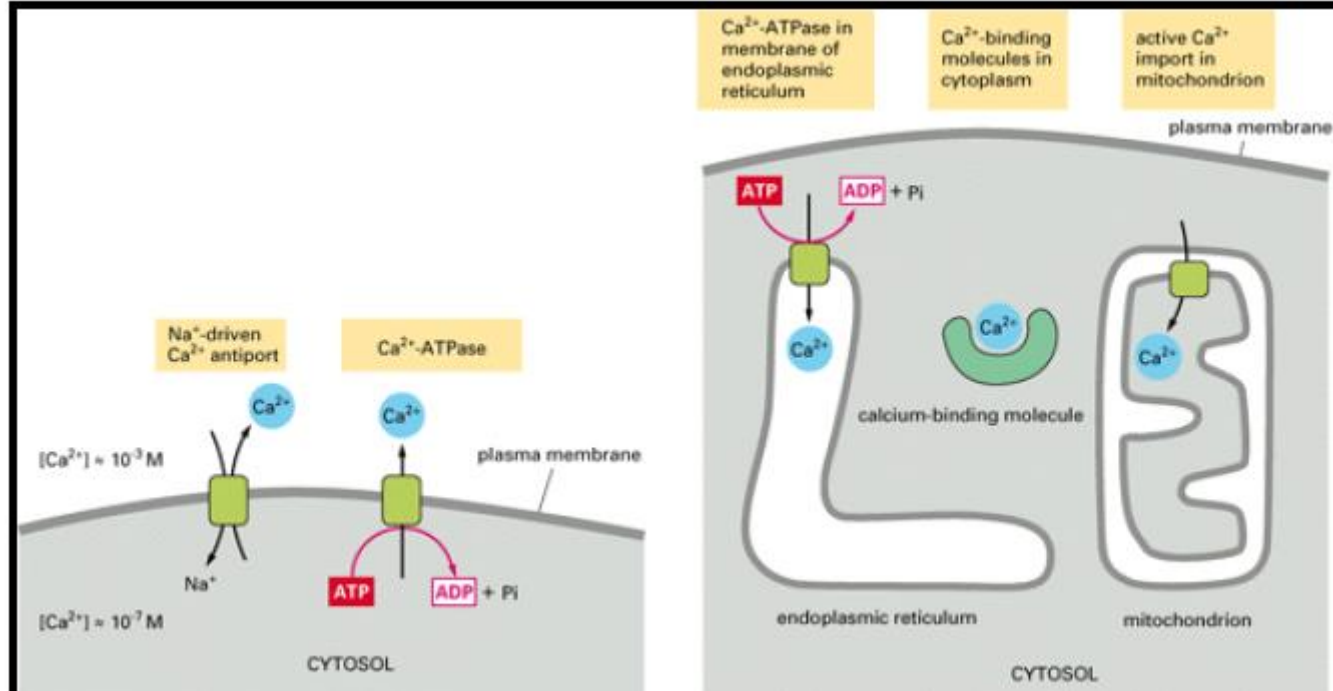
- Three main types of Ca^{2+} channels can mediate this Ca^{2+} signaling:

1. ***Voltage-dependent Ca^{2+} channels*** in PM open in response to membrane depolarization and allow, e.g., Ca^{2+} to enter activated nerve terminals and trigger neurotransmitter secretion.
2. ***IP_3 -gated Ca^{2+} -release channels*** allow Ca^{2+} to escape from the ER when the inositol phospholipid signaling pathway is activated.
3. ***Ryanodine receptors*** (so called because they are sensitive to plant alkaloid ryanodine) react to a change in plasma membrane potential to release Ca^{2+} from the sarcoplasmic reticulum and thereby stimulate the contraction of muscle cells; they are also present in ER of many non-muscle cells, including neurons, where they can contribute to Ca^{2+} signaling.

Ca²⁺ concentration in cytosol is kept low in resting cells by several mechanisms.

Eukaryotic cells have:

- A Ca²⁺-pump in their PM that uses energy from ATP hydrolysis to pump Ca²⁺ out of cytosol.
- An additional Ca²⁺ transport protein (exchanger) in PM that couples efflux of Ca²⁺ to influx of Na⁺, on PM of cells that extensively use Ca²⁺ signaling such as muscle and nerve cells.
- A Ca²⁺ pump in ER membrane also has an important role in keeping cytosolic Ca²⁺ concentration low: this Ca²⁺-pump enables ER to take up large amounts of Ca²⁺ from cytosol against a steep concentration gradient, even when Ca²⁺ levels in cytosol are low.
- A low-affinity, high-capacity Ca²⁺ pump in the inner mitochondrial membrane has an important role in returning the Ca²⁺ concentration to normal after a Ca²⁺ signal; it uses the electrochemical gradient generated across this membrane during electron-transfer steps of oxidative phosphorylation to take up Ca²⁺ from cytosol.



The Frequency of Ca²⁺ Oscillations Influences a Cell's Response

Ca²⁺-sensitive fluorescent indicators, as aequorin or fura-2, are used to monitor cytosolic Ca²⁺ in individual cells after inositol phospholipid signaling pathway has been activated. The initial Ca²⁺ signal is often seen to be small and localized to one or more discrete regions of the cell. These signals have been called Ca²⁺ blips, quarks, puffs, or sparks, and they are thought to reflect the local opening of individual (or small groups of) Ca²⁺-release channels in the ER and to represent elementary Ca²⁺ signaling units. **If the extracellular signal is sufficiently strong and persistent, this localized signal can propagate as a regenerative Ca²⁺ wave through the cytosol, much like an action potential in an axon. Such a Ca²⁺ "spike" is often followed by a series of further spikes, each usually lasting seconds.**

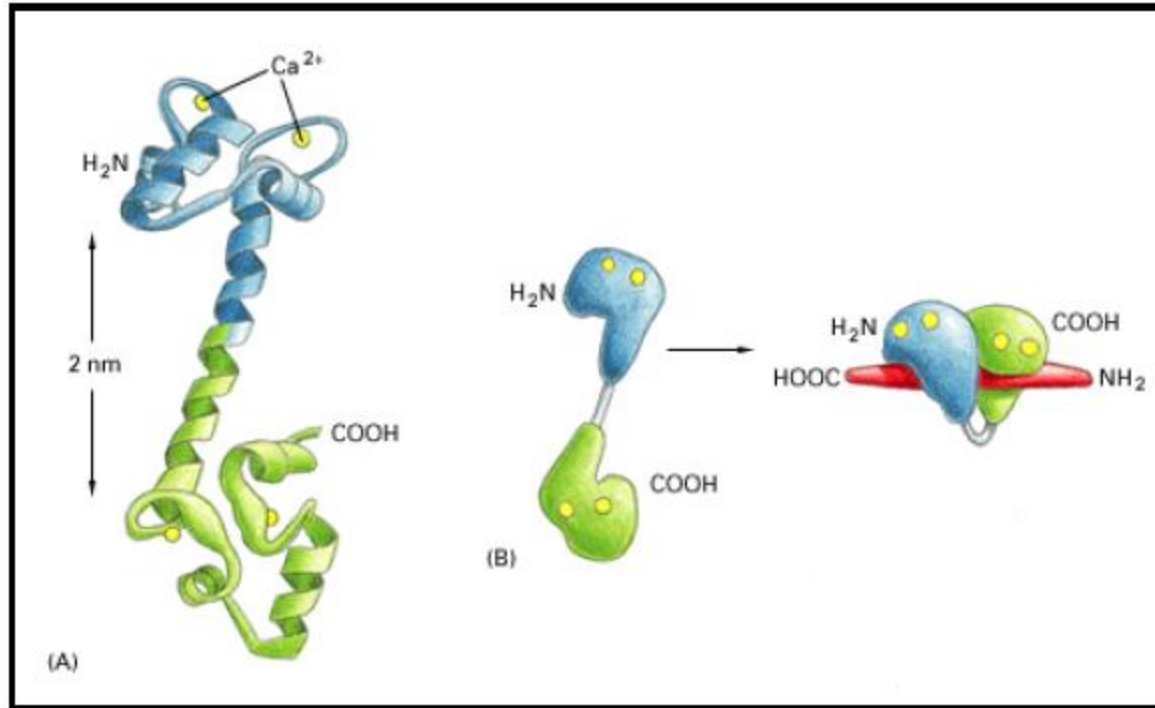
Waves and the oscillations depend, in part at least, on a combination of positive and negative feedback by Ca²⁺ on both the IP₃-gated Ca²⁺-release channels and the ryanodine receptors: the released Ca²⁺ initially stimulates more Ca²⁺ release. But then, as its concentration gets high enough, the Ca²⁺ inhibits further release. The frequency of the Ca²⁺ oscillations reflects the strength of the extracellular stimulus, and it can be translated into a frequency dependent cell response. In some cases, the frequency-dependent response itself is also oscillatory.

Ca²⁺/Calmodulin-dependent Protein Kinases (CaMKinases) Mediate Actions of Ca²⁺ within Animal Cells

- *Ca²⁺-binding proteins serve as transducers of the cytosolic Ca²⁺ signal.*
- *1st such protein discovered was *troponin C* in skeletal muscle cells (role in muscle contraction).*
- *A closely related Ca²⁺-binding protein, k/a **calmodulin**, is found in all eucaryotic cells, where it can constitute as much as 1% of the total protein mass.*
- *Calmodulin functions as a **multipurpose intracellular Ca²⁺ receptor**, mediating many Ca²⁺-regulated processes. It consists of a highly conserved, single polypeptide chain with four high-affinity Ca²⁺-binding sites. When activated by binding Ca²⁺, it undergoes a conformational change. Because two or more Ca²⁺ ions must bind before calmodulin adopts its active conformation, the protein responds in a switchlike manner to increasing concentrations of Ca²⁺: a tenfold increase in Ca²⁺ concentration, e.g., causes a 50-fold increase in calmodulin activation.*
- *In some cases, calmodulin serves as a permanent regulatory subunit of an enzyme complex, but mostly the binding of Ca²⁺ enables calmodulin to bind to various target proteins in the cell to alter their activity.*
- *When an activated molecule of Ca²⁺/calmodulin binds its target protein, it undergoes a marked change in conformation. Many targets as enzymes and membrane transport proteins calmodulin binding. For example, Ca²⁺/calmodulin binds to and activates PM Ca²⁺-pump that pumps Ca²⁺ out of cells. Thus, whenever Ca²⁺ concentration in cytosol rises, the pump activates, and restores normal cytosolic Ca²⁺ levels.*

- Many effects of Ca^{2+} , are more indirect and are mediated by protein phosphorylations catalyzed by a family of **Ca^{2+} /calmodulin-dependent protein kinases (CaM-kinases)**. These kinases, just like PKA and PKC, **phosphorylate serines or threonines** in proteins, and, as with PKA and PKC, the response of a target cell depends on which CaM-kinase-regulated target proteins are present in the cell.
- The first CaM-kinases to be discovered is *myosin light-chain kinase*, which activates smooth muscle contraction. Another CaM kinase, the *phosphorylase kinase*, which activates glycogen breakdown have narrow substrate specificities.
- A number of CaM-kinases, however, have much broader specificities, and these seem to be responsible for mediating many of the actions of Ca^{2+} in animal cells.
- Some phosphorylate gene regulatory proteins, such as the CREB protein, and in this way activate or inhibit the transcription of specific genes.

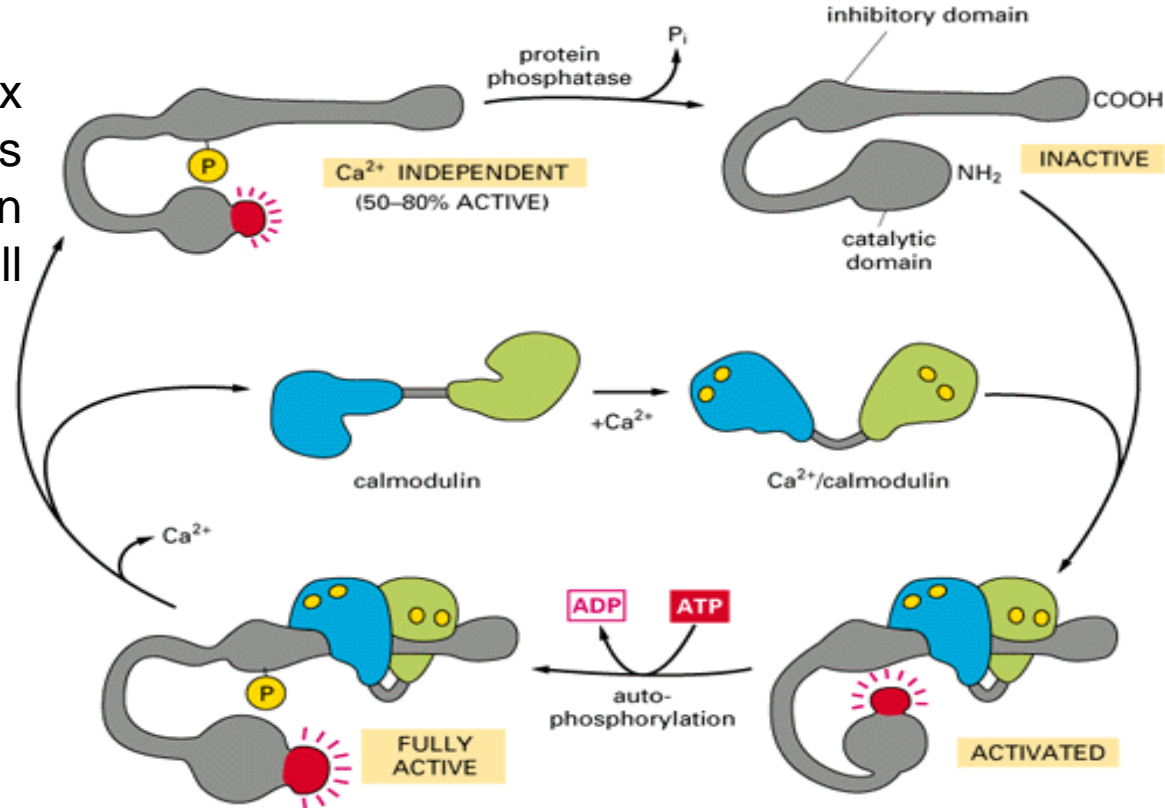
- The best-studied example of such a *multifunctional CaM-kinase* is **CaM-kinase II**, which is found in all animal cells and is especially enriched in the nervous system.
- It constitutes up to 2% of the total protein mass in some regions of brain, and it is highly concentrated in synapses.



The structure of Ca²⁺/calmodulin. (A) The molecule has a "dumbbell" shape, with 2 globular ends connected by a long, exposed α -helix. Each end has 2 Ca²⁺-binding domains, each with a loop of 12 amino acids, in which aspartic acid and glutamic acid side chains form ionic bonds with Ca²⁺. The 2 Ca²⁺-binding sites in carboxyl-terminal part of molecule have a 10-fold higher affinity for Ca²⁺ than the two in amino-terminal part. In solution, the molecule is flexible, displaying a range of forms, from extended to more compact. (B) The major structural change in Ca²⁺/calmodulin that occurs when it binds to a target protein.

Activation of CaM-kinase II.

CaM-kinase II is a protein complex of about 12 subunits. The subunits (α , β , γ and δ), are expressed in different proportions in different cell types.



CaM-kinase II has at least 2 remarkable properties that are related.

- 1st: it can function as a **molecular memory device**, switching to an active state when exposed to Ca²⁺/calmodulin and then remaining active even after the Ca²⁺ signal has decayed. This is because the kinase phosphorylates itself (*autophosphorylation*) as well as other cell proteins when it is activated by Ca²⁺/calmodulin. In its autophosphorylated state, the enzyme remains active even in absence of Ca²⁺, thereby prolonging duration of kinase activity beyond that of initial activating Ca²⁺ signal. CaM-kinase II activation can thereby serve as a memory trace of a prior Ca²⁺ pulse, and has an important role in some types of memory and learning in the vertebrate nervous system.

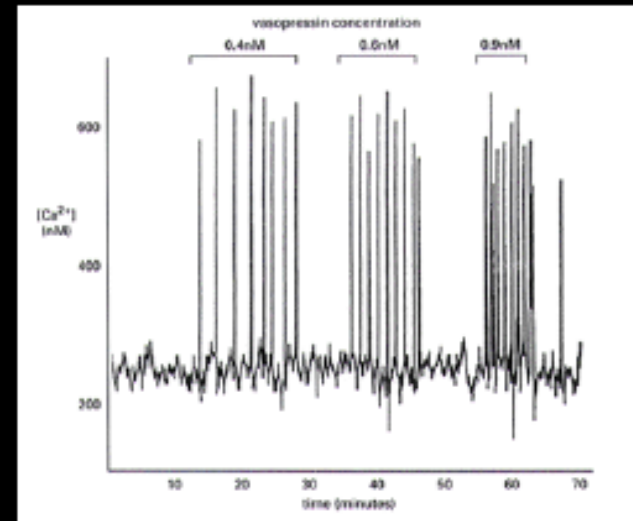
- 2nd remarkable property of CaM-kinase II : it can use its memory mechanism to **act as a frequency decoder of Ca²⁺ oscillations**. This property is especially important at nerve cell synapse, where changes in intracellular Ca²⁺ levels in activated postsynaptic cell can lead to long-term changes in subsequent effectiveness of that synapse.

Frequency of calcium oscillations influences a cell's response

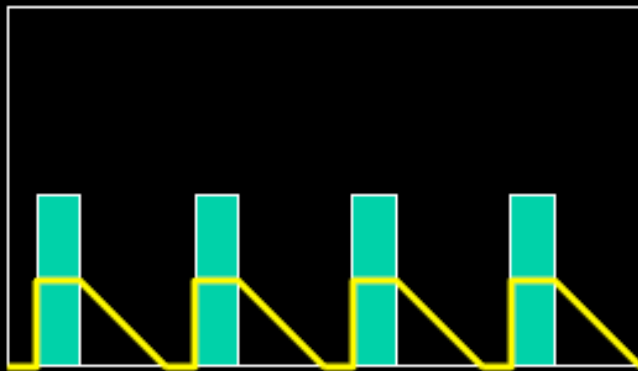
CaM-kinase uses memory mechanism to decode frequency of calcium spikes.

Requires the ability of the kinase to stay active after calcium drops. This is accomplished by autophosphorylation.

Alberts 15-39,42

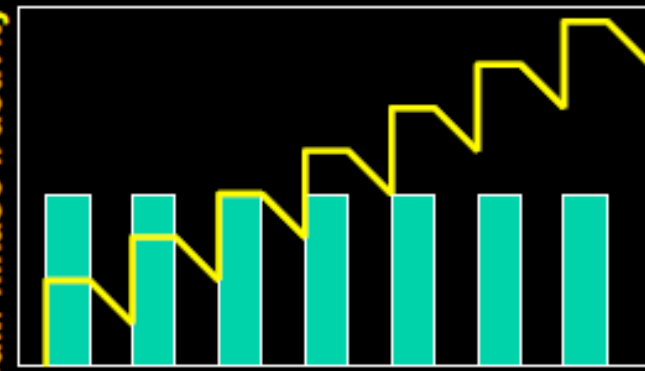


CaM-kinase II activity



Low frequency Ca²⁺ oscillations

CaM-kinase II activity



High frequency Ca²⁺ oscillations

(Note: All the original contributors of the concept and findings published elsewhere are gratefully acknowledged while preparing the E-content for the purpose of student reading material in convenient form for biochemistry and allied discipline).

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