Cell signaling
Chapter 15

Signaling Through G-Protein-Coupled Receptors

Ca2+ Functions as a Ubiquitous Intracellular Mediator

- Many extracellular signals, and not just those that work via G proteins, trigger an increase in cytosolic Ca^{2+} concentration.
- In muscle cells, Ca^{2+} triggers **contraction**, and in many secretory cells, including nerve cells, it triggers **secretion**.
- Ca^{2+} has numerous other functions in a variety of cell types.

- Ca^{2+} is such an effective signaling mediator because its concentration in the cytosol is normally very low (~10^{-7} M), whereas its concentration in the extracellular fluid (~10^{-3} M) and in the lumen of the ER [and sarcoplasmic reticulum (SR) in muscle] is high. Thus, there is a large gradient tending to drive Ca^{2+} into the cytosol across both the plasma membrane and the ER or SR membrane.

- When a signal transiently opens **Ca^{2+} channels** in these membranes, Ca^{2+} rushes into the cytosol, and the resulting 10–20-fold increase in the local Ca^{2+} concentration activates **Ca^{2+}-responsive proteins** in the cell.

- ✓ Some stimuli, including membrane depolarization, membrane stretch, and certain extracellular signals, activate Ca^{2+} channels in the plasma membrane, resulting in Ca^{2+} influx from outside the cell.
- ✓ Other signals, including the GPCR-mediated signals, act primarily through **IP3 receptors** to stimulate Ca^{2+} release from intracellular stores in the ER.
- ✓ The ER membrane also contains a second type of regulated Ca^{2+} channel called the **ryanodine receptor** (so called because it is sensitive to the plant alkaloid ryanodine), which opens in response to rising Ca^{2+} levels and thereby amplifies the Ca^{2+} signal.

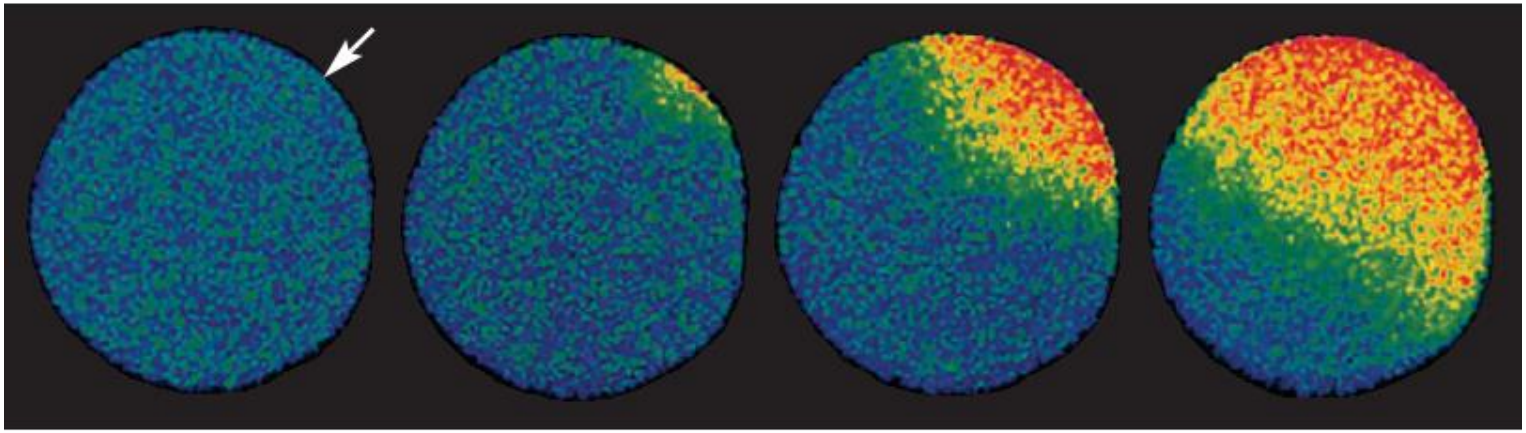
Several mechanisms rapidly terminate the Ca^{2+} signal and are also responsible for keeping the concentration of Ca^{2+} in the cytosol low in resting cells:
Most importantly, there are **Ca^{2+}-pumps** in the plasma membrane and the ER membrane that use the energy of ATP hydrolysis to pump Ca^{2+} out of the cytosol.
Cells such as muscle and nerve cells, which make extensive use of Ca^{2+} signaling, have an additional **Ca^{2+} transporter (a Na^{+}-driven Ca^{2+} exchanger)** in their plasma membrane that couples the efflux of Ca^{2+} to the influx of Na^{+}.

Feedback Generates Ca2+ Waves and Oscillations

- The IP3 receptors and ryanodine receptors of the ER membrane have an important feature: they are both stimulated by low to moderate cytoplasmic Ca^{2+} concentrations.

- This **Ca^{2+}-induced calcium release (CICR)** results in positive feedback, which has a major impact on the properties of the Ca^{2+} signal.

- The importance of this feedback is seen clearly in studies with Ca^{2+}-sensitive fluorescent indicators, such as **aequorin** or **fura-2**, which allow researchers to monitor cytosolic Ca^{2+} in individual cells under a microscope.



The fertilization of an starfish egg by a sperm triggers a wave of cytosolic Ca2+. This starfish egg was injected with a Ca^{2+}-sensitive fluorescent dye before it was fertilized. A wave of cytosolic Ca^{2+} (red), released from the ER, sweeps across the egg from the site of sperm entry (arrow). This Ca^{2+} wave changes the egg cell surface, preventing the entry of other sperm, and it also initiates **embryonic development**. The initial increase in Ca^{2+} is thought to be caused by a sperm-specific form of PLC (PLCζ) that the sperm brings into the egg cytoplasm when it fuses with the egg; the PLCζ cleaves PI(4,5)P2 to produce IP3, which releases Ca^{2+} from the egg ER. The released Ca^{2+} stimulates further Ca^{2+} release from the ER, producing the spreading wave.

Feedback Generates Ca2+ Waves and Oscillations

- When cells carrying a Ca^{2+} indicator are treated with a small amount of an extracellular signal molecule that stimulates IP3 production, tiny bursts of Ca^{2+} are seen in one or more discrete regions of the cell.

- These Ca^{2+} puffs or sparks reflect the **local opening of small groups of IP3-gated Ca^{2+} -release channels** in the ER.

- Because various Ca^{2+}-binding proteins act as **Ca^{2+} buffers** and **restrict the diffusion of Ca^{2+}**, the signal often remains localized to the site where the Ca^{2+} enters the cytosol.

- If the extracellular signal is **sufficiently strong and persistent**, however, the local Ca^{2+} concentration can reach a **sufficient level to activate nearby IP3 receptors** and **ryanodine receptors**, resulting in a **regenerative wave of Ca^{2+} release** that moves through the cytosol, much like an action potential in an axon.

Positive and negative feedback produce Ca2+ waves and oscillations

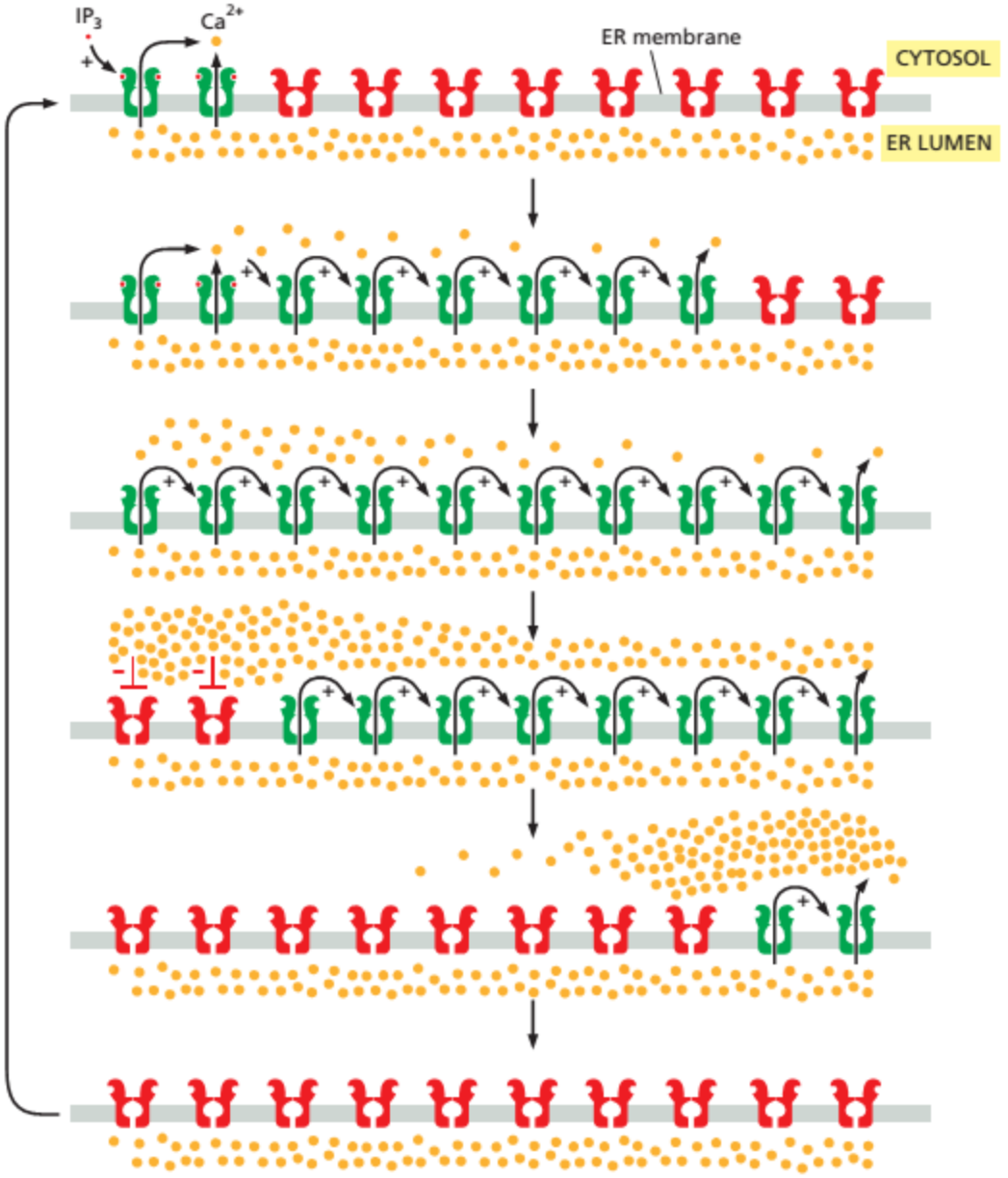
This diagram shows IP3 receptors and ryanodine receptors on a portion of the ER membrane: active receptors are in **green**; inactive receptors are in **red**.

When a small amount of cytosolic IP3 activates a cluster of IP3 receptors at one site on the ER membrane (top), the local release of Ca^{2+} promotes the opening of nearby IP3 and ryanodine receptors, resulting **in more Ca^{2+} release**.

This positive feedback (indicated by positive signs) produces a **regenerative wave** of Ca^{2+} release that spreads across the cell.

These waves of Ca^{2+} release move more quickly across the cell than would be possible by simple diffusion.

Also, unlike a diffusing burst of Ca^{2+} ions, which will become more dilute as it spreads, the regenerative wave produces a **high Ca^{2+} concentration** across the entire cell.



Positive and negative feedback produce Ca2+ waves and oscillations

Eventually, the local Ca^{2+} concentration inactivates IP3 receptors and ryanodine receptors (middle; indicated by red negative signs), shutting down the Ca^{2+} release. Ca^{2+}-pumps reduce the local cytosolic Ca^{2+} concentration to its normal low levels.

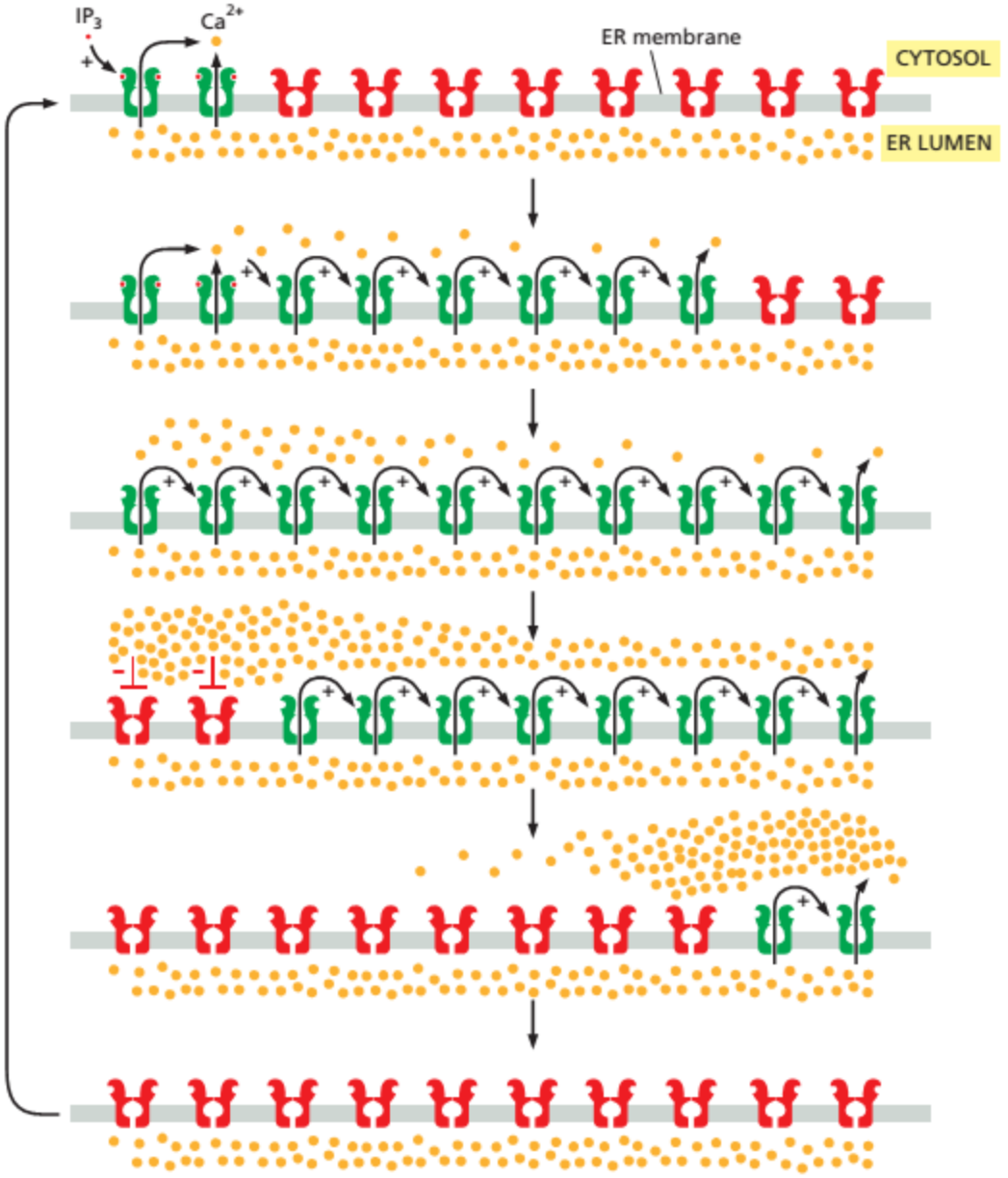
The result is a **Ca^{2+} spike**:
positive feedback drives a rapid rise in cytosolic Ca^{2+}, and
negative feedback sends it back down again.

The Ca^{2+} channels remain refractory to further stimulation for some period of time, delaying the generation of another Ca^{2+} spike (bottom).

Eventually, the negative feedback wears off, allowing IP3 to trigger another Ca^{2+} wave.

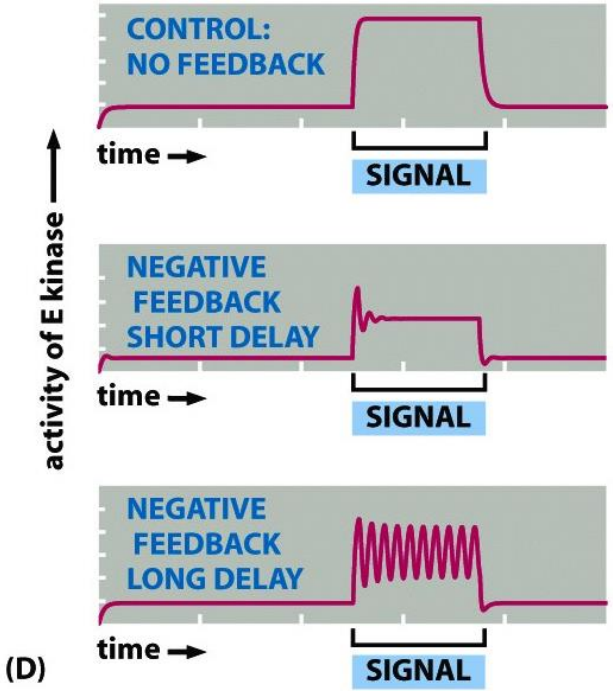
The end result is repeated Ca^{2+} oscillations.

Under some conditions, these oscillations can be seen as repeating narrow waves of Ca^{2+} moving across the cell.



Positive and negative feedback produce Ca2+ waves and oscillations

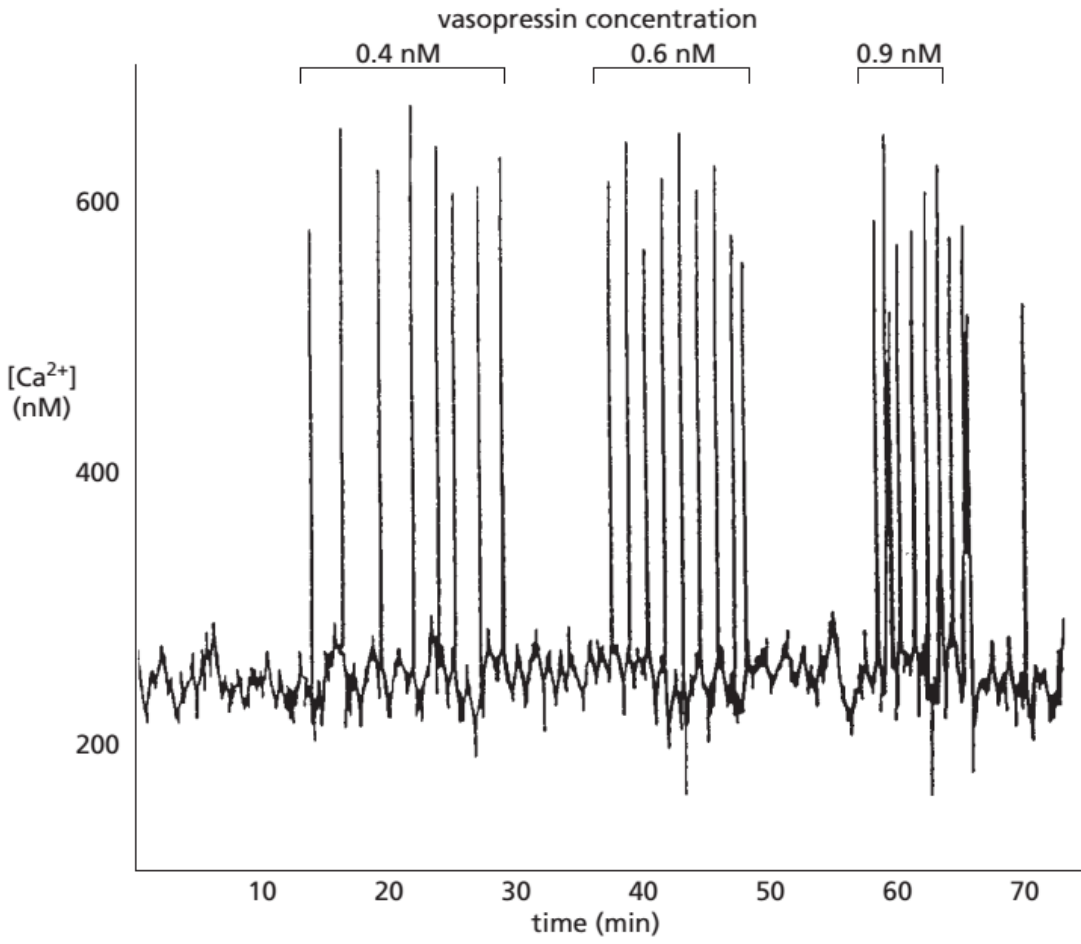
- Another important property of IP3 receptors and ryanodine receptors is that they are **inhibited**, after some delay, by **high** Ca^{2+} **concentrations** (a form of negative feedback):
- Thus, the rise in Ca^{2+} in a stimulated cell leads to inhibition of Ca^{2+} release;
- because **Ca^{2+} pumps** remove the cytosolic Ca^{2+}, the Ca^{2+} concentration falls.
- The decline in Ca^{2+} eventually relieves the negative feedback, allowing cytosolic Ca^{2+} to rise again.
- As in other cases of **delayed negative feedback**, the result is an oscillation in the Ca^{2+} concentration.



Positive and negative feedback produce Ca2+ waves and oscillations

These oscillations persist for as long as receptors are activated at the cell surface, and their frequency reflects the strength of the extracellular stimulus.

The frequency, amplitude, and breadth of oscillations can also be modulated by other signaling mechanisms, such as **phosphorylation**, which influence the **Ca^{2+} sensitivity of Ca^{2+} channels** or affect **other components** in the signaling system.



Vasopressin-induced Ca^{2+} oscillations in a liver cell. The cell was loaded with the Ca^{2+}-sensitive protein aequorin and then exposed to increasing concentrations of the peptide signal molecule vasopressin, which activates a GPCR and thereby PLCβ.
Note that the frequency of the Ca^{2+} spikes increases with an increasing concentration of vasopressin but that the amplitude of the spikes is not affected. Each spike lasts about 7 seconds.

Positive and negative feedback produce Ca2+ waves and oscillations

The frequency of Ca^{2+} oscillations can be translated into a frequency-dependent cell response:

-In some cases, the frequency-dependent response itself is also oscillatory: in hormone-secreting pituitary cells, for example, stimulation by an extracellular signal induces repeated Ca^{2+} spikes, each of which is associated with a burst of hormone secretion.

-In other cases, the frequency-dependent response is non-oscillatory: in some types of cells, for instance, one frequency of Ca^{2+} spikes activates the transcription of one set of genes, while a higher frequency activates the transcription of a different set.

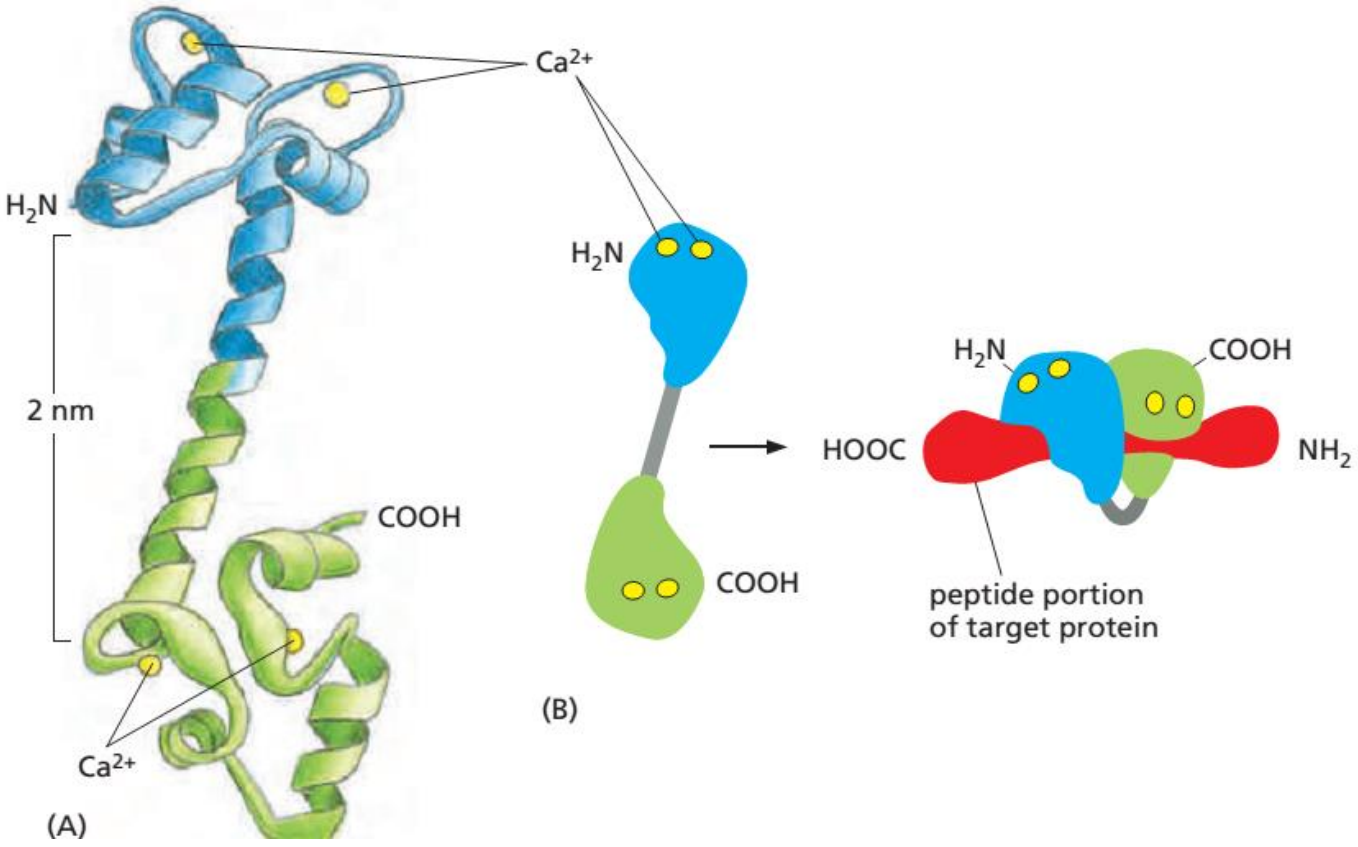
How do cells sense the frequency of Ca^{2+} spikes and change their response accordingly?

The mechanism presumably depends on **Ca^{2+}-sensitive proteins** that change their activity as a function of Ca^{2+}-spike frequency.

A protein kinase that acts as a **molecular memory device** seems to have this remarkable property.

Ca^{2+} /Calmodulin-Dependent Protein Kinases Mediate Many Responses to Ca^{2+} Signals

- Various Ca^{2+}-binding proteins help to relay the cytosolic Ca^{2+} signal.
- The most important is **calmodulin**, which is found in all eukaryotic cells and can constitute as much as 1% of a cell's total protein mass.
- Calmodulin functions as a multipurpose intracellular Ca^{2+} receptor, governing 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 Ca^{2+} binding, it undergoes a **conformational change**.
- Because two or more Ca^{2+} ions must bind before calmodulin adopts its active conformation, the protein displays a **sigmoidal response** to increasing concentrations of Ca^{2+}.



Ca^{2+} /Calmodulin-Dependent Protein Kinases Mediate Many Responses to Ca^{2+} Signals

- The allosteric activation of calmodulin by Ca^{2+} is analogous to the activation of PKA by cyclic AMP, except that Ca^{2+} /calmodulin has no enzymatic activity itself but instead acts by binding to and activating other proteins.

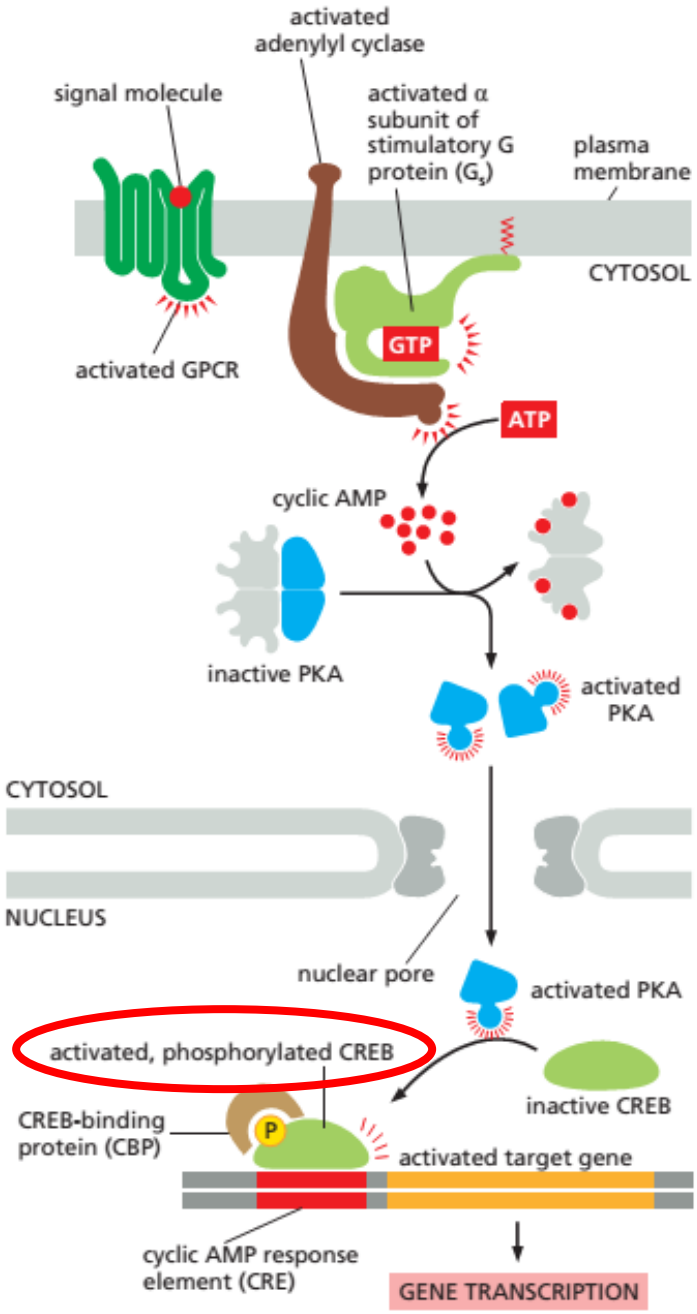
- In some cases, calmodulin serves as a permanent regulatory subunit of an enzyme complex, but usually the binding of Ca^{2+} instead enables calmodulin to bind to various target proteins in the cell to alter their activity.

- When an activated molecule of Ca^{2+}/calmodulin binds to its target protein, the calmodulin further changes its conformation, the nature of which depends on the specific target protein.

- Among the many targets calmodulin regulates are **enzymes** and **membrane transport proteins**:
- As one example, Ca^{2+}/calmodulin binds to and activates the plasma membrane Ca^{2+}-pump that uses ATP hydrolysis to pump Ca^{2+} out of cells. Thus, whenever the concentration of Ca^{2+} in the cytosol rises, the pump is activated, which helps to return the cytosolic Ca^{2+} level to resting levels.

- Many effects of Ca^{2+}, however, are more indirect and are mediated by **protein phosphorylations** catalyzed by a family of protein kinases called **Ca^{2+}/calmodulin-dependent kinases (CaM-kinases).**

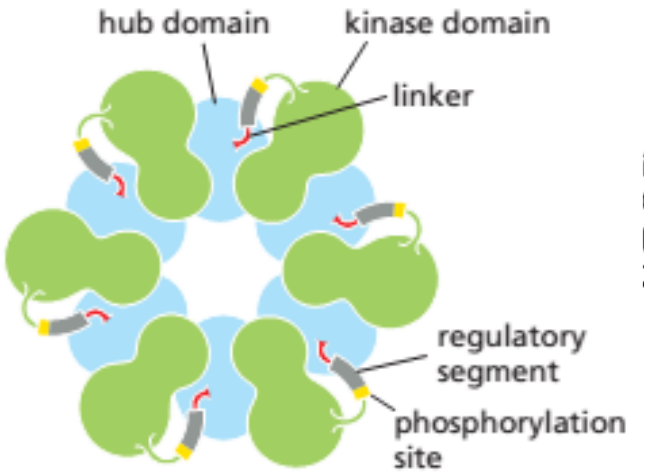
- Some CaM-kinases phosphorylate transcription regulators, such as the CREB protein and in this way **activate or inhibit the transcription of specific genes**.


activated
adenylyl cyclase
signal molecule
activated α
subunit of
stimulatory G
protein (G_s)
plasma
membrane
CYTOSOL
GTP
activated GPCR
ATP
cyclic AMP
inactive PKA
activated
PKA
CYTOSOL
NUCLEUS
nuclear pore
activated PKA
activated, phosphorylated CREB
inactive CREB
CREB-binding
protein (CBP)
P
activated target gene
cyclic AMP response
element (CRE)
GENE TRANSCRIPTION

Ca^{2+} /Calmodulin-Dependent Protein Kinases Mediate Many Responses to Ca^{2+} Signals

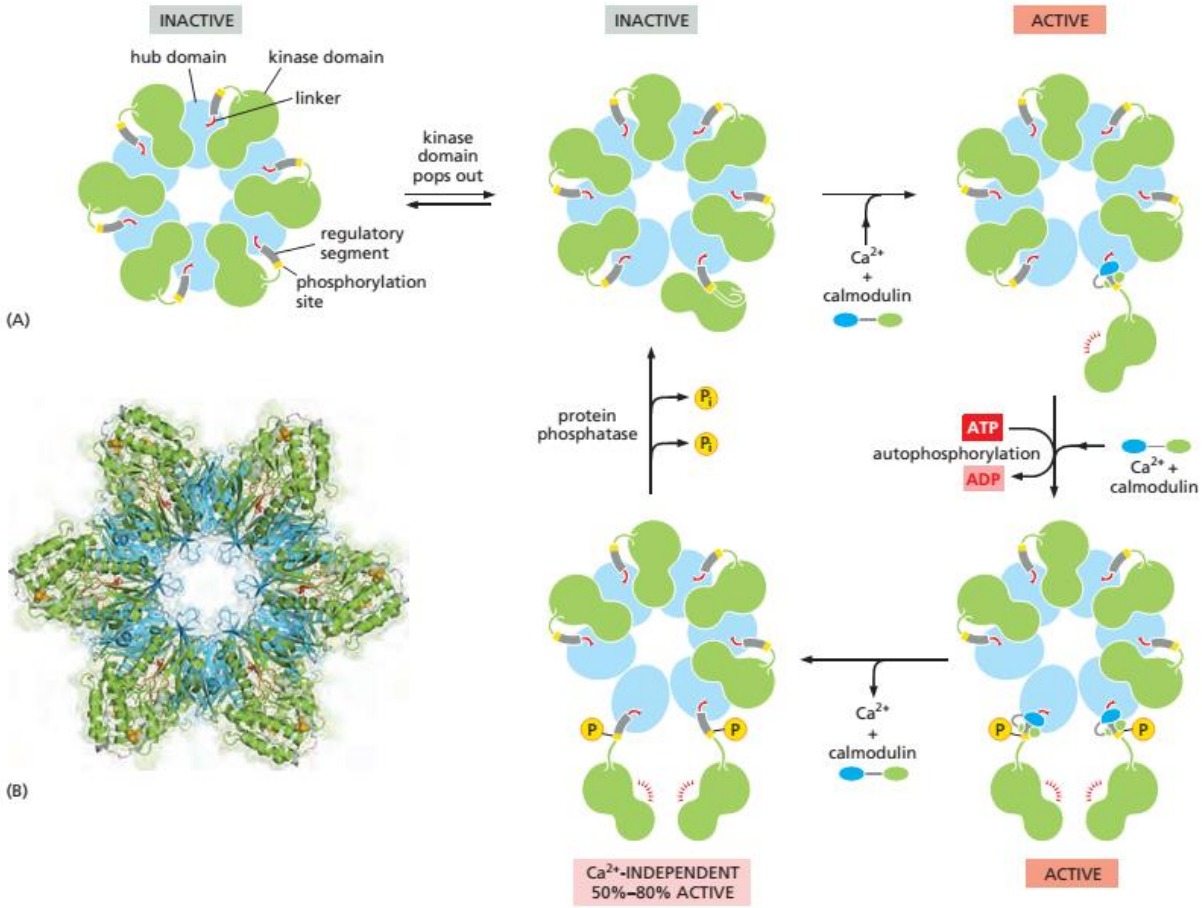
- One of the best-studied CaM-kinases is **CaM-kinase II**, which is found in most animal cells but is especially enriched in the nervous system.
- It constitutes up to 2% of the total protein mass in some regions of the brain, and it is highly concentrated in **synapses**.
- CaM-kinase II has several remarkable properties.
- It has a **spectacular quaternary structure**: twelve copies of the enzyme are assembled into a stacked pair of rings, with kinase domains on the outside linked to a central hub.

-Each CaM-kinase II protein has two major domains: an amino-terminal kinase domain (**green**) and a carboxyl-terminal hub domain (**blue**), linked by a **regulatory segment**.
-Six CaM-kinase II proteins are assembled into a giant ring in which the hub domains interact tightly to produce a central structure that is surrounded by kinase domains.
-The complete enzyme contains two stacked rings, for a total of 12 kinase proteins, but only one ring is shown here for clarity.



Ca^{2+} /Calmodulin-Dependent Protein Kinases Mediate Many Responses to Ca^{2+} Signals

- This structure helps the enzyme 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 adjacent kinase subunits can phosphorylate each other (a process called **autophosphorylation**) when Ca^{2+}/calmodulin activates them.
- Once a kinase subunit is autophosphorylated, it remains active even in the absence of Ca^{2+}, thereby prolonging the duration of the kinase activity beyond that of the initial activating Ca^{2+} signal.
- The enzyme maintains this activity until a protein **phosphatase** removes the autophosphorylation and shuts the kinase off.



The stepwise activation of CaM-kinase II

When the enzyme is inactive, the ring exists in a dynamic equilibrium between two states:

-The first (upper left) is a **compact state**, in which the kinase domains interact with the hub, so that the regulatory segment is buried in the kinase active site and thereby **blocks catalytic activity**.

-In the second inactive state (upper middle), a kinase domain has **popped out** and is linked to the central hub by its regulatory segment, **which continues to inhibit the kinase but is now accessible to Ca^{2+}/calmodulin**.

-If present, Ca^{2+}/calmodulin will bind the regulatory segment and prevent it from inhibiting the kinase, thereby locking the kinase in an active state (upper right).

-If the adjacent kinase subunit also pops out from the hub, it will also be activated by Ca^{2+}/calmodulin, and the two kinases will then phosphorylate each other on their regulatory segments (lower right).

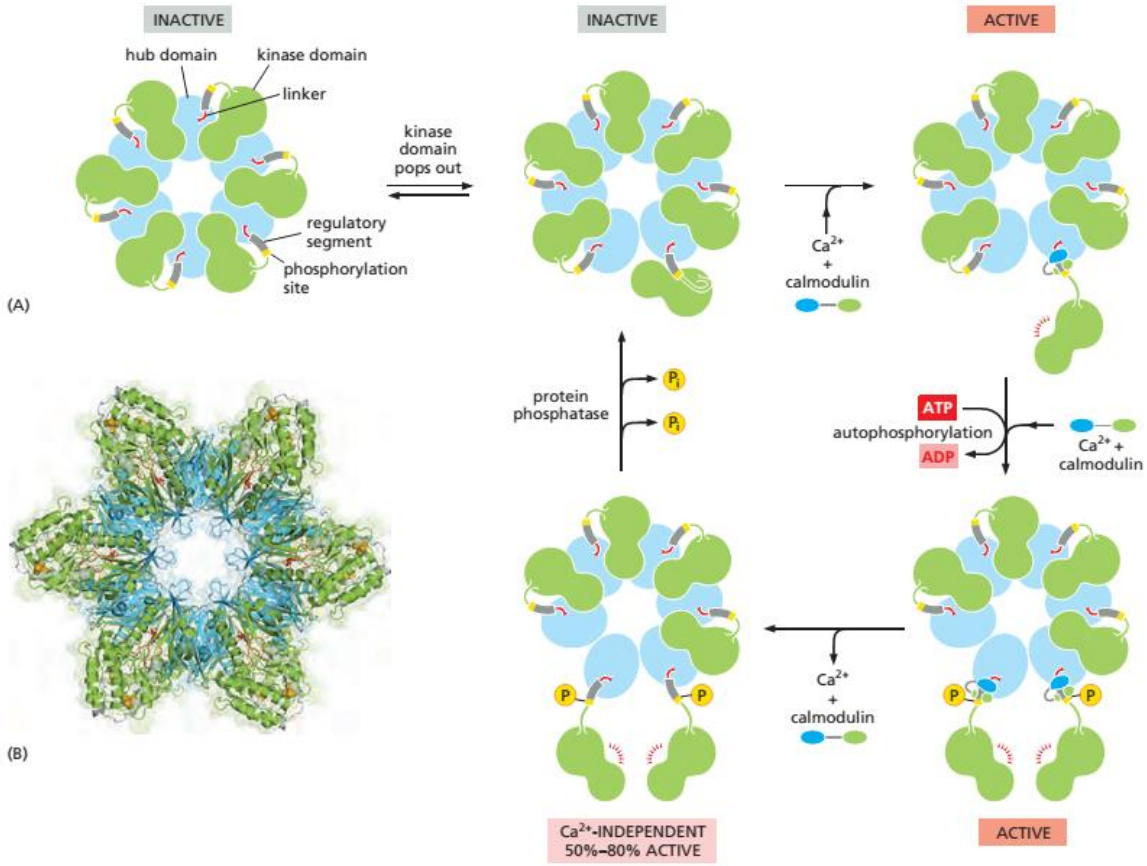
This **autophosphorylation** further activates the enzyme.

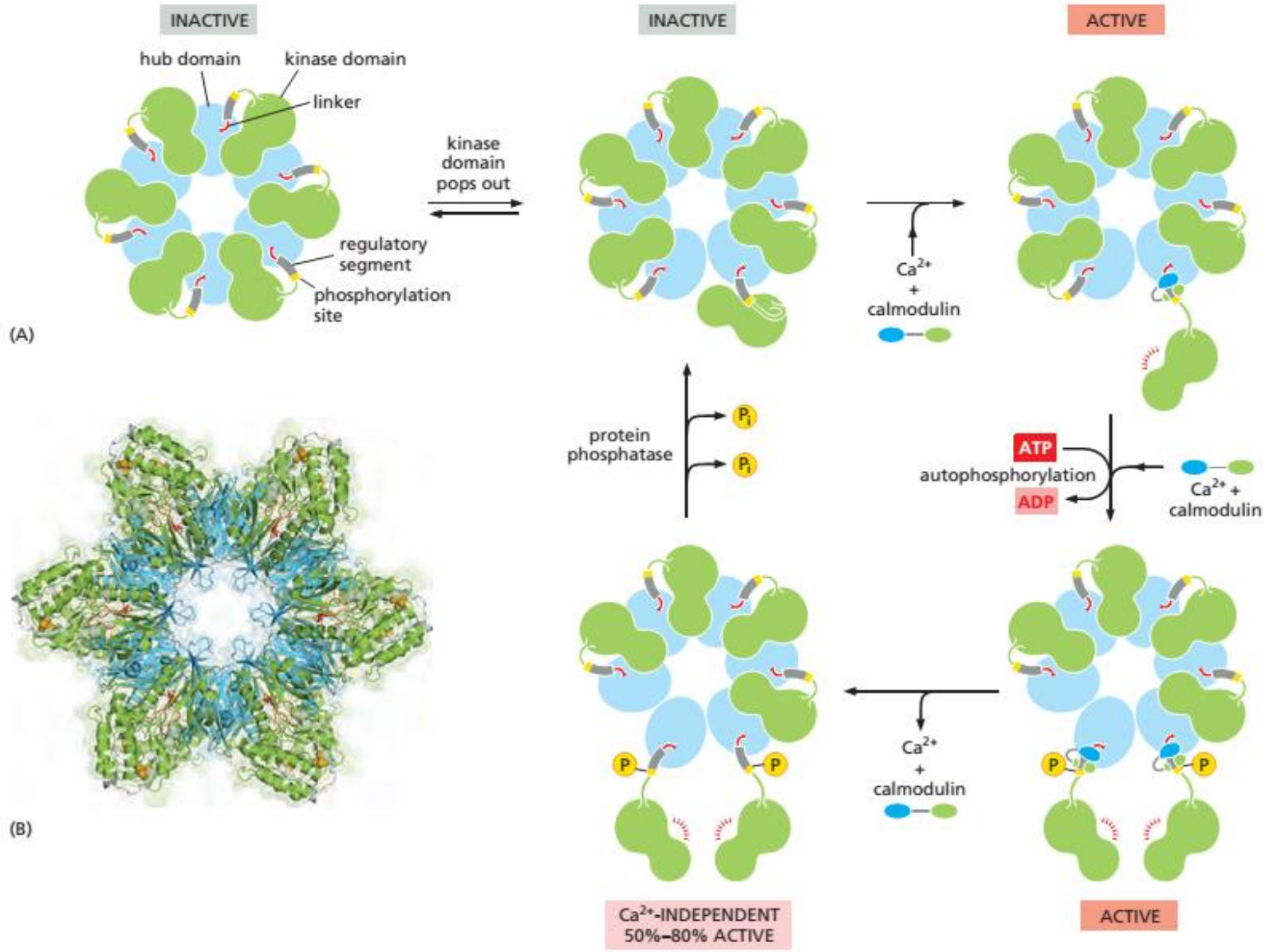
It also prolongs the activity of the enzyme in two ways:

-First, it traps the **bound Ca^{2+}/calmodulin** so that it does not dissociate from the enzyme until cytosolic Ca^{2+} levels return to basal values for at least 10 seconds (not shown).

-Second, it converts the enzyme to a **Ca^{2+}-independent form**, so that the kinase remains active even after the Ca^{2+}/calmodulin dissociates from it (lower left).

This activity continues until the action of a protein phosphatase overrides the autophosphorylation activity of CaM-kinase II.





The behavior of CaM-kinase II is also controlled by the length of the linker segment between the kinase and hub domains. The linker is longer in some isoforms of the enzyme; in these isoforms, the kinase domains tend to pop out of the ring more frequently, making it more sensitive to Ca^{2+}.

These and other mechanisms allow the cell to tailor the responsiveness of the enzyme to the needs of different types of neurons.

Ca^{2+} /Calmodulin-Dependent Protein Kinases Mediate Many Responses to Ca^{2+} Signals

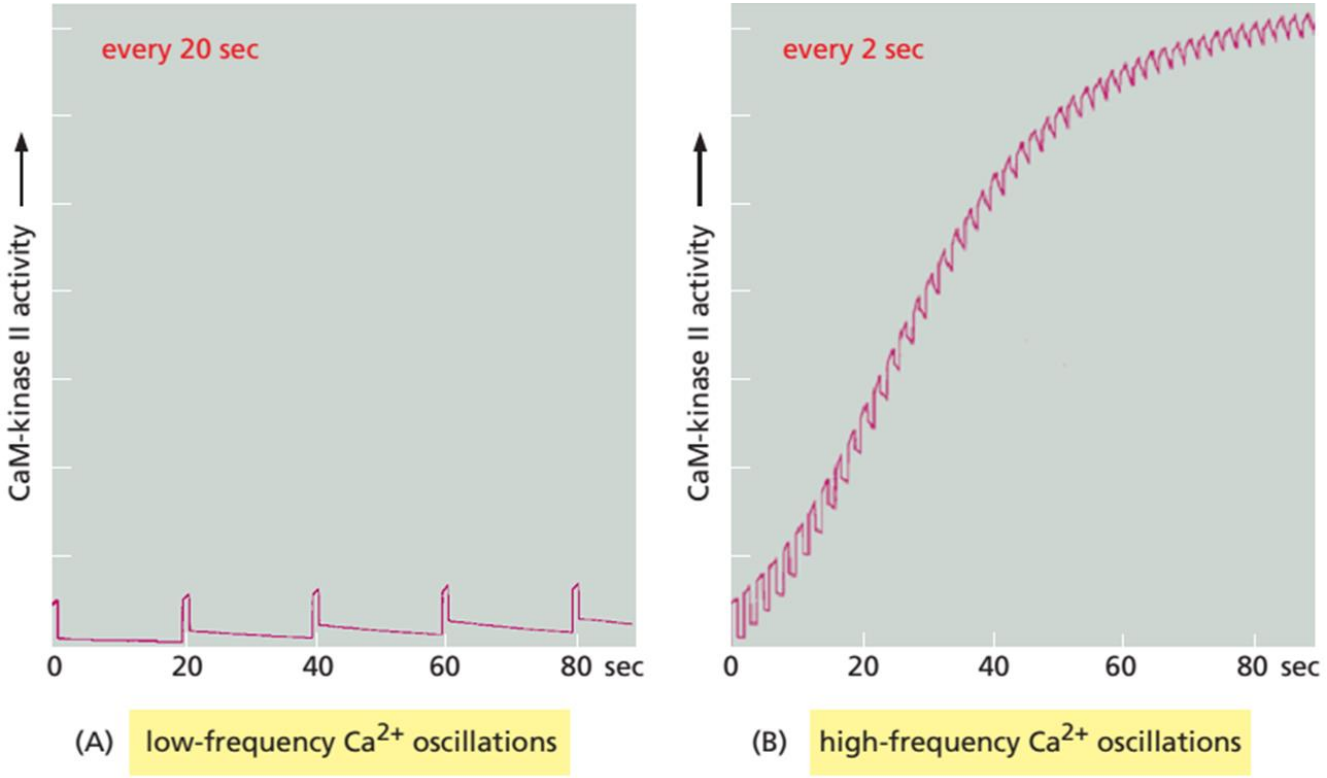
- CaM-kinase II activation can thereby serve as **a memory trace of a prior Ca^{2+} pulse**, and it seems to have a role in some types of memory and learning in the vertebrate nervous system.

Mutant mice that lack a brain-specific form of the enzyme have specific defects in their ability to remember where things are.

Another remarkable property of CaM-kinase II is that the enzyme can use its intrinsic memory mechanism to decode the frequency of Ca^{2+} oscillations:

-At **low frequencies of Ca^{2+}** spikes, the enzyme becomes inactive after each spike, as the autophosphorylation induced by Ca^{2+}/calmodulin binding does not maintain the enzyme's activity long enough for the enzyme to remain active until the next Ca^{2+} spike arrives.

-At **higher spike frequencies**, the enzyme fails to inactivate completely between Ca^{2+} spikes, so its activity ratchets up with each spike.



(A) low-frequency Ca^{2+} oscillations

(B) high-frequency Ca^{2+} oscillations

Ca^{2+} /Calmodulin-Dependent Protein Kinases Mediate Many Responses to Ca^{2+} Signals

- If the spike frequency is high enough, this progressive increase in enzyme activity will continue until the enzyme is autophosphorylated on all subunits and is therefore maximally activated.

- Although not shown, once enough of its subunits are autophosphorylated, the enzyme can be maintained in a highly active state even with a relatively low frequency of Ca^{2+} spikes (**a form of cell memory**).

- The binding of Ca^{2+}/ calmodulin to the enzyme is enhanced by the CaM-kinase II autophosphorylation (**an additional form of positive feedback**), helping to generate a more switchlike response to repeated Ca^{2+} spikes.