Chapter 1
Calcium Signaling: From Basic to Bedside

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Abstract Calcium signaling and its interacting networks are involved in mediating numerous processes including gene expression, excitation-contraction coupling, stimulus-secretion coupling, synaptic transmission, induction of synaptic plasticity, and embryonic development. Many structures, organelles, receptors, channels, calcium-binding proteins, pumps, transporters, enzymes, and transcription factors are involved in the generation and decoding of the different calcium signals in different cells. Powerful methods for measuring calcium concentrations, advanced statistical methods, and biophysical simulations are being used for modelling calcium signals. Calcium signaling is being studied in many cells, and in many model organisms to understand the mechanisms of many physiological processes, and the pathogenesis of many diseases, including cancers, diabetes, and neurodegenerative disorders. Studies in calcium signaling are being used for understanding the mechanisms of actions of drugs, and for discovery of new drugs for the prevention and treatment of many diseases.

Keywords Calcium signaling · Excitation-contraction coupling · Stimulus-secretion coupling · Calcium and gene expression · Calcium and diabetes · Calcium and cancer · Calcium channels · Calcium binding proteins · Calcium oscillations · Calcium pumps · Calcium-sensing receptor

Concentration of Ca^{2+} in the cytoplasm ($[Ca^{2+}]_c$) is >20, 000 times lower than that outside the cell. This is achieved by the Ca^{2+}-transporting ATPases, Na^+/Ca^{2+} exchangers, and Ca^{2+}-binding proteins. When activated by a variety of external stimuli, cells respond by an increase in the $[Ca^{2+}]_c$, in the form or Ca^{2+}-spikes and

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oscillations, which allosterically regulate many proteins leading to alterations of numerous processes like gene expression, meiotic resumption, gastrulation, somitogenesis, early embryonic development of different organs, left-right asymmetry, muscle contraction, exocytosis, synaptic transmission, and induction of synaptic plasticity, to name just a few.

The discovery of the method for measuring $[Ca^{2+}]_c$ by fluorescent Ca^{2+}-indicators [1] that are made temporarily membrane permeable [2] started a revolution in biology, and today many laboratories are using fluorescent Ca^{2+}-indicators for measuring $[Ca^{2+}]_c$. Since these indicators bind Ca^{2+}, it is necessary to assess to what extent the indicators attenuate the Ca^{2+} signal. At low concentration of the indicators, it is more likely that an un-attenuated Ca^{2+} signal is being measured, whereas at high concentration of the indicators, it is more likely that Ca^{2+}-flux is being measured [3]. Many investigators are using a variety of genetically encoded Ca^{2+} indicators suitable for measuring Ca^{2+} in different organelles and subcellular compartments [4]. The technology has advanced to the point that we can measure highly localized Ca^{2+} changes in femtoliter volume by high resolution laser microscopy. We can also measure the physiological changes in the membrane potential, and consequent changes in physiological Ca^{2+} currents, in living cells, in their native environment, by using fluorescence-based optical techniques. Pharmaceutical companies are using high- throughput fluorescence-based assays using a variety of Ca^{2+}-indicators, for screening of ion-channels and G-protein coupled receptor as drug targets, and for identifying novel lead compounds.

Plasma membrane Ca^{2+} transport ATPase (PMCA), Na^+/Ca^{2+} exchanger, and sarco/endoplasmic reticulum (SR/ER) Ca^{2+}-ATPase (SERCA) maintain $[Ca^{2+}]_c$ at a normal low level. More than 20 variants of PMCA, with different regulatory properties, cell-type-specific expressions, different localizations, and interactions with different signaling molecules, not only maintain Ca^{2+} homeostasis, but also shape the Ca^{2+} signals. Mutations or genetic variations in the PMCA genes have been associated with diseases like hypertension, preeclampsia, and neural disorders. Twelve isoforms of SERCA proteins encoded by three genes, are expressed in different patterns in different tissues. Impaired Ca^{2+} homeostasis and Ca^{2+} signaling caused by impaired functions of SERCA pumps have been implicated in the pathogenesis of Darier disease, and some neuropsychiatric and neurodegenerative disorders.

Cells contain numerous Ca^{2+}-binding proteins, some of which act as Ca^{2+}-sensors, others as Ca^{2+}-buffers, and some as both. Ca^{2+} is buffered by rapid binding to Ca^{2+}-binding proteins that vary in their Ca^{2+}-binding and -dissociation kinetics, their concentrations in different locations inside the cell, and in their diffusional mobility. Ca^{2+}-buffers make the $[Ca^{2+}]_c$ changes transient, and, thus, finely tune the timing and spatial extension of Ca^{2+} signaling.

Receptor activation increases incorporation of P^{32} into the phospholipids [5], due to activation of phospholipase C. Thirteen family members of phospholipase C, and their isoforms are regulated by numerous agonists in isozyme-selective manner; they perform distinct functions in signal transduction, and mediate a variety of

cellular responses, in almost every cells of the body. Activation of phospholipase C leads to the formation of the second messengers inositol 1,4,5 trisphosphate, and diacylglycerol [6, 7], which activates protein kinase C [8]. Inositol 1,4,5 trisphosphate activates 1,4,5 trisphosphate receptor, and releases Ca^{2+} from the ER [9]. In addition to mediating Ca^{2+} release, the three 1,4,5 trisphosphate receptors that form homotetramers, and heterotetramers, interact with >100 proteins, and signaling molecules. The expression of different isoforms of 1,4,5 trisphosphate receptor in different tissues, and their interactions with other proteins add to the complexity and diversity of the regulation of Ca^{2+} signaling in mediating many processes including apoptosis, autophagy, and cancer development.

The critical components of excitation-contraction coupling are the ryanodine receptors, the L-type voltage-gated Ca^{2+} channels and the junctophilins. From evolutionary perspective, Ca^{2+}-induced-Ca^{2+} release (CICR) (as in the cardiomyocytes) is the earliest form of excitation-contraction coupling, whereas depolarization-induced Ca^{2+} release from SR (DICR) through the type 1 ryanodine receptor mediated by direct protein-protein interaction between the ryanodine receptor and the L-type voltage-gated Ca^{2+} channel, (as in skeletal muscle cells) is a later development in the vertebrates. Four types of junctophilins link the ER to the plasma membrane, and support both CICR and DICR. Ca^{2+} regulates the three ryanodine receptors both positively and negatively (depending on concentration), by binding to the receptors, directly or indirectly through the Ca^{2+}-binding proteins, both from the cytosolic side and the luminal side. Release of Ca^{2+} from the ER /SR where Ca^{2+} is present in the form or "Ca^{2+}-lattice" bound to proteins, is a highly regulated process that prevents depletion of Ca^{2+} stores and consequent activation of ER stress response.

The pyridine nucleotide metabolite cyclic ADP ribose (cADPR) releases Ca^{2+} from the intracellular stores by mechanisms that may involve activation of the ryanodine receptors, but it is not clear how this happens, and to which protein cADPR binds. In heart, cADPR increases the gain of CICR. Another pyridine nucleotide metabolite, NAADP also releases Ca^{2+} from some acidic lysosomal Ca^{2+} stores by mechanisms that involve activation of the two pore channels (TPC). It is not clear whether NAADP binds to proteins other than TPCs. Stimulation of β-adrenergic receptors increases formation of NAADP, which releases Ca^{2+} from the acidic stores by binding to TPCs, but numerous questions remain unanswered. Endosomes and lysosomes are acidic organelles that contain Ca^{2+} in high concentration in readily releasable form. Ca^{2+} is loaded into these stores by the actions of vacuolar H^+-ATPase, and Ca^{2+}/H^+ exchange. Ca^{2+} can be released from these stores through the TRPML channel or the TPCs.

Receptor activation not only releases Ca^{2+} from the ER, but also leads to Ca^{2+} entry through the plasma membrane channels [10]. The stromal interaction molecule 1 (STIM1) senses the Ca^{2+} concentration in the ER Ca^{2+} stores, and it regulates the CRAC (Ca^{2+} release-activated Ca^{2+} channel) formed by Orai1, a highly calcium-selective channel located in the plasma membrane. STIM1 also regulates store-operated Ca^{2+} channel formed by TRPC1. Mutations in the STIM1/Orai1 have been associated with diseases like severe combined immune deficiency, Stormorken

syndrome, and tubular aggregate myopathy. Disturbances in store-operated Ca^{2+} entry have been implicated in promoting angiogenesis, tumor growth, muscle differentiation, and progression from cardiac hypertrophy to heart failure.

Mitochondria plays important roles in Ca^{2+} signaling. Mitochondria-associated ER membrane (MAM) provides a mechanism for communication between the ER and the mitochondria. The Sigma-1 receptor, a chaperone protein located in the MAM, is involved in Ca^{2+} exchange between the ER and the mitochondria. Ca^{2+} enters into the mitochondria through the mitochondrial Ca^{2+}-uniporter. Efflux of Ca^{2+} from the mitochondria is mediated by the Na^{+}/Ca^{2+} exchanger. Ca^{2+} regulates mitochondrial respiration, and ATP synthesis, but mitochondrial Ca^{2+} overload triggers the apoptosis pathways. The Sigma-1 receptor is also located near the plasma membrane, and it interacts with many other proteins. Mutations of the Sigma-1 receptor may lead to diseases like amyotrophic lateral sclerosis, and distal hereditary neuropathy.

The discovery of the transient receptor potential channels was helped by the clues obtained from the photoreceptor cells of drosophila mutants [11, 12]. These channels act as molecular sensors, and they participate in numerous cellular processes. Many cells express many of the TRP channels, and these channels appear to be involved in the pathogenesis of many diseases. In lungs, TRPC3 appears to be involved in mediating airway hyper-responsiveness seen in asthma. Dysregulation of several TRP channels has been implicated in promoting cancer growth, metastasis, and in determining sensitivity or resistance to chemotherapy. Some TRP channels that regulate tumorogenesis or tumor progression, are themselves targets of specific microRNAs, which are expressed in many cancer cells, and which function in RNA silencing. Manipulation of the TRP/miRNA interactive network is a potential way to treat cancer.

Ca^{2+} signals are decoded by numerous proteins, including, many ion channels, enzymes, transcription factors, and exocytotic proteins, which can be activated or inactivated by Ca^{2+}. Activation of protein kinase C [8] and multifunctional calcium/calmodulin stimulated protein kinases (CaMK) by Ca^{2+}, mediates a variety of cellular processes. The CaMKs, which are expressed in numerous cells, are activated following a variety of stimuli. Specificity of the functions of these kinases is determined by "molecular targeting" mechanisms mediated by some specific binding proteins. CaMK-II remains active in proportion to the frequency and amplitude of the Ca^{2+} signals, and the activation persists for some time even after $[Ca^{2+}]_c$ returns to the normal basal level. CaMK-II plays important roles in decoding Ca^{2+} signals to activate specific events during the embryonic development. Ca^{2+} singling regulates expression of many genes by acting at the level of gene transcription, gene translation, regulation of alternative splicing, and by regulating the epi-genetic mechanisms. Alteration in the expression of many genes can alter the so called "Ca^{2+} homeostasome" [13].

The Ca^{2+}-microdomains comprised of Ca^{2+} channels, Ca^{2+}-activated Ca^{2+} channels, Ca^{2+}-buffers, and other molecules, are fundamental elements of Ca^{2+} signaling. Different simulation strategies including stochastic method, deterministic method, Gillespie's method, and hybrid methods in multi-scale simulations, have been used for modeling of the Ca^{2+} signaling systems. Ca^{2+} signals occur in the

form of Ca^{2+} spikes, and Ca^{2+} oscillations, which are stochastic events. Advanced statistical approaches, and biophysical simulations are being used to obtain insight into the dynamics of Ca^{2+} oscillation, including the processes underlying the generation and decoding of the oscillations.

In biology, Ca^{2+} signaling is almost universal. Even bacteria use Ca^{2+} as a signal; they sense Ca^{2+} by using the so called two component regulatory systems consisting of a sensor kinase and a response element. To understand different biological phenomena, and many human diseases, Ca^{2+} signaling is being studied in many model organisms including *Drosophila melanogaster* [14], *Saccharomyces cerevisiae* [15], *Caenorhabditis elegans* [16], and zebrafish, and such researches have led to important discoveries. Study of Ca^{2+} imaging in the zebrafish has helped our understanding of the development processes, many other physiological processes, and the roles of disease-related genes in a vertebrate system.

Extracellular Ca^{2+} functions as charge carrier, and regulates neuromuscular excitability. Extracellular Ca^{2+} is sensed by a G-protein coupled receptor (calcium-sensing receptor), which regulates secretion of parathyroid hormone, and can be inhibited by the calcimimetic drug cinacalcet used in the treatment of hyperparathyroidism. In the bone marrow high extracellular Ca^{2+} leads to predominant osteoblast formation by acting through calcium-sensing receptor. High Ca^{2+} in the bone marrow also inhibits the differentiation and the bone-resorbing function of the osteoclasts. Extracellular matrix is the largest Ca^{2+} store in animals. The macromolecules of extracellular matrix interact with the receptor on the plasma membrane, and by that way regulate the $[Ca^{2+}]_c$ through complex mechanisms. Ca^{2+} also controls cell-extracellular matrix interaction through focal adhesions.

Study of Ca^{2+} signaling is helpful in understanding the pathogenesis of many diseases including that of diabetes, and the neurodegenerative diseases, and in understanding the mechanisms of action of drugs used in the treatment of these diseases. Many calcium channel blockers are being extensively used in the treatment of hypertension and atrial fibrillation. Over 400 million people in the world have diabetes. Studies of Ca^{2+} signaling have increased our understanding of the mechanisms underlying stimulus-secretion coupling in the β-cells, which is impaired in type 2 diabetes. Some of the commonly used antidiabetic drugs act by altering Ca^{2+} signaling in the β-cells [17]. It is likely that studies of Ca^{2+}-signaling and its interacting networks, will lead to new breakthroughs that will increase our understanding of the molecular mechanisms of many cellular processes that we do not fully understand today.

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