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SUBJECT NAME	**CELL BIOLOGY**
PROGRAM NAME	**FOR M.Sc. And Ph.D. STUDENTS**
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SUBTOPIC NAME	**Signal transduction : Calcium signaling**
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SEARCH KEYWORDS	**Calcium signaling**

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 (~10^{-7} M), whereas
-its concentration in the extracellular fluid (~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.

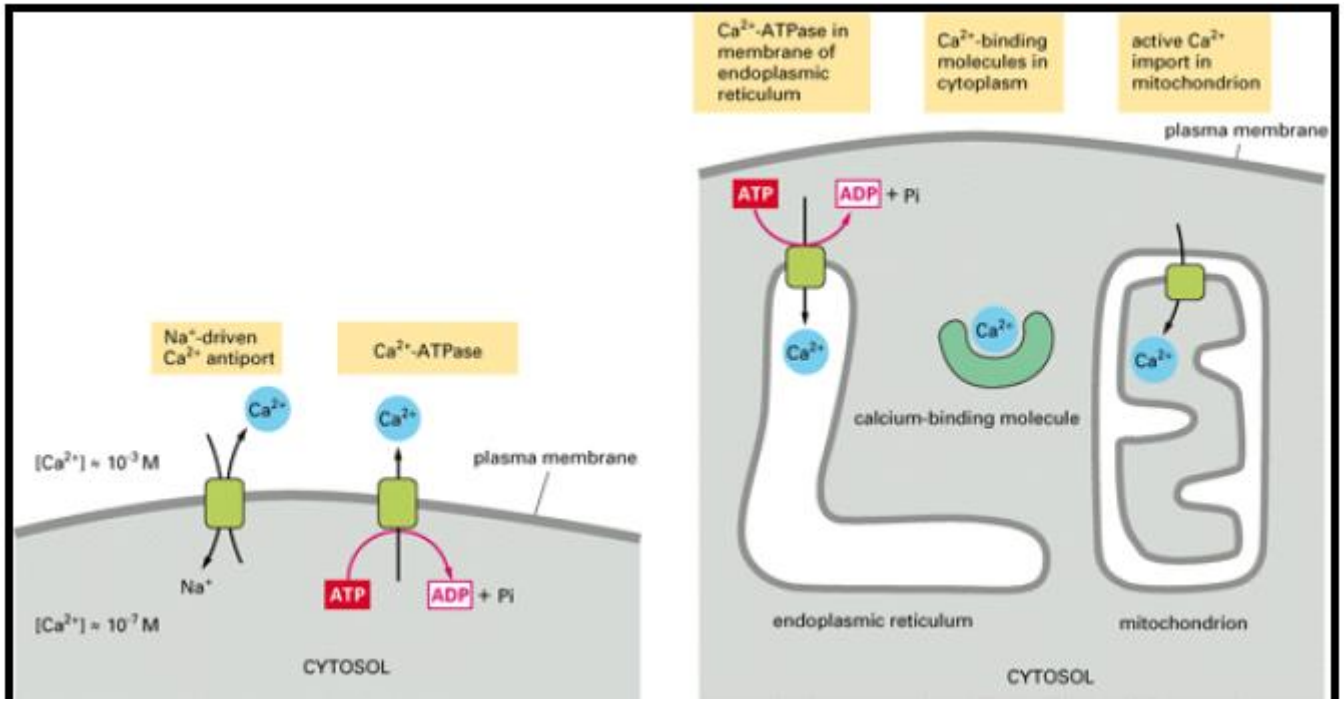
•When a signal transiently opens Ca^{2+} channels in either of these membranes, Ca^{2+} rushes into the cytosol, increasing the local Ca^{2+} concentration by 10-20 fold and triggering Ca^{2+}-responsive proteins in the cell.

•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. *IP3 -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.

Ca^{2+} concentration in cytosol is kept low in resting cells by several mechanisms.

Eukaryotic cells have:

- A *Ca^{2+}-pump in their PM* that uses energy from ATP hydrolysis to pump Ca^{2+} out of cytosol.
- An additional *Ca^{2+} transport protein (exchanger) in PM* that couples efflux of Ca^{2+} to influx of Na^{+}, on PM of cells that extensively use Ca^{2+} signaling such as muscle and nerve cells.
- A *Ca^{2+} pump in ER membrane* also has an important role in keeping cytosolic Ca^{2+} concentration low: this Ca^{2+}-pump enables ER to take up large amounts of Ca^{2+} from cytosol against a steep concentration gradient, even when Ca^{2+} levels in cytosol are low.
- A low-affinity, high-capacity *Ca^{2+} pump in the inner mitochondrial membrane* has an important role in returning the Ca^{2+} concentration to normal after a Ca^{2+} signal; it uses the electrochemical gradient generated across this membrane during electron-transfer steps of oxidative phosphorylation to take up Ca^{2+} from cytosol.



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

Ca^{2+}-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 Ca^{2+} signal is often seen to be small and localized to one or more discrete regions of the cell. These signals have been called Ca^{2+} blips, quarks, puffs, or sparks, and they are thought to reflect the local opening of individual (or small groups of) Ca^{2+}-release channels in the ER and to represent elementary Ca^{2+} 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 Ca^{2+} "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 Ca^{2+} on both the IP3-gated Ca^{2+}-release channels and the ryanodine receptors: the released Ca^{2+} initially stimulates more Ca^{2+} release.But then, as its concentration gets high enough, the Ca^{2+} inhibits further release. The frequency of the Ca^{2+} 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.

Ca^{2+}/Calmodulin-dependent Protein Kinases (CaMKinases) Mediate Actions of Ca^{2+} within Animal Cells

- *Ca^{2+}-binding proteins serve as transducers of the cytosolic Ca^{2+} signal.*
- Ist such protein discovered was *troponin C in skeletal muscle cells* (role in muscle contraction).
- A closely related Ca^{2+}-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 Ca^{2+} receptor***, mediating many Ca^{2+}-regulated processes. It consists of a highly conserved, single polypeptide chain with four high-affinity Ca^{2+}-binding sites. When activated by binding Ca^{2+}, it undergoes a conformational change. Because two or more Ca^{2+} ions must bind before calmodulin adopts its active conformation, the protein responds in a switchlike manner to increasing concentrations of Ca^{2+}: a tenfold increase in Ca^{2+} 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 Ca^{2+} enables calmodulin to bind to various target proteins in the cell to alter their activity.
- When an activated molecule of Ca^{2+}/calmodulin binds its target protein, it undergoes a marked change in conformation. Many targets as enzymes and membrane transport proteins calmodulin binding. For example, Ca^{2+}/calmodulin binds to and activates PM Ca^{2+}-pump that pumps Ca^{2+} out of cells. Thus, whenever Ca^{2+} concentration in cytosol rises, the pump activates, and restores normal cytosolic Ca^{2+} levels.

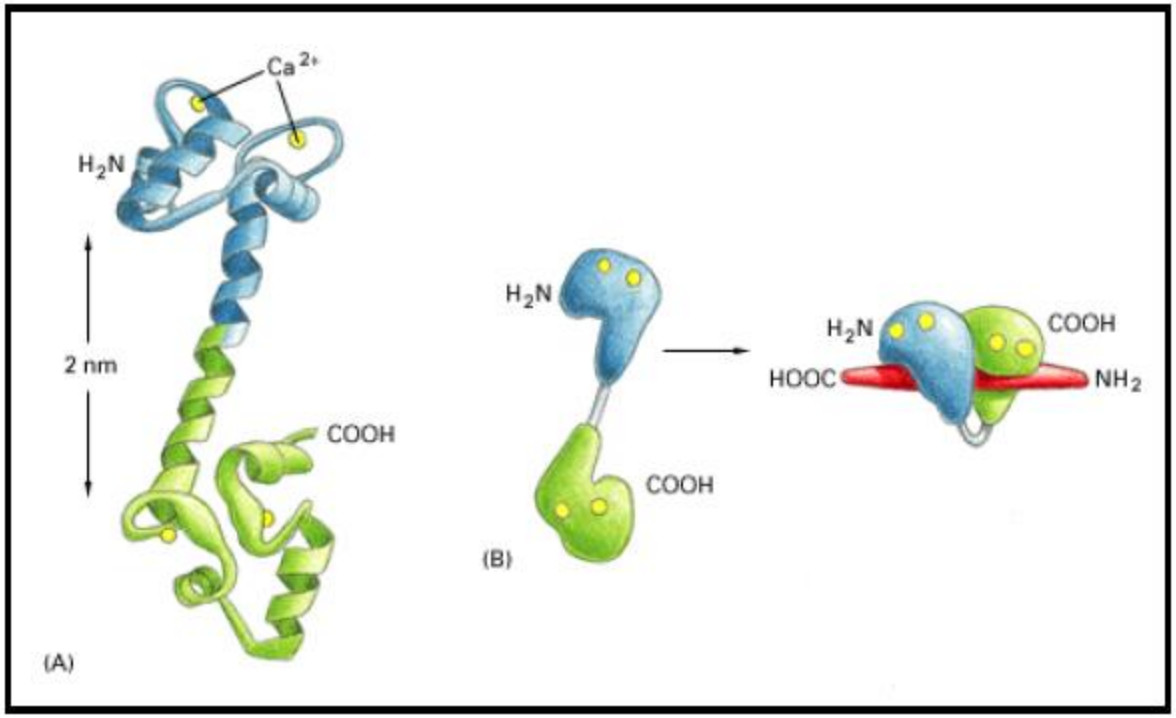
•Many effects of Ca^{2+}, are more indirect and are mediated by protein phosphorylations catalyzed by a family of **Ca^{2+}/calmodulin-dependent protein kinases (CaM-kinases).** These kinases, just like PKA and PKC, **phosphorylate serines or threonines** in proteins, and, as with PKA and PKC, the response 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.

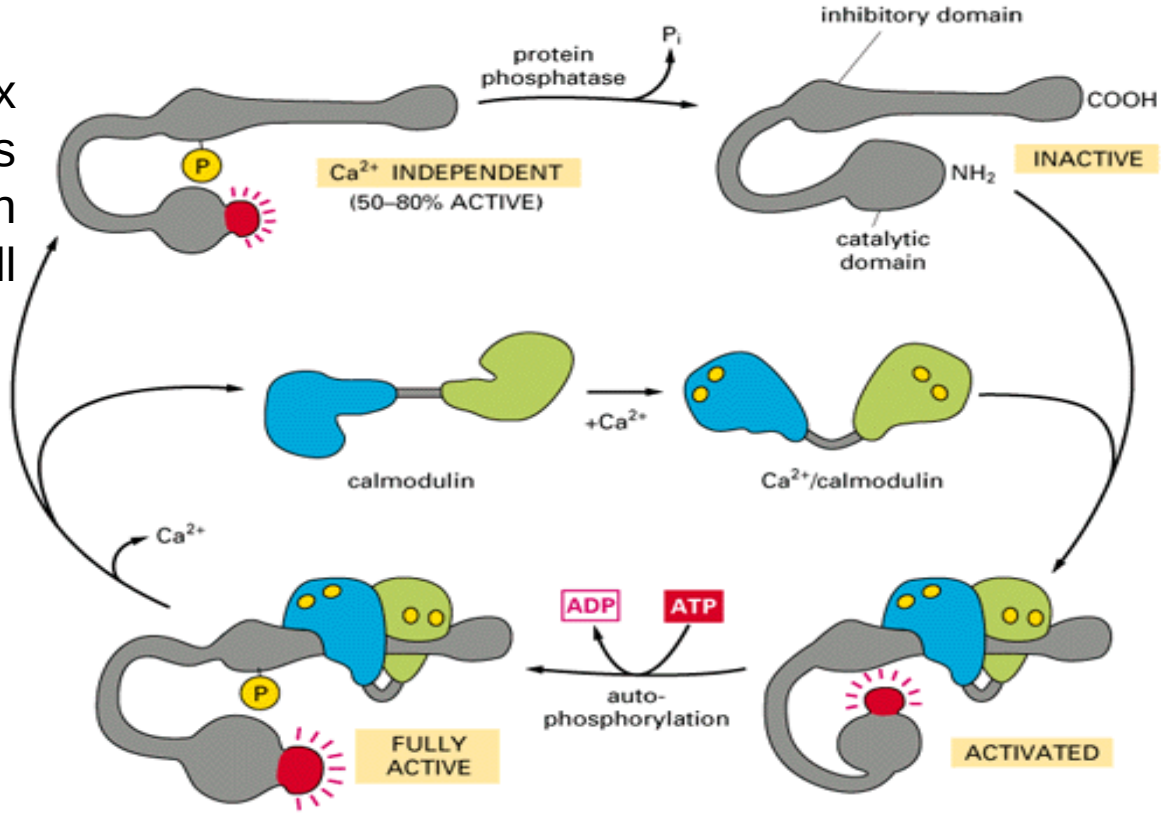
•The best-studied example of such a *multifunctional CaM-kinase is* ***CaM-kinase*** **II**, which is found in all animal cells and is especially enriched in the nervous system.
•It constitutes up to 2% of the total protein mass in some regions of brain, and it is highly concentrated in synapses.



The structure of Ca^{2+}/calmodulin. (A) The molecule has a "dumbbell" shape, with 2 globular ends connected by a long, exposed α-helix. Each end has 2 Ca^{2+}-binding domains, each with a loop of 12 amino acids, in which aspartic acid and glutamic acid side chains form ionic bonds with Ca^{2+}. The 2 Ca^{2+}-binding sites in carboxyl-terminal part of molecule have a 10-fold higher affinity for Ca^{2+} 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 Ca^{2+}/calmodulin that occurs when it binds to a target protein.

Activation of CaM-kinase II.

CaM-kinaseII 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.

•Ist: it can function as a **molecular memory device**, switching to an active state when exposed to Ca^{2+}/calmodulin and then remaining active even after the Ca^{2+} signal has decayed. This is because the kinase phosphorylates itself (*autophosphorylation) as well as other cell proteins when it is activated by* Ca^{2+}/calmodulin. In its autophosphorylated state, the enzyme remains active even in absence of Ca^{2+}, thereby prolonging duration of kinase activity beyond that of initial activating Ca^{2+} signal. CaM-kinase II activation can thereby *serve as a memory trace of a prior* Ca^{2+} *pulse*, and has an important role in some types of memory and learning in the vertebrate nervous system.

•IInd 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 Ca^{2+} levels in activated postsynaptic cell can lead to long-term changes in subsequent effectiveness of that synapse.

(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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