Calcium-related proteins involving signal transduction pathways

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Abstract

Calcium plays various roles in cellular functioning. Besides well-known functions as osteogenesis, muscle contraction and blood coagulation, calcium is essential for intracellular and intercellular signal transduction, which deals with almost every cell, including neural cells. Fluorescent dyes are used for quantification of intracellular calcium concentration. Calcium is absorbed from the small intestine by binding with calbindin and incorporated to cells via calcium channels. Intracellular calcium is mainly stored in the endoplasmic/sarcoplasmic reticulum. Stimulus to the cell such as a binding hormone with a hormone receptor on the cell membrane causes production of phosphatidylinositol-4,5-diphosphate which is cleaved into diacylglycerol and inositol-1,4,5-triphosphate (IP3) catalyzed by phospholipase C coupled with G protein. Diacylglycerol activates protein kinase C and IP3 binds with its receptor on the endoplasmic reticulum and releases calcium. Elevated intracytoplasmic calcium concentration activates calmodulin kinase.

Introduction

Various cell functions are supported by calcium. Classically known biological roles of calcium include bone and tooth formation (osteogenesis, odontogenesis), cardiac and skeletal muscle contraction and blood coagulation (required for activation of blood coagulation factors IX, X, III, V and XIII). In addition, calcium is involved in nerve conduction, activation of lymphocytes, regulation of hormone secretion as insulin, and is a crucial cofactor for numerous enzymes. Recent studies revealed that calcium is a principal messenger in signal transduction pathways. In signal transduction processes, numerous proteins and accompanying molecules participate in very strict order, and many of those proteins specifically bind with calcium ions in the cytoplasm. Knowledge concerning those cytoplasmic proteins and other related molecules which work with calcium ions is essential for better understanding of dynamic cell functions and those disorders. Experimentally, quantification of intracellular calcium is achieved using fluorescent dyes such as Quin-2, Fluo-3, Rhod-2, Indo-1 and Fura-2 as well as aequorin. Aequorin is a luminescent protein extracted from jellyfish Aequorea victoria, and was first discovered and extracted by Shimomura.

1. Regulation of intracellular calcium ion concentration

In eukaryotes, calcium ions enter the cytoplasm either from outside of the cell through the specific channel molecules on the cell membrane, or are supplied from some internal calcium storage sites, such as endoplasmic/sarcoplasmic reticulum and mitochondria.

In mammals, intracellular calcium concentration is regulated (lowered) by transport proteins that remove calcium ions from the cytoplasm. For example, the
sodium-calcium exchanger requires energy generated from the electrochemical gradient of sodium by exporting calcium out of the cell in exchange for the imported sodium. Energy required for the plasma membrane calcium ATPase to export cytoplasmic calcium out of the cell is produced by hydrolyzing adenosine triphosphate.

Calcium ions can damage cells if they enter the cytoplasm excessively. As for actual clinical examples, in neurodegenerative diseases or as sequelae of brain trauma or cerebral apoplexy, overexcitation of neural circuits called excitotoxicity may take place. Excessive entry of calcium into a cell occasionally causes apoptosis or cell necrosis. Substances such as excitatory neurotransmitter glutamate induce excitotoxicity. Glutamate facilitates calcium influx into a cell via N-methyl D-aspartate (NMDA) receptor. Excitotoxicity also occurs when alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are over-activated. Besides glutamate, substances binding these receptors as NMDA and kainic acid, can similarly cause excitotoxicity by influx of massive calcium ions to the cell. Calcium influx into cells causes activation of numerous intracellular enzymes that act as to damage cells, as phospholipases, endonucleases, and proteases.

a. Cell membrane

1) Voltage-dependent calcium channel (VDCC)

Voltage-dependent calcium channels (VDCC) are voltage-gated ion channels found in excitable cells (neurons, glial cells, muscle cells, etc.) that permit influx of calcium ion into cells. VDCC is solely activated by a depolarizing current and induces calcium influx under static membrane potential. This character is the source of the name “voltage-dependent.” VDCCs are involved in various cellular functions as the release of neurotransmitters and hormones, muscular contraction, excitation of neurons and gene expression. Another group of calcium channels are ligand-gated calcium channels such as inositol 1,4,5-triphosphate (IP3) receptor and ryanodine receptor on the endoplasmic/sarcoplasmic reticulum. Receptor-operated calcium channels or ligand-gated calcium channels has also been revealed on the cell membrane.

VDCCs are composed of a complex of heterogeneous subunits as α1, α2δ, β1-4, and γ. The α1 subunit forms the ion-conducting pore and other subunits associated have several functions including modulation of gating.

Types of high voltage-gated calcium channels include types L (long-lasting), P (Purkinje)/Q, N (neural), R (residual), and T (transient). Specific inhibitors against L-type channel, such as nifedipine, diltiazem and verapamil, inhibit calcium influx specifically and are therapeutically used for coronary vasodilators. The identical channel molecules may have a different function depending on the types of cells. The L-type calcium channel in the skeletal muscle transduces voltage change to ryanodine receptor as a voltage sensor, not act as a calcium channel. On the contrary, in cardiac muscle, the L-type calcium channel acts as a calcium channel in the usual manner, followed by calcium-induced calcium release. Those channel molecules are structurally homologous but not structurally identical. They can be precisely classified by studying their physiological roles and/or inhibition by specific toxins. Among high voltage-gated calcium channels, N-type channel is blocked by ω-conotoxins, while P/Q-type channel is blocked by ω-agatoxins. The dihydropyridine-sensitive L-type channels are involved in the contraction of skeletal, smooth, and cardiac muscle and in hormone secretion in endocrine cells.

2) Store-operated calcium channel (SOCCK)

Store-operated calcium channels are found in the cell membrane of any cells including endocrine cells. They incorporate extracellular calcium ion into the cytoplasm. The epithet “store-operated” is given because they are activated when calcium ion is depleted from intracellular calcium stores as the endoplasmic reticulum.

3) Calcium pump and sodium-calcium exchanger

Calcium-pump is occasionally confused with calcium channel. Essentially calcium-pump literally pumps out an excessive cytoplasmic calcium ion, whereas calcium channel incorporates calcium ion into cytoplasm. Since cell membrane itself is not permeable to calcium ion, calcium ion is incorporated solely through calcium channel. Conversely, cytoplasmic calcium ion is actively pumped out through calcium pump by using ATP. Alternative way to pump out calcium ion is via sodium-calcium exchanger (not be included in the type of pump that uses the energy, but powered by electrochemical gradient called secondary active transport) that exchange one calcium ion with three sodium ions. As a result, elevated cytoplasmic sodium concentration is compensated by transmembranous exchanging of sodium and potassium through sodium-potassium pump (elevated intracellular potassium concentration is not harmful to the cell). Calcium pumps are also found in endoplasmic reticulum, sarcoplasmic reticulum and mitochondria (described later).

b. Endoplasmic reticulum and sarcoplasmic reticulum

The sarcoplasmic reticulum is a special type of smooth endoplasmic reticulum found in myocytes. The only difference from the smooth endoplasmic reticulum is the protein components, otherwise, both bound to their membranes and drifting within their lumens. This difference correlates the difference among their functions, i.e. the smooth endoplasmic reticulum...
Table  Calcium-related proteins: Subcellular localization and their principal functions

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<td>calsequestrin</td>
<td>sarcoplasmic reticulum</td>
<td>retain calcium in sarcoplasmic reticulum</td>
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<td>calnexin</td>
<td>membrane of endoplasmic reticulum</td>
<td>retain unfolded or unassembled proteins in endoplasmic reticulum as a molecular chaperone</td>
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<td>annexin (= lipocortin)(A1, A2, A5)</td>
<td>cell membrane (occasionally secreted?)</td>
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<td>cadherin (E-, N-, P- and T-cadherins)</td>
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<td>osteocalcin, osteonectin and osteopontin</td>
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synthesizes protein molecules, while the sarcoplasmic reticulum stores and transports calcium ions. The sarcoplasmic reticulum possesses large amount of calcium in a sequestered manner, and releases it in case of cell depolarization⁹. This type of action is especially crucial for triggering muscle contraction. Ryanodine receptor (stated below) and IP3 receptors are known as calcium channels which release calcium ion from sarcoplasmic/endoplasmic reticulum to cytosol, while calcium pump reuptakes calcium ion from cytoplasm.

1) Ryanodine receptor
Ryanodine receptor is a receptor channel that is activated by calcium ion influxed from VDCCs. In skeletal muscles, under physiological conditions, the
channel is activated not by calcium ion but by "charge movement". The molecular mechanisms of "charge movement" are still unclear. Ryanodine is an alkaloid found in the South American plant Ryania speciosa and locks the ryanodine receptor in a half-open state at low concentration. Simultaneous release of calcium from numerous ryanodine receptors causes a rapid increment of cytoplasmic calcium concentration. This phenomenon is called calcium spark.

Disorders in ryanodine receptor cause diseases. Receptor 1 mutations may cause malignant hyperthermia (a specific antagonist, Dantrolene, is used for treatment) and central core disease. Receptor 2 mutations relate to stress-induced polymorphic ventricular tachycardia and arrhythmogenic right ventricular dysplasia. Anti-ryanodine receptor autoantibody has been found in the serum of patients with myasthenia gravis.

2) Inositol 1,4,5-triphosphate (IP3) receptor
IP3 induces calcium release also from the intracellular calcium store such as endoplasmic/sarcoplasmic reticulum through IP3 receptor which is a calcium channel and activated with IP3 synthesized via G-protein coupling pathways. This type of calcium release is called IP3-induced calcium release (ICR).

3) Calcium-pump
Sarcoplasmic reticulum calcium pump (sarcoplasmic calcium ATPase) reuptakes calcium ion that is previously released by myocytes during muscle contraction into the sarcoplasmic reticulum using energy provided by ATP hydrolysis. This pump yields 90% of membrane protein of sarcoplasmic reticulum. Endoplasmic calcium pump resembles to sarcoplasmic reticulum calcium pump, though, less abundant than that of sarcoplasmic reticulum.

4) Various proteins associated with endoplasmic reticulum
The endoplasmic reticulum serves many general functions, including the facilitation of protein folding and the transport of synthesized proteins into cisternae. Correct folding of newly-made proteins is made possible by several endoplasmic reticulum chaperone proteins, including protein disulfide isomerase, ERP29, the Hsp70 family member Grp78, calnexin, calreticulin, and the peptidylprolyl isomerase family. Only correctly folded proteins are permitted to be transported from rough endoplasmic reticulum to Golgi complex. Storage and release of calcium from the sarcoplasmic reticulum is associated with the high-capacity, low-affinity calcium-binding protein, called calsequestrin.

i) Calsequestrin
Calsequestrin is a calcium-binding protein of the sarcoplasmic reticulum. This protein facilitates recruitment of calcium in the sarcoplasmic reticulum after a muscle contraction, even though the concentration of calcium in the sarcoplasmic reticulum is much higher (approximately 10,000 times) than in the cytoplasm. Thus, it can be said that this protein augments the effect of sarcoplasmic/endoplasmic reticulum calcium—ATPase (SERCA) to reduce the concentration of free calcium in the sarcoplasmic reticulum.

ii) Calnexin
Calnexin is a 90 kDa integral protein of the endoplasmic reticulum. It consists of a large (50 kDa) N-terminal calcium-binding luminal domain, a single transmembrane helix and a short (90 residues) acidic cytoplasmic tail. Calnexin is a kind of chaperone, which is defined by its principal function to assist protein folding and ensuring that only properly folded and assembled proteins can proceed to processing and secretion.

As a member of chaperone protein, calnexin retain unfolded or unassembled N-linked glycoproteins in the endoplasmic reticulum. Calnexin binds only those N-glycoproteins that have GlcNAc2Man9Glc1 oligosaccharide. GlcNAc2Man9Glc1 oligosaccharide is added to nitrogen atom of asparagine residues if the nascent proteins in the endoplasmic reticulum possess tripeptide with a structure Asn-X-Ser/Thr. By the sequential action of glucosidases I and II, the monoglucosylated oligosaccharides that are recognized by calnexin are produced. Further action of glucosidase II, the third and last glucose residue is removed and transported to Golgi apparatus as a molecule with GlcNAc2Man8 residues. In case the glycoprotein is not properly folded, an enzyme UDP-glucose: glycoprotein glucosyltransferase will add the glucose residue back onto the oligosaccharide, regenerating the glycoprotein ability to bind to calnexin. The glycoprotein chain, which hinders proper folding in the endoplasmic reticulum, is removed with MNS1 (alpha-mannosidase) by removing its mannoside residue. ATP and calcium ion work as the cofactors involved in substrate binding for calnexin.

iii) Calreticulin and calnexin
Calreticulin is located in association with the endoplasmic reticulum and lowers the intracellular calcium ion concentration by binding calcium ion to the molecules. It possesses a large binding capacity for calcium with low affinity and releases calcium upon a signal. Synonyms for calreticulin include calregulin, CPR55, CaBP3 and calsequestrin-like protein and probably mobilin.

Calreticulin binds to misfolded proteins to keep them in the endoplasmic reticulum and prevents them to be transferred to the Golgi apparatus.

Calnexin is also a chaperone molecule with the same function as calreticulin. They both degrade incomplete molecules by binding to oligosaccharides possessing...
terminal glucose residues. However, removal of glucose residues from the core oligosaccharide (GlcNAc2Man9Glc1) that is previously added during N-linked glycosylation is a normal part of protein processing, if residues are misfolded, proteins within the rough endoplasmic reticulum will re-add glucose residues so that other calreticulin/calnexin can bind to these proteins and prevent them from proceeding to the Golgi apparatus.

iv) Annexin
Annexins include proteins previously called as lipocortins (calpactins, which is an abbreviation of "calcium-dependent phospholipid- and actin-binding protein")

endonexins (Named after its localization to the endoplasmic reticulum and its similarity to synexin and calelectrin). Annexins bind to phospholipid membranes calcium-dependently. They are ubiquitously found among eukaryotes. However, prokaryotes such as bacteria do not possess annexins. Some human annexins (Annexin A1, Annexin A2 and Annexin A5) exist extracellularly as in blood. Although the reason why glucocorticoids such as cortisol inhibit inflammation is because extracellular calcium can be usually imported to organelles homeostasis by cell membrane and intracellular organelles.

The calcium current that is induced by release of stored calcium reserves is named as calcium-release-activated calcium current (ICRAC). Recent studies propose phospholipase A2 beta, nicotinic acid adenine dinucleotide phosphate, and the protein STIM are possible mediators of ICRAC.

Changes in the cytoplasmic free calcium concentration ([Ca\(^{2+}\)\(_{cyt}\)] are one of the main transduction pathways of extracellular signals. The signal is transferred by its magnitude, location and duration of the changes in [Ca\(^{2+}\)\(_{cyt}\)]. Increases in [Ca\(^{2+}\)\(_{cyt}\)] are usually initiated by the binding of an agonist to its receptors on a cell surface. However, such signals can arise either from the release of stored calcium or the calcium influx across the cell membrane, or from both routes. A common mechanism by which such cytoplasmic calcium signals are generated involves receptors that are coupled to activate phospholipase C. Phospholipase C generates IP3, IP3 releases calcium from intracellular calcium stores such as the endoplasmic reticulum. Usually, a decrement in calcium concentration in the calcium-storing organelles subsequently activates plasma membrane calcium channels. Such transmembranous calcium influx across plasma membrane channels has been called capacitative calcium entry, or store-operated calcium entry. This type of influx is more important for non-excitable cells because capacitative calcium entry is the major means of regulated influx of calcium. As a second messenger, capacitative calcium entry induces both short term cellular responses, such as protein-protein interactions, granule secretion and longer-term cellular responses such as gene-transcription that support cell growth, apoptosis, differentiation or activation.

In vitro, the activation of gene-transcription factor via calcium can be induced by calcium ionophores such as ionomycin.

The positive feedback mechanism, i.e., increased intracellular calcium concentration facilitates further
release of calcium from the endoplasmic/sarcoplasmic reticulum via IP3 or influx of calcium from a membrane-bound calcium channel produces periodic increment/decrement of intracellular calcium concentration. Such a phenomenon is called calcium oscillation. IP3 oscillation synchronizing calcium oscillation was experimentally visualized using green fluorescent protein (GFP)\(^{(2)}\).

A close relation between mitochondria and endoplasmic reticulum is noted. Calcium ion released from IP3 receptor is incorporated into mitochondria. And calcium uptake into mitochondria also plays a role on the change in intracellular calcium concentration during depolarization of neuron. Excessively elevated intracellular calcium concentration is rapidly normalized by calcium incorporation into mitochondria through mNCE, and then released later through mNCE. Those mechanisms are extremely essential because they protect the cell from damage due to excessive calcium as well as maintaining various cell functions by maintaining intracellular calcium concentration.

2. Calcium-related signal transduction in the cells

Calcium ions are one of the most ubiquitous second messengers used in signal transduction. For example, various neurotransmitters bind to membrane-bound receptor protein molecules as acetylcholine, adrenaline, dopamine, histamine and 5-hydroxytryptamine (serotonin) receptors. The binding of the neurotransmitter to the receptor changes the conformation of receptor and causes it to expose a binding site for a guanine nucleotide (guanosine diphosphate and guanosine triphosphate) binding protein abbreviated as G-protein and composed of three subunits and bound to the internal membrane of a cell. When the G-protein binds to the receptor, it becomes able to exchange a guanosine diphosphate molecule on its alpha subunit for a guanosine triphosphate molecule. After this exchange occurs, the alpha subunit of the G-protein transducer dissociates from the beta and gamma subunits, all of the remaining parts are membrane-bound. The alpha subunit slides (movable) along the inner membrane, and eventually contacts another membrane-bound protein.

G-protein activates phospholipase C that cleaves PIP2 into diacylglycerol and IP3. Diacylglycerol activates protein kinase C, which phosphorylates various protein residues and induces various cellular responses and assists in the activation of cyclic adenosine monophosphate (cAMP), which increases calcium ion permeability of the membrane. IP3 binds to IP3 receptor on the endoplasmic/sarcoplasmic reticulum to release calcium ion from endoplasmic/sarcoplasmic reticulum into the cytoplasm. Active G-protein opens calcium channels and facilitates influx of calcium ion.

G-protein also activates adenylate cyclase to facilitate synthesis of cAMP, which activates cAMP-dependent protein kinases (protein kinase A)(PKA). PKAs include a family of enzymes whose activity is dependent on cellular concentration of cAMP.

PKA phosphorylates phospholamban. Phospholamban is an integral membrane protein that regulates the calcium pump in cardiac muscle cells. Dephosphorylated phospholamban interacts with the sarcoplasmic/endoplasmic reticulum calcium-ATPase (SERCA) to decrease its activity and sensitivity to calcium, and thus decreases calcium uptake into the sarcoplasmic reticulum. When phospholamban is phosphorylated, its interaction with SERCA is reduced, resulting in an increase in calcium transport into the sarcoplasmic reticulum. Since phospholamban is a substrate for protein kinase A, which is activated with beta-adrenergic stimulation, one would expect relaxation to be favored when PKA is activated. However, this requires more calcium stored in the SR to be available for release. Gene knockout animals of phospholamban show hyperdynamic hearts, with little apparent negative consequence\(^{(2024)}\).

A sudden increment of calcium ion concentration in the cytoplasm due to opening of channels in the endoplasmic reticulum and/or cell membrane activates various proteins as troponin C (muscle contraction), protein tyrosine kinase, IP3-kinase, and protein kinase C. In addition, the activation of myosin light chain kinase, phosphorylase kinase, calcium phosphatase, calcium/calmodulin-dependent protein kinase, nitric oxide synthase, adenylyl cyclase and phosphodiesterase is mediated by activation of calmodulin (phosphorylase kinase). Those result in various cell responses; as in secretory cells release vesicles with their secretion, muscle cells contract, synapses release synaptic vesicles and go into processes of synaptic plasticity, etc. Long-term potentiation is a persistent increase in the chemical strength of a synapse that continues from minutes to several days.

a. Calmodulin and calmodulin binding proteins

1) Calmodulin

Calmodulin is a relatively small acidic protein composed of approximately 148 amino acids and its molecular weight is 16,706 Dalton. It contains four EF-hand motifs or domains, which bind with calcium ions; therefore, calmodulin belongs to one of the two main groups of calcium-binding proteins, called EF-hand proteins. The proteins of the other group, possessing EF-hand domains are called annexins, which bind calcium and phospholipids (e.g., lipocortin). Many other proteins bind calcium, although binding calcium may not be considered their principal function in the cell. Inhibitors of calmodulin include chlorpromazine, trifluoperazine; substances used therapeutically as antipsychotic agents (major tranquilizers).
Calmodulin is a ubiquitous calcium-binding protein that binds to and regulates various different proteins, thereby affecting numerous cellular functions such as inflammation, metabolism, apoptosis, muscle contraction, intracellular movement, short-term and long-term memory, nerve growth and immune response. Calmodulin is expressed in numerous cell types and has different intracellular locations, including the cytoplasm, within organelles, or associated with the plasma or organelle membranes. Many of the calmodulin-binding proteins are unable to bind calcium themselves but use calmodulin as a calcium sensor and signal transducer molecule. Calmodulin can also make use of the calcium stores in the endoplasmic/sarcoplasmic reticulum. Calmodulin causes a conformational change by binding to calcium, and enables binding to specific proteins for a specific response. Calmodulin can bind up to four calcium ions, and can undergo post-translational modifications. Calmodulin can also bind to edema factor toxin from the anthrax bacteria.

2) Myosin kinase and calmodulin kinase II

Calcium/calmodulin-dependent protein kinases (calmodulin kinases) are serine/threonine-specific protein kinase and are primarily regulated by the calcium/calmodulin complex. These kinases show a memory effect on activation. There are 2 types of calmodulin kinases exist:

i) Specialized calmodulin kinases. As the myosin light chain kinase that phosphorylates myosin, causing smooth muscles to contract and regulating contractility of striated muscles.

ii) Multifunctional calmodulin kinases. Also collectively called calmodulin kinase II, which play a role in many processes, such as neurotransmitter secretion, transcription factor regulation, and glycogen metabolism. Between 1% and 2% of the proteins in the brain are calmodulin kinase II.

3) Caldesmon

Caldesmon is a regulatory protein of smooth muscle contraction, corresponding troponin I in the cardiac and skeletal muscles. It is named for actin and calmodulin-binding protein since the Greek term desmos means to bind. Caldesmon can bind with calmodulin or actin alternatively. When caldesmon binds with calcium-calmodulin, actin is apart from caldesmon and binds with myosin. On the contrary, when caldesmon binds with actin, actin-myosin binding is disconnected.

4) Calponin

Calponin is a guanosine triphosphate-binding protein (34 kDa) of the actin filament of the smooth muscle corresponding troponin C, T and I and possesses calmodulin-binding sites. Calponin is named because it binds with calmodulin and possesses cross-reactivity with troponin T. Three isoforms are found, basic (h1) binds with tropomyosin and actin, neutral (h2) relates with intercellular adhesion, and acidic which is expressed in the brain. The role is to maintain the inactivated state of actin filament as troponin. Calponin also has been recently known to have interactions with signal transduction system as protein kinase C and extracellular kinase (ERK) besides binding with well-known contractile proteins such as actin, myosin and tropomyosin.

5) Calcineurin

Calcineurin is a calcium/calmodulin-dependent serine/threonine dephosphatase also known as protein phosphatase 2B. The molecule is a heterodimeric protein composed of calcineurin A (61 kDa) and calcineurin B (19 kDa) subunits. Calcineurin is responsible for activating the transcription of interleukin 2, which stimulates the growth and differentiation of the T cell response. In immunosuppressive therapy, immunosuppressive agents, such as cyclosporin, pimecrolimus and tacrolimus inhibit calcineurin. These drugs bind with immunophilins specific to each compound and those complex molecules bind to the catalytic site via the calcineurin B subunit (regulatory subunit) to cause steric hindrance. Calcineurin dephosphorylates NFAT (nuclear factor of activated T-cells), a transcription factor that can then go into the nucleus and turn on genes involved in IL-2 synthesis.

When T-helper cell’s receptor interacts with an antigen, the intracellular concentration of calcium in the cell rises. This increase activates calcineurin, by binding a regulatory subunit and activating calmodulin binding. Calcineurin induces different transcription factors (NF-ATs) that are important in the transcription of IL-2 genes. IL-2 activates T-helper lymphocytes and induces the production of other cytokines. In this way, it governs the action of cytotoxic lymphocytes and NK cells. The amount of IL-2 being produced by the T-helper cells is believed to greatly influence the extent of the immune response.

Calcineurin is linked to receptors for two neurotransmitters distributed in the brain, NMDA and dopamine. Experimentally, genetically altered mice who could not produce calcineurin showed similar symptoms as in humans with schizophrenia: impairment in working memory, attention deficits, aberrant social behavior and several other abnormalities characteristic of schizophrenia.

b. Troponin

Troponin is a complex of three proteins that is integral to muscle contraction in skeletal and cardiac muscle, but not smooth muscle. Troponin is attached to the protein tropomyosin and lies within the groove between actin filaments in muscle tissue. In a relaxed muscle, tropomyosin blocks the attachment site for the myosin heads, thus preventing contraction. When the
muscle cell is stimulated to contract by an action potential, calcium channels open and release calcium into the sarcoplasm. Some of this calcium attaches to troponin, causing a conformational change that moves tropomyosin out of the way so that the myosin heads can attach to actin and form cross-bridges resulting in muscle contraction.

Troponin is found in both skeletal muscle and cardiac muscle, but the specific types of troponin differ between types of muscle. A recent study on the structure of troponin molecule revealed that the “regulatory head” (composed of TnC and Tnl) has one calcium-binding site in cardiac muscle whereas there are two binding sites in the skeletal muscle, and the “IT-arm” (composed of TnC, Tnl and TnT) has two calcium binding sites commonly in both cardiac and skeletal muscle. Troponin is an EF-hand protein. Discussions of troponin often pertain to its functional characteristics and/or to its usefulness as a diagnostic marker for various heart disorders. Both cardiac and skeletal muscles are controlled by changes in the intracellular calcium concentration. When calcium increases, the muscles contract, and when calcium decreases, the muscles relax.

Troponin is a component of thin filaments (along with actin and tropomyosin), and is the protein to which calcium binds to accomplish this regulation. Troponin has three subunits, TnC (C stands for calcium), Tnl (I stands for inhibitory), and TnT (T stands for tropomyosin)\(^{(27)}\). When calcium is bound to specific sites on TnC, the structure of the thin filament changes in such a manner that myosin (a molecular motor organized in muscle thick filaments) attaches to thin filaments and produces force and/or movement. In the absence of calcium, tropomyosin interferes with this action of myosin, and therefore muscles remain relaxed. Troponin I has also been shown to inhibit angiogenesis in vivo and in vitro.

Each subunit serves different functions:

1. Troponin C binds to calcium ions to produce movement.
2. Troponin T binds to tropomyosin, interlocking with them to form a troponin-tropomyosin complex.
3. Troponin I binds to actin in thin myofilaments to hold the troponin-tropomyosin complex in place.

**c. Protein kinase C**

Protein kinase C is a family of plasma membrane-bound serine/threonine protein kinases consisting of approximately 10 isozymes\(^{(28)}\). The name usually refers to the entire family of isoforms. They are divided into three subfamilies, based on their second messenger requirements: conventional (or classical), novel, and atypical\(^{(29)}\). Conventional PKCs require calcium ion, diacylglycerol (DAG), and a phospholipid for activation. Novel PKCs require DAG, but do not require calcium ion for activation. On the other hand, atypical PKCs require neither calcium ion nor diacylglycerol for activation. The term “protein kinase C” usually refers to the entire family of isoforms. A similar amino acid sequence is shared among protein kinase A, B, and C. The consensus sequence of protein kinase C enzymes is similar to that of protein kinase A, since it contains basic amino acids close to the serine/threonine to be phosphorylated.

Various types of protein kinase C are found among various cells. For example, they mediate stimuli by binding adrenaline, acetylcholine, 5-hydroxytryptamine, prostaglandin and angiotensin with each corresponding receptor on the smooth muscle, nerve, ependymal cells, excretory glands. Upon activation, protein kinase C enzymes are translocated to the plasma membrane by RACK (Receptor for Activated C-Kinase) proteins.

d. S-100 proteins

S-100 proteins are a type of low molecular weight (approximately 20 kDa) protein found in vertebrates characterized by two calcium-binding sites of the helix-loop-helix (EF-hand type) conformation. The first EF-hand in a pair of S-100 proteins is called a pseudo EF-hand, not the canonical EF-hand that has a 12 residue loop as seen in calmodulin, whereas the pseudo EF-hand has 14 residue binding loops. The second EF-hand is the canonical EF-hand including S-100 proteins.

There are at least 21 different types of S-100 proteins. The name is derived from the fact that the protein is 100% Soluble in ammonium sulfate at neutral pH.

S-100 proteins seem to involve with various cell functions mainly interactions with nucleus. Interestingly, a tandem-bound dimer molecule of S-100 protein, nominated as p260, binds with calcium ion in olfactory cells and cilia in bronchial epithelial cells\(^{(30)}\).

i) Calgranulin

Calgranulin is an S-100 calcium-binding protein that is expressed in multiple cell types, including renal epithelial cells and neutrophils. Some in vitro evidence suggests that calgranulin can inhibit the precipitation of calcium oxalate in a urine-like environment at calgranulin concentrations below physiological concentrations\(^{(31)}\). Thus, it may also function in vivo as an inhibitor of calcium oxalate kidney stone formation. However, the role of calgranulin in the stone formation process has not been confirmed.

e. Calpain

Calpains are a family of calcium-dependent, nonlysosomal cysteine proteases shared ubiquitously among animal cells\(^{(32)}\). The name calpain means calcium related cysteine protease. As papain and bromelain, a postfix “-pain” means the molecule belongs to cysteine...
protease. The calpain proteolytic system includes the calpain proteases, the small regulatory subunit, and the endogenous calpain-specific inhibitor, calpastatin.

Roles of calpains still have not been clarified, though, they have been shown to participate in processes such as cell mobility and cell cycle progression, as well as cell-type specific functions such as long-term potentiation in neurons and cell fusion in myoblasts, regulating clotting and the diameter of blood vessels, and playing a role in memory. Calpains have been implicated in apoptotic cell death, and appear to be an essential component of necrosis. Excessive amounts of calpain can be activated due to calcium influx after ischemic cerebrovascular accident or some types of traumatic brain injury such as diffuse axonal injury. The sustained influx of calcium into the cell results in calpain hyperactivation, unregulated proteolysis of both target and non-target proteins and consequent irreversible tissue damage.

Excitotoxicity may be involved in stroke, traumatic brain injury and neurodegenerative diseases of the central nervous system such as multiple sclerosis, Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease and Huntington's disease.

f. Cadherin

Cadherins are a superfamily of transmembrane cell-adhesion molecules expressed in various types of cells. They share the extracellular calcium-binding domains called cadherin repeats. A superfamily includes E-cadherins found in epithelium; N-cadherins in neurons; P-cadherins in the placenta. A remaining group of cadherin molecule is the T-cadherins, which lacks the transmembrane and cytoplasmic domains and is tethered to the cell membrane through the glycosylphosphatidylinositol (GPI) anchor. A cell expressing one of those types of molecule binds only with cells expressing the same type of molecule. A cytoplasmic domain of the molecule (except T-cadherins) binds with actin filaments, a ubiquitous cytoskeleton molecule via catenin and vinculin molecules. Loss or decrement of E-cadherin expression loosens the cellular unity, as frequently found in cancer invasion and metastasis. In fact, expression of E-cadherin is often decreased among cancer cells.

3. Calcium metabolism

a. Absorption

Calcium is usually taken from food orally and absorbed in the small intestine. Vitamin D is an important co-factor in the intestinal absorption of calcium, as it increases the number of calcium binding proteins, involved in calcium absorption through the apical membrane of enterocytes in small intestine.

1) Vitamin D-dependent calcium-binding proteins (Calbindin, Calbindin-D)

The name calbindin designates for calcium binding proteins. Those proteins were initially described as the vitamin D-dependent calcium binding proteins in the intestine and kidney. Hence calbindin is composed of various molecules. The name calbindin-D is described in some articles, a suffix -D emphasizes a relation with vitamin D. Vitamin D-dependent calcium binding proteins were discovered in the cytosolic fractions of chicken intestine, and later in mammalian intestine and kidney, by researchers including Wasserman in 1966. Calbindins bind to calcium with extremely high affinity. Expression of calbindin is extremely dependent to vitamin D concentration as it is decreased in vitamin D-deficient animals and increased by treating animals with vitamin D derivatives. The vitamin D receptor recognition sequence is identified as upstream of calbindin gene. Two different sizes with molecular weights of approximately 9 kDa and 28 kDa were found. They were renamed as calbindin-D9k and -D28K, respectively. Calbindin-D9k is found in mammalian intestine and calbindin-D28k is found in avian intestine and in mammalian kidney. They do not share immunological cross-reactivity.

Calcium ion from the luminal side of the small intestine (especially proximal as the jejunum) is initially incorporated by binding with membrane calcium channel TRPV6 on the apical brush border of the mammalian intestinal epithelial cells. The expression of TRPV6 is facilitated by vitamin D. Incorporated calcium is transported by binding with calbindin-D9K. Since calcium-binding capacity of calbindin-D9K is extremely large, calbindin transports calcium without increasing cytoplasmic calcium concentration. Cytoplasmic calcium concentration should be maintained low (approximately 0.1 micromolar) to avoid noise in signal transduction and to facilitate calcium incorporation from the intestine. It is true that calbindin plays a role to avoid increase of cytoplasmic calcium concentration, though its principal role is as a carrier molecule of incorporated calcium ions in the intestine. Transported cytoplasmic calcium is pumped out to the blood (portal vein in human) via plasma membrane calcium ATPase (PMCA) 1 at the basolateral side of the cell. Calbindin may stimulate PMCA 1.

Calbindin-D28k (calbindin 1) is found in the avian intestine, mammalian kidney and some of the neuroendocrine cells as in the cerebellum and possesses a similar role. There is no homology between calbindin-D28k and calbindin-D9k, except both possess calcium binding domains (called EF-hands). Calbindin-D9k has two EF-hands and binds two calcium ions, and calbindin-D28k has six but binds four calcium ions.
2) Calretinin (calbindin 2)

Calbindin 2 molecule is 29 kDa EF-hand family calcium binding protein and also called as calretinin, known as a marker of mesothelial cells.

b. Reabsorption

Circulating calcium is either in the free, ionized form or bound to serum proteins such as albumin. The parathyroid hormone regulates the resorption of calcium from bone and elevates blood calcium concentration. A counterpart hormone calcitonin (a polypeptide composed of 32 amino acids with its molecular weight 3,500 kDa) secreted from the parafollicular cells of the thyroid gland decreases blood calcium concentration. Stored calcium is mostly calcium phosphate and partially calcium sulphate in the bones and cartilages among vertebrates. In plants, calcium oxalate is stored in plastids (thus causing renal stone in vegetarians) and calcium can construct plant tissue structures and is involved in signal transduction pathways as well as in animal cells.

c. Bone metabolism and calcium

Bones are the major calcium-storing organs, as they serve as important storage points for calcium, containing 99% (approximately 1 kg) of the total body calcium. Calcium release from bone is regulated by parathyroid hormone. Calcitonin stimulates incorporation of calcium in bone, although this process is largely independent of calcitonin. Primarily calcium is regulated by the actions of Vitamin D, parathyroid hormone (PTH) and calcitonin. The only real regulatory organ is the parathyroid gland. The parathyroid glands are located behind the thyroid, and produce parathyroid hormone in response to low calcium levels.

The parafollicular cells of the thyroid produce calcitonin in response to high calcium levels, but its significance is much smaller than that of PTH.

There are some bone-specific calcium binding proteins:

1) Osteocalcin

Osteocalcin (bone gamma-carboxyglutamic acid protein, BGP) is a protein found in bone and dentin. It is secreted by osteoblasts and thought to play a role in mineralization and calcium ion homeostasis. The C-terminus of the molecule facilitates chemotaxis as an osteoclast. It has been reported that osteocalcin may also function as a negative regulator of bone formation, although its exact role is unknown. A recent study shows that osteocalcin acts as a hormone in the body, causing beta cells in the pancreas to facilitate insulin release, and simultaneously directing fat cells to release adiponectin, the hormone, which increases sensitivity to insulin.

2) Osteonectin

Osteonectin is a glycoprotein in the bone that binds calcium. It is secreted by osteoblasts during bone formation, initiating mineralization and promoting mineral crystal formation. Osteonectin also shows affinity for collagen in addition to bone mineral calcium. Some current research shows a correlation between osteonectin over expression and ampullary cancers and chronic pancreatitis.

3) Osteopontin

Osteopontin is a glycoprotein first identified in 1986 in osteoblasts. Osteopontin is an extracellular structural protein and therefore an organic component of bone. Synonyms for this protein include sialoprotein I and 44K BPP (bone phosphoprotein). Osteopontin is biosynthesized by a variety of tissue types including preosteoblasts, osteoblasts, osteocytes, extraosseous cells in the inner ear, brain, kidney, deciduum, placenta, odontoblasts, some bone marrow cells, hypertrophic chondrocytes, macrophages, smooth muscle, and endothelial cells. Synthesis of osteopontin is stimulated by calcitriol (1,25-dihydroxy-vitamin D3).

4. Body function and calcium

As mentioned throughout this article, calcium involves in numerous function, though, involvement in muscle contraction and blood coagulation are briefly noted below as classically known representatives of calcium-related functions.

a. Muscle contraction

Contribution to muscle contraction might be the first recognized biological role of calcium. Elevated concentration of cytoplasmic calcium is essential for initiation of any type of muscle including the striated and smooth muscles. A common intrinsic calcium store among muscles is sarcoplasmic reticulum. Released calcium binds with surrounding calcium-binding molecules as troponin-C in striated muscles and calmodulin in smooth muscles then produce muscle contraction.

b. Blood coagulation

Calcium ion is essential for activation of blood coagulation factors IX (Christmus factor), X (Stuart-Prower factor), III (Tissue factor), V (proaccelerin) and XIII (fibrin-stabilizing factor). Calcium is required for coagulation factors to bind to phospholipid. Moreover calcium ion is a member of the blood coagulation factor; formerly known as factor IV. Calcium mediates the binding of the complexes via the terminal gamma-carboxy residues on factor Xa and factor IXa to the phospholipid surfaces expressed by platelets as well as procoagulant microparticles or microvesicles shedded from them. Moreover, calcium is extremely involved in the signal-transduction of platelet that supports platelet functions such as release of various factors required for aggregation.

Conclusion

Calculus ions play important roles in cellular response.
to various stimuli by transducing intracellular signals with numerous calcium-related proteins. Knowledge of those signal transduction pathways is essential to better understanding of living cell functions.

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