Russian Journal of Plant Physiology, Vol. 52, No. 2, 2005, pp. 249–270. Translated from Fiziologiya Rastenii, Vol. 52, No. 2, 2005, pp. 282–305.

REVIEWS

Calcium Signaling System in Plants

S. S. Medvedev

Department of Plant Physiology and Biochemistry, St. Petersburg State University, Universitetskaya nab. 7/9, St. Petersburg, 199034 Russia; fax: 7 (812) 328-9703; e-mail: ssmedvedev@mail.ru

Received August 31, 2004

Abstract—Calcium ions have unique properties and universal ability to transmit diverse signals that exert primary action on cells, such as hormones, pathogens, light, gravity, and stress factors. The principal elements in the system of calcium signaling of plant cells are different Ca^{2+} channels, Ca^{2+}-ATPases, Ca^{2+}/H^+ antiporters, Ca^{2+}-binding and Ca^{2+}-dependent proteins. The system of calcium signaling also includes receptors, the cascades of amplifying Ca^{2+} signals, and transcription factors. The process of transmitting the calcium signal within the cell consists of at least two stages. At the first stage, the cytosolic calcium concentration undergoes temporal and usually local increase due to its entry through the Ca^{2+} channels. The second stage is related to the signal "decay" and represents the active removal of calcium excess from the cytosol to the extracellular medium or organelles (vacuoles, endoplasmic reticulum, mitochondria) by means of Ca^{2+}-ATPases and/or Ca^{2+}/H^+ antiporters. The primary intracellular targets of calcium are various calcium-binding proteins. Some of these proteins ensure Ca^{2+} transport, others serve as a calcium buffer, and the others (e.g., calmodulin or Ca^{2+}-dependent protein kinases) translate the calcium signal to intracellular operational mechanisms and initiate Ca^{2+}-dependent physiological processes. An important feature of the calcium signal transduction is that this signal originates and propagates in the pulse mode. Such way of information transmission is not only faster than the diffusion but it also ensures the spatiotemporal regulation of cell functions, because the signal encoding can be realized via amplitude- and frequency-modulated oscillations in cytosolic calcium concentration.

Key words: Calcium - Ca^{2+}-channels - Ca^{2+}-ATPases - Ca^{2+}/H^+ antiporters - Ca^{2+}-binding proteins - calmodulin - Ca^{2+}-dependent protein kinases - calcium spikes, waves, and oscillations - signal transduction

INTRODUCTION

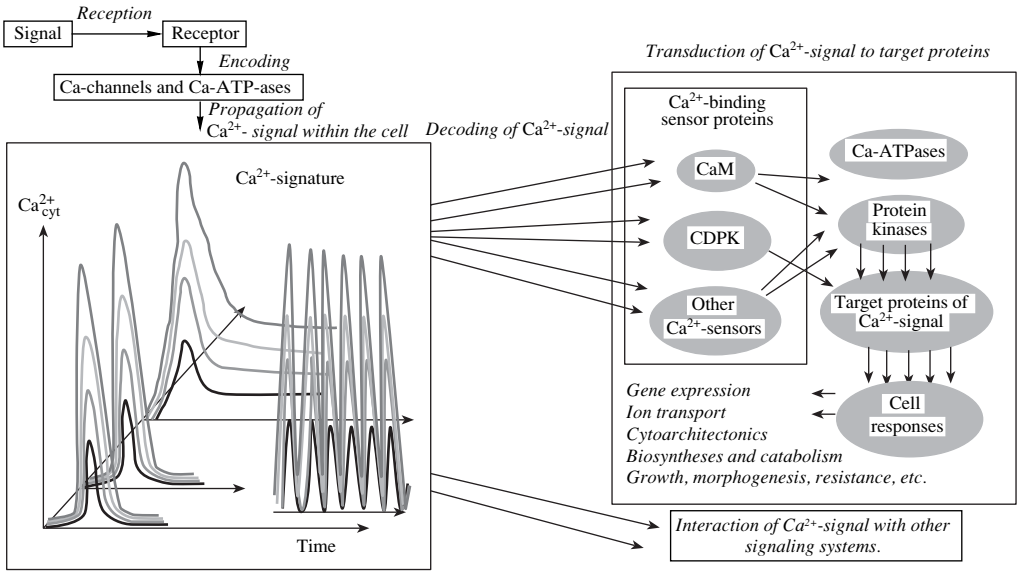
The British physiologist Ringer [1] was the first to reveal in 1883 the significance of calcium as an intracellular regulator. He found that the heart muscle activity is drastically reduced in the absence of Ca^{2+} ions. Nevertheless, it was almost 100 years later that Rasmussen [2, 3] formulated a notion that calcium functions as a second messenger during the transmission of signals arriving from the external and internal media of the organism. Subsequent studies showed that calcium is a ubiquitous "signaling" ion in animals [4–8] and plants [9–15].

Calcium ions belong to most important elements in the system of intracellular signaling in plants. Each known system of signal transduction engages Ca^{2+} as an important second messenger [16, 17]. Calcium is an effective regulator of metabolism in all cell systems capable of responding to changes in Ca^{2+} concentration. The preferable intracellular targets of Ca^{2+} action are various calcium-binding proteins that either change their own activity or relay the effect of Ca^{2+} on diverse molecular targets.

Being a second messenger, calcium is required at all stages of plant growth and development. It is involved in the control of such processes as growth and differentiation [18, 19], photomorphogenesis [20–22] and embryogenesis [23], the self-incompatibility responses in pollen–pistil interactions [14, 24, 25], perception of symbiotic signals [26, 27], hypersensitive responses induced by pathogens and elicitors [13, 28–32], gravitropism [33–37] and phototropism [38], assembling and disassembling of cytoskeleton elements [39], perception of red [20, 40] and blue light [41], cyclosis [18], and movement of stomatal cells [42–45].

Calcium plays a fundamental role in regulation of polar growth of cells and tissues [10, 11, 18, 19]. The apex-localized gradient of cytoplasmic Ca^{2+} (Ca^{2+}_{cyt}) is a general feature always observed in cells with a tip growth, such as pollen tubes [25, 46, 47], root hair cells [48–50], and rhizoidal protrusion in the *Fucus* zygote

Abbreviations: CaM—calmodulin; CDPK—calcium-dependent protein kinase; IP_3—inositol-1,4,5-*tris*phosphate; PIP_2—phosphatidylinositol-4,5-diphosphate; cADPR—cyclic ADP-ribose; CICR—calcium-induced calcium release; Ca^{2+}_{cyt} —concentration of ionized calcium in the cytoplasm; MP—membrane potential; Nod-factors—symbiotic signals.



Schematic view of generation and transduction of the calcium signal in the plant cell (modified from [14]).

Ca^{2+}_{cyt} —concentration of ionized calcium in the cytoplasm; CaM—calmodulin; CDPK—Ca^{2+}-dependent protein kinase.

[51–53]. Calcium participates in adaptation to various stress factors [54]. Signals associated with chilling stress [28, 55–57] and heat shock [58], salinity and drought [56, 57, 59], anoxia [60–62] and elicitors [13, 28, 30, 31, 63], osmotic shock [52, 64–66] and mechanical stimulation [28, 67], as well as oxidative stress [68, 69], induce a transient increase in the level of cytoplasmic Ca^{2+}. Calcium is the principal component in the transduction of hormonal signals induced by gibberellic acid [70–72], ABA [45, 73–75], IAA [76–80], and cytokinins [81–83].

The main elements in the system of calcium signaling in plant cells are various types of Ca^{2+} channels, Ca^{2+}-ATPases, Ca^{2+}/H^+ antiporters, Ca^{2+}-binding sensor proteins, Ca^{2+}-binding proteins acting as Ca^{2+} buffers, and Ca^{2+}-regulated ion channels (figure). The calcium signaling system comprises also various receptors and second messengers (inositol-1,4,5-*tris*phosphate, IP_3; cyclic ADP-ribose, cADPR), cascades amplifying the Ca^{2+} signals, various types of protein kinases and protein phosphatases, Ca^{2+}-regulated enzymes and cytoskeletal proteins, transcription factors and Ca^{2+}-regulated genes. The distinctive feature of Ca^{2+}-mediated information transmission is the wave-like mode of signal transmission. The Ca^{2+} signaling is largely based on Ca^{2+} waves and Ca^{2+} oscillations arising in particular cell regions.

Several recent reviews presented extensive analysis of the Ca^{2+} function as a second messenger during signal transduction in plant cells [11–15, 84]. This article focuses on the following questions. Why is calcium ideally suited for the key role of universal second messenger in cell responses to environmental cues? What are the mechanisms of maintaining the required calcium level in the cytoplasm? Which components are the main intracellular targets for calcium? How is the calcium signal transmitted through membranes and within the cytoplasm?

PROPERTIES OF Ca^{2+} AS A SECOND MESSENGER

Why it was calcium that became a principal and ubiquitous second messenger in cell responses to environmental changes and, furthermore, the only ionic messenger? First, the information transmission should rely on small-sized substances that are readily available in the cell and extracellular medium and are capable of functioning as mediators during signal transduction. Second, the cell needs a well-organized transport system for signal substances to ensure fine and operative regulation of their levels in different cellular compartments. Several candidates are principally suitable for the role of ionic second messengers. However, large sizes of anions and the unit electric charge of K^+ and Na^+ hamper their strong interactions with protein molecules. The Nature had to make the choice between Ca^{2+} and Mg^{2+} [85].

It is known that coordination bonds between calcium and ligands (from six to nine) are more numerous than those of magnesium (six at most). In the case of Mg^{2+}, some coordination bonds are reserved for interactions with water molecules, which lowers the affinity of magnesium to protein ligands. Remarkably, the length of bonds formed by calcium is prone to larger variations than that formed by magnesium. For example, the length of Ca–O bond varies from 0.23 to 0.26 nm, whereas the length of Mg–O bond is always

kept at 0.21 nm. Magnesium usually forms regular six-coordination octahedral complexes with bonds of fixed length. In contrast, calcium forms labile complexes with a higher and variable coordination numbers and with variable length of bonds. Owing to this property, calcium adjusts itself to the binding site of the ligand molecule and can be exchanged in this complex 1000 times faster as compared to magnesium [85].

The energy consumption allotted for maintaining nonequilibrium distribution of major ions between the cell and the external medium constitutes 20–30% of the total energy expenditures in the cell. The maintenance of calcium homeostasis in the cell requires much less energy (about 1%). The plasma membrane and the cellular organelles possess a finely organized system of Ca^{2+} transport represented by various types of calcium channels and pumps; this system ensures elaborate and effective regulation of free calcium level in cellular compartments [86]. According to Trewavas [16], the origin of Ca^{2+} signaling system is related to high toxicity of Ca^{2+}, which prompted cells to develop a mechanism ensuring low level of this cation in the cytoplasm. Subsequent evolution of these detoxication mechanisms gave rise to Ca^{2+}-based signaling pathways.

MEASUREMENTS OF Ca^{2+} CONCENTRATION

The studying of Ca^{2+} role in signal transduction made a great advance when Tsien synthesized a highly sensitive fluorescent calcium probe quin-2 and developed a principally novel method of loading this dye into the cell [87]. This new methodological approach allowed researchers to monitor the Ca^{2+} dynamics in any cytoplasmic region within the live cell. The syntheses of quin-2 and calcium probes of the second (fura-2, indo-1), third (fluo-3, rhod-3), and fourth (calcium green, calcium orange, Oregon green-488) generations were based on the use of Ca^{2+} chelators—EGTA or BAPTA—that were linked with a chromophore group capable of emitting fluorescence upon illumination with UV or visible light [13]. In some cases measurements of free calcium are based on the use of aequorin, a protein from jellyfish *Aequorea aequorea*, whose fluorescence increases with Ca^{2+} concentration in the medium. Recently so-called "chameleon" probes have been introduced for measuring calcium in plant cells. These probes, obtained in the Tsien's laboratory [88], represent a combination of calmodulin (CaM) and green fluorescent protein (GFP). Such a transformed CaM can effectively bind Ca^{2+} in the range from 10^{-8} to 10^{-2} M. Further information on measurements of free calcium with fluorescent methods can be found in a review [89] and a book [90].

Sometimes the changes in Ca^{2+} concentration are recorded with vibrating Ca^{2+}-selective electrodes [91, 92]. However, calcium electrodes cannot resolve rapid changes in Ca^{2+} concentration and have comparatively low cationic selectivity. In electrophysiological experiments the patch clamp method is more common, as a means of assessing Ca^{2+}-dependent changes of the membrane potential (MP). Neher and Sakmann [93] were the first to introduce the patch-clamp recordings of ionic currents as a research tool. The patch-clamp method allows local (point-wise) clamping of MP in very small cells (3–10 μm) and provides for measurements of single channel currents. Patch-clamp recordings of the transmembrane Ca^{2+} currents is a suitable tool to investigate rigorously very small, periodic, or local changes in cytosolic Ca^{2+} that cannot be recorded with fluorescent probes.

Ca^{2+} Levels in Cell Compartments

The concentration of Ca^{2+} in the cytosol is lower than in the cell walls or vacuoles by 3–4 orders of magnitude. In the cytoplasm of resting cells, the concentration of free calcium is exceptionally low, ranging from 100 to 200 nM [9, 10]. A 1 μm^3 sample of cytoplasm contains 60 to 120 Ca^{2+} ions. The intracellular organelles contain much higher amounts of free calcium. The Ca^{2+} concentration in the endoplasmic reticulum and mitochondria equals to 1 mM, whereas Ca^{2+} content in the cell wall and vacuoles reaches 1–10 mM [9, 10, 16]. There is a Ca^{2+} gradient between the nucleus and the cytoplasm, which indicates the presence of regulatory mechanisms controlling calcium transport through the nuclear membrane. It was shown that changes in cytosolic and nuclear Ca^{2+} levels are controlled by independent mechanisms [94]. Thus, the plasma membrane, the tonoplast, the membranes of mitochondria and endoplasmic reticulum maintain steep gradients of ionized calcium, which are much higher than the gradients of other ions. The Ca^{2+} gradients are maintained by various calcium pumps [95–97] that extrude Ca^{2+} from the cytoplasm to the external medium or sequester it in the organelles.

The discovery of sharp gradients of ionized calcium at the cell membranes led to a series of important assumptions eventually proved by experiments. (1) The Ca^{2+} permeability of the membrane lipid layer is very low. (2) Calcium enters the cytoplasm through Ca^{2+}-selective channels. (3) The plasma membrane and the membranes of cellular organelles are endowed with specific Ca^{2+} pumps (Ca^{2+}-ATPases) extruding calcium to the external medium or depositing it in the organelles. (4) Ca^{2+} is an effective regulator of metabolism in those cells that contain systems responding to changes of its concentration (in the range from 0.1 to 10 μM). The ability of plant organisms to ensure fine and rapid regulation of Ca^{2+} level in cellular compartments is determined by the concerted operation of Ca^{2+}-transporting membrane systems including various Ca^{2+} channels, Ca^{2+}-ATPases, and Ca^{2+}/H^+ antiporters [86].

Ca^{2+}-PERMEABLE CHANNELS

Functioning of the system of Ca^{2+} channels is the most important element in the process of generation,

encoding, and propagation of Ca^{2+} signals in the plant cell [10–12, 14, 45]. The occurrence of Ca^{2+}-permeable channels in the plasma membrane, tonoplast, the membranes of endoplasmic reticulum, chloroplasts, and the nuclear envelope of plant cells was revealed with electrophysiological, biochemical, and molecular–genetic approaches [98–101]. Unlike Ca^{2+} channels of animals, the major part of cation channels in plant cells are low selective with respect to mono- and divalent cations. These channels, often termed nonselective cation channels, represent a rather large and nonuniform group of channels. In addition to Ca^{2+} channels, these cation channels constitute an important element in generation and encoding Ca^{2+} signals [100]. These channels account for the low-affinity transport of cations, including Ca^{2+} ions [100, 102–104].

The known types of Ca^{2+}-permeable channels are classified in two main groups: voltage-gated and ligand-gated channels. Accordingly, these channels turn opened upon changes in membrane potential or upon interaction of some ligand (second messenger, hormone) with a specific receptor (this receptor can be a channel constituent or independent entity) [4]. The classification of channels into voltage-gated and ligand-gated types is quite arbitrary, because many voltage-dependent channels are directly controlled by receptors. On the other hand, the activity of ligand-gated channels may also depend on MP level [98, 99, 105]. The activity of Ca^{2+}-permeable cation channels can be also regulated by mechanical stimulation, interactions with cytoskeleton elements, as well as by phosphorylation and dephosphorylation [99, 100].

Ca^{2+}-Permeable Channels of the Plasma Membrane

The Ca^{2+}-permeable channels activated by depolarization of the plasma membrane turn open when the MP rises above –135 mV [106]. These Ca^{2+} channels display maximum activation upon depolarization of the plasma membrane towards the MP range from –60 to –100 mV, depending on given experimental conditions and plant material. The classic depolarization-activated Ca^{2+} currents were observed in protoplasts from the suspension culture of carrot cells [107, 108] and on protoplasts from the root cells of *Arabidopsis* [98, 109]. These Ca^{2+} currents appeared when the MP was more positive than –140 mV and disappeared when the MP was far more negative. The channel responsible for this current was permeable to divalent cations including Ca^{2+}, Ba^{2+}, Sr^{2+}, and Mg^{2+} [109].

In root cells of wheat and rye, two types of Ca^{2+}-permeable depolarization-activated channels were observed. The channels of the first type were termed *rca* (an abbreviation derived from $\underline{Ca}^{2+}$-permeable channels from wheat roots [110–112]), and the second type was called *maxi cation channel* [98, 102, 113, 114]. These depolarization-activated Ca^{2+}-permeable channels are thought to account for the Ca^{2+} currents arising upon cell depolarization. Both the maxi cation channel and the rca-channel are permeable to a wide range of mono- and divalent cations, including Ca^{2+}. However, the rca-channel is predominantly selective to Ca^{2+} when the ionic composition of the medium simulates physiological conditions [112]. This Ca^{2+}-permeable channel is blocked by micromolar concentrations of Al^{3+}, La^{3+}, Gd^{3+}, verapamil, diltiazem, ruthenium red, but is insensitive to 1,4-dihydropyridines [98, 111]. The pore in the rca-channel of plants is structurally different from that in the L-type Ca^{2+} channel of animals [112]. This distinction probably arises from different ionic compositions of the media and from different MP values characteristic of plant and animal cells. The depolarization-activated Ca^{2+} channels play an important role in plant responses to environmental factors. A range of signals, including blue and red light, phytohormones, symbiotic factors, and fungal elicitors, induce fast depolarization of the membrane that suffices for opening Ca^{2+}-permeable channels in the plasma membrane [98–100, 105, 114].

The Ca^{2+}-permeable channels activated by hyperpolarization were observed in the cells of root hairs, endoderm, epidermis, and cortex of *Arabidopsis* roots [115, 116]. These channels are more specific to Ca^{2+} than to K^+. These channels are similar in electrophysiological properties to Ca^{2+} channels of stomatal guard cells; the latter are activated by hyperpolarization and have different permeabilities to Ca^{2+}, Mg^{2+}, and K^+ [69, 98, 117, 118]. These channels are activated by ABA, which is apparently due to direct interaction of the hormone with the channel [118]. The hyperpolarization-activated Ca^{2+}-permeable channels may participate in the transduction of plant responses to elicitors and pathogens [119]. Tomato cells were shown to possess Ca^{2+} channels that turn open upon hyperpolarizing shift of the MP below –120 mV [120]. The activity of these channels was decreased upon depolarization. These Ca^{2+} channels were more permeable to Ca^{2+} and Ba^{2+} than to K^+ and Cl^-. The Ca^{2+} channels of this type are presumably significant for interactions of tomato plants with the pathogenic fungus *Cladosporium fulvum* [121].

The ligand-gated (receptor-regulated) Ca^{2+}-permeable channels are responsible for the increase in Ca^{2+} concentration in the cytoplasm upon binding of ligands (hormones, elicitors) to the receptors [4, 105]. The earliest response of stomatal guard cells to ABA treatment is the elevation of cytosolic Ca^{2+} level [45, 122]. This process is supposedly related to activation of voltage-gated nonselective Ca^{2+}-permeable channels in the plasma membrane [117, 123]. At the same time, the amount of inositol trisphosphate (IP_3) increases, which initiates the release of Ca^{2+} from the intracellular stores.

In 1988 Felle *et al.* [77] had already discovered that the auxin treatment induces oscillations of membrane potential, Ca^{2+}, and pH in the cytoplasm of epidermal cells from maize coleoptiles. Direct measurements of Ca^{2+}_{cyt} using the fluorescent Ca^{2+} probe fluo-3 and a confocal microscope revealed that the treatment of

maize coleoptile cells with the auxin 2,4-D over 4 min led to an increase in cytosolic Ca^{2+} level from 280 to 380 nM [38].

In our studies, we analyzed the action of auxin on Ca^{2+} transport by loading the probe indo-1 into the plasmalemma vesicles from the maize coleoptile cells [78–80]. The fluorescence of indo-1 entrapped in the vesicles was found to increase upon the addition of IAA (10 μM), which proved the induction of calcium permeability of vesicles by auxin. The stimulating effect of auxin was inhibited by Ni^{2+} ions (1 μM) known to inhibit receptor-operated ion channels in animal cells [4]. Experiments with the probe fura-2 revealed that 1-NAA induced calcium permeability of the plasma membrane in the protoplasts from wheat leaves [124]. The observed effects were supposedly due to the ability of auxin to activate Ca^{2+}-permeable channels—either receptor-operated [78] or nonspecific cation channels [124]. However, it should be kept in mind that the stimulating effect of auxin on calcium fluxes could be also related to the potential-dependent Ca^{2+} channels activated by depolarization. The activation of anion channels is known to be the most effective mechanism for depolarization of plant cells. The effects of auxin on potential- and ATP-dependent anion channels were also observed on tobacco protoplasts [125] and stomatal guard cells [126]. Hence, the activating effect of IAA on calcium fluxes can be mediated by auxin-induced activation of anion channels and by the membrane depolarization.

The earliest responses of plants to pathogens consist in changes of cytoplasmic calcium level and pH [13, 28]. Bach *et al.* studied the action of elicitors from the fungus *Pythium aphanidermatum* on ion transport in carrot protoplasts. The treatment of protoplasts with elicitors induced the influx of $^{45}Ca^{2+}$, which was associated with the efflux of K^+ to the medium. The authors proposed that this process involves nonselective Ca^{2+} channels. Further studies revealed two classes of elicitor-activated Ca^{2+} channels in the plasma membrane. The first elicitor-activated channel was observed in the plasma membrane of tomato protoplasts [120, 121]. This channel was inhibited by La^{3+} and nifedipine at micromolar concentrations. The second channel, termed large-conductance elicitor-activated channel (LEAC) was discovered in parsley protoplasts [127]. The binding of elicitors with the protoplast cell wall induced the influx of Ca^{2+} into the cell and the efflux of anions, which led to depolarization of the plasma membrane. Activation of these nonselective Ca^{2+}-permeable channels was the earliest response to the elicitor treatment. Furthermore, it was found that the elicitors activated nonselective Ca^{2+}-permeable channels indirectly, via some intracellular second messenger. The operation of LEAC was independent of MP (in the potential range from –30 to –150 mV) and was inhibited by La^{3+} and Gd^{3+} at micromolar concentrations.

The presence of mechanosensitive channels involved in Ca^{2+} transport was demonstrated for the plasma membrane and endoplasmic reticulum of plant cells [98, 105, 128–130]. These channels feature rather high Ca^{2+} specificity (the ratio of Ca^{2+}- to K^+-currents varied from 7 : 1 to 17 : 1 in different plant materials) and comparatively low single-channel conductance. The activity of mechanosensitive Ca^{2+} channels depended on membrane potential and pH. Mechanosensitive Ca^{2+}-permeable channels may participate in the transduction of mechanical stimuli associated with gravity-stimulation, membrane tension or shrinkage, touching or bending, and changes in osmotic potential of the cytoplasm and/or external medium. Ding and Pickard [131] discovered such channels in cells of onion epidermis.

The genome of *Arabidopsis* contains a series of genes encoding Ca^{2+}-permeable channels [101]. This group comprises the genes coding for the two-pore channel *AtTPC1* [132], the cation transporter of wheat LCT1 [133, 134], the channels regulated by cyclic nucleotides [135], and glutamate receptors endowed with ionophore functions [136]. The *Arabidopsis* genome contains a gene *At4g03560* encoding a highly selective Ca^{2+} channel activated by depolarization. The homologs of this gene termed *ATPC1* (*Arabidopsis* two-pore channel) are present in many plant species [132]. The gene *AtTPC1* encodes Ca^{2+} channel that consists of 12 transmembrane domains and includes two CaM-binding sites and two pores allowing the passage of Ca^{2+} ions [132]. The protein AtTPC1 is homological to α1- subunit of the L-type potential-dependent Ca^{2+} channel of animals [137]. The expression of *ATPC1* gene was particularly active in green seed pods and developing seeds of *Arabidopsis*; this gene was also expressed in leaves, stems, and root tissues [132]. When the expression of *ATPC1* gene was reduced, the leaf cells lost their ability to increase the cytosolic Ca^{2+} level in response to sucrose treatment [132].

The *Arabidopsis* genome contains about 20 genes encoding ion channels gated by cyclic nucleotides (CNGC, cyclic nucleotide-gated channel) [135, 138]. The *CNGC*-type genes were first isolated from the cells of barley aleurone [139] and tobacco leaves [140]. The CNG-channels of plants are permeable to mono- and divalent cations. Some of these channels have domains that bind cyclic nucleotides (cAMP, cGMP) and CaM, which allows the integration of signals arriving from different signaling pathways [135, 141–143]. The CNG-channels are supposed to fulfill functions in senescence and programmed death of plant cells [144]. In animals, CNG-channels are permeable to mono- and divalent cations.

The activation of ion channels by glutamate or other amino acids is a key event in the transmission of the nerve pulse through the synapse in animals [145]. The postsynaptic membrane contains Ca^{2+}-permeable cation channels formed by ionotropic glutamate receptors

(iGluR) [137]. The treatment of *Arabidopsis* seedlings with L-glutamate initiates Ca^{2+} currents through the plasma membrane of root cells, thereby leading to the increase in cytosolic Ca^{2+} concentration [146]. These Ca^{2+} channels were also permeable to monovalent cations (Na^+, Cs^+, and K^+) [100]. In the *Arabidopsis* genome, about 20 nucleotide sequences are homological to animal genes *iGluR* coding for the ionotropic glutamate-like receptor GLR [136]. Apart from *Arabidopsis*, homologs of *iGluR* animal genes were found in other plant species [136, 147, 148]. The predominant part of cloned *Arabidopsis* GLR is attributed to the plasma membrane. However, some glutamate-gated channels were present in the endomembranes. Glutamate plays a key role as a signaling molecule capable of interorganelle transport; it is engaged in synthesis and catabolism of amino acids and in photorespiration [100]. Apparently, glutamate takes part in the intracellular coordination of photosynthesis and nitrogen metabolism by controlling the glutamate receptor activity and, thereby, ion fluxes between cellular compartments.

Ca^{2+}-Permeable Channels of the Tonoplast

The central vacuole that occupies up to 90% of the cell volume is the main source of intracellular calcium. The Ca^{2+} flow from vacuole may account for elevation of cytosolic calcium concentration in response to a variety of environmental factors. Four types of calcium channels were identified in the vacuolar membrane [149]. Two of them are voltage-dependent, and two others are ligand-gated channels. One of the voltage-dependent Ca^{2+} channels is activated by hyperpolarization of the tonoplast [150–152]; the other, by depolarization [153–155].

The class of depolarization-activated Ca^{2+} channels includes slow vacuolar (SV) channels that were observed in many plant tissues [149, 156, 157]. These channels are activated when the tonoplast membrane potential ranging between –30 and –50 mV is shifted to +10–+30 mV and upon the increase in Ca^{2+}_{cyt} to 0.5–5.0 μM or above. The calcium-binding site is located on the cytoplasmic side of the channel protein. Therefore, it is thought [153, 158] that these channels can be involved in Ca^{2+}-induced calcium release (CICR). However, the occurrence of CICR in *in vivo* system has not been shown up to date. Therefore, it appears that SV channels are not involved in CICR [159]. The SV cation channels are nonselective; they are permeable to Ca^{2+}, Ba^{2+}, and Mg^{2+}, and, to a lesser extent, K^+, Na^+, Rb^+, and Cs^+ [98, 154, 155, 157, 159, 160].

The tonoplast Ca^{2+}-permeable channels activated by hyperpolarization display a higher permeability to divalent cations and provide for a substantial Ca^{2+} flux from the vacuole into the cytoplasm. These channels turn open at tonoplast MP values from –20 to –80 mV. These channels were also identified in the vacuoles from *Beta vulgaris* roots [150, 151] and in the guard cells of *Vicia faba* [152]. Two types of hyperpolarization-activated tonoplast channels were revealed. The first type channel is inhibited at Ca^{2+}_{cyt} concentrations above 1 μM [151]. This channel is permeable to Ca^{2+}, Ba^{2+}, and Sr^{2+}; it is insensitive to IP_3 and inhibited by nifedipine applied from the vacuolar side and by verapamil and La^{3+} applied from the cytoplasmic side [152]. The second type channel is insensitive to Ca^{2+}_{cyt} and is permeable to Ca^{2+}, Ba^{2+}, Sr^{2+}, Mg^{2+}, and K^+. The probability of the open state (P_o) of this channel increases with elevation of vacuolar Ca^{2+} concentration [152, 161].

The ligand-gated Ca^{2+} channels of the tonoplast comprise channels activated by such second messengers as IP_3 [162, 163] and cyclic ADP-ribose (cADPR) [164]. These Ca^{2+} channels are highly selective to divalent cations compared to monovalent cations. The treatment of isolated vacuoles and tonoplast vesicles with IP_3 and cADPR induced the Ca^{2+} efflux in preparations from various tissues of many plant species [149, 164, 165]. Experiments with microinjection of IP_3 and cADPR into stomatal guard cells demonstrated the ability of these substances to increase the cytosolic Ca^{2+} level, which supplies evidence for the occurrence and functioning of IP_3- and cADPR-sensitive Ca^{2+} channels in plant cells [42, 166].

The IP_3-dependent vacuolar channels are involved in turgor regulation during hyperosmotic stress, in stomatal closure, self-incompatibility response, and reorientation of pollen tube growth [14, 25, 98, 149, 165, 167]. The half-maximum activation of channels is attained at 200 nM IP_3. The probability of the open state for IP_3-gated channels (P_o) increases with hyperpolarization of the tonoplast [98, 167]. The opening of IP_3-gated channels is insensitive to Ca^{2+}_{cyt} oscillations (in the range from 0.1 μM to 1 mM). These channels are highly selective to divalent cations, i.e., Ca^{2+}, Ba^{2+}, and Sr^{2+}; they are inhibited by verapamil and heparin but are insensitive to ruthenium red and ryanodine [98, 149, 165, 167].

Experiments on red beet roots [149, 165] and stomatal guard cells [166] showed that cADPR initiates Ca^{2+}-dependent electric currents from the vacuole to the cytoplasm. The channels allowing passage of these currents are comparatively selective to Ca^{2+} and less permeable to K^+. The pharmacology of these channels is similar to cADPR-activated ryanodine receptors in endomembranes of animal cells. These channels are activated by ryanodine and caffeine but are suppressed by ruthenium red and procaine. In the stomatal guard cells, the vacuolar cADPR-channels are inhibited at Ca^{2+} concentrations above 600 nM, which implies that these channels are uninvolved in the Ca^{2+}-induced calcium release (CICR) [168].

Heparin and TMB8, specific inhibitors of IP_3-dependent channels, had no effect on cADPR-gated

channels. The cADPR channels are thought to participate in ABA-dependent signal transduction [45, 75].

The propagating Ca^{2+} wave in animal organisms originates from the Ca^{2+} release proceeding through the Ca^{2+}-channels of endomembranes. These channels are activated not only by second messengers such as IP_3 and cADPR, but also by low concentrations of Ca^{2+} (CICR phenomenon) [168]. When the Ca^{2+} concentration is increased to a certain level, these channels close and prevent calcium efflux from the intracellular stores. In animals, it is CICR mediated by IP_3-dependent channels of endoplasmic reticulum, that provides enhancement of Ca^{2+} signal and allows the propagation of excitation wave over the cytoplasm [169]. The attempts to reveal a similar mechanism in plant cells were unsuccessful so far. The IP_3- and cADPR-induced calcium currents across the vacuolar membrane are not activated by cytosolic calcium [149, 166, 167]. Thus, the question on mechanisms underlying the generation of Ca^{2+} waves in plant cells remains open.

Ca^{2+}-Permeable Channels of Endomembranes

Two classes of potential-dependent Ca^{2+}-permeable channels were identified in the membranes of endoplasmic reticulum. One of these, BCC1 (*Bryonia* calcium channel 1) was detected in mechanosensitive hairs of *Bryonia dioica* [130, 170], while the other one, LCC1 (*Lepidium* calcium channel 1) was found in the apex of *Lepidium sativum* roots [171]. Both channels displayed a rectifying conductance; they were more selective to Ca^{2+} than to K^+, and were permeable to Ba^{2+}, Sr^{2+}, and Mg^{2+}. The membrane potential range required for channel activation was strictly dependent on the transmembrane Ca^{2+} gradient. Both BCC1 and LCC1 channels were blocked by micromolar concentrations of Gd^{3+}, La^{3+}, and erythrosine B applied from the lumenal side of endoplasmic reticulum. Verapamil had no effect on LCC1 but blocked BCC1. The conductance and open-state probability (P_o) for BCC1 channel increased with Ca^{2+} concentration in the lumen of endoplasmic reticulum and also rose upon acidification of the cytoplasm [170].

Using membrane vesicles of endoplasmic reticulum isolated from cauliflower inflorescences (*Brassica oleracea*), a group of researchers demonstrated the release of Ca^{2+} under the action of IP_3 [172], cADPR [173], and nicotinic acid adenine dinucleotide phosphate (NAADP) [174]. At the same time, NAADP had no effect on Ca^{2+} transport in the tonoplast vesicles. These results provide evidence for the existence in endoplasmic reticulum of cADPR-, NAADP-, and IP_3-dependent Ca^{2+}-permeable channels. The high-affinity binding of IP_3 to endoplasmic reticulum also points to the existence of IP_3-gated Ca^{2+} channels [175]. The voltage-dependent channels permeable for Ca^{2+}, Mg^{2+}, and K^+ were revealed on thylakoids of spinach chloroplasts [176]. The voltage-gated channels permeable for K^+, Na^+, Cs^+, and Ca^{2+} were detected in nuclear membranes isolated from red beet [177].

ACTIVE TRANSPORT OF Ca^{2+} IN PLANT CELLS

Bush [9] was the first to recognize a possible role of Ca^{2+} pumps in signaling processes. The systems of active Ca^{2+} transport fulfill at least four functions in plant cells: (1) they replenish intracellular calcium stores to be used for channel-mediated Ca^{2+} release during signal transduction; (2) they maintain low Ca^{2+} concentration in the cytoplasm and account for spatiotemporal patterns of Ca^{2+} signal; (3) they supply Ca^{2+} for biochemical processes in cellular organelles; and (4) they furnish Ca^{2+} for membrane interactions, such as vesicle transport, membrane fusion, and secretion. The active Ca^{2+} transport is realized via Ca^{2+}/H^+ antiporters and Ca^{2+}-ATPases [95–97]. Both carriers utilize ATP as an energy source, but the operation of the Ca^{2+}/H^+ antiporter depends also on the transmembrane pH gradient.

Mitochondria, chloroplasts, and vacuoles are capable of accumulating calcium ions in large amounts (up to 1 mM). In membranes of these organelles, the Ca^{2+}-transporting systems are abundant but they have low affinity to Ca^{2+}. The operation of Ca^{2+} carriers in vacuoles, mitochondria, and especially chloroplasts ensures effective, though poorly selective removal of excessive Ca^{2+} from the cytosol. The Ca^{2+} pumps of the plasma membrane and endoplasmic reticulum provide more precise correction of the cytosolic calcium level owing to their higher Ca^{2+} affinity [178].

Ca^{2+}-ATPases

The Ca^{2+}-ATPases are engaged in fine regulation of cytoplasmic Ca^{2+} at the submicromolar level. The Ca^{2+}-ATPases (together with Ca^{2+} channels) participate in creating specific spatiotemporal pattern (coding) of Ca^{2+} signals. During the signal transmission, Ca^{2+}-ATPases restore the cytosolic calcium concentration to the initial level by pumping Ca^{2+} out of the cell or by pumping it into organelles, such as vacuoles and endoplasmic reticulum [178]. The calcium pumps belong to a superfamily of P-type ATPases, because the enzyme-mediated Ca^{2+} transfer includes the formation of phosphorylated intermediate (E ~ P). These ATPases are inhibited by *ortho*-vanadate and utilize ATP energy for ion movement. Erythrosine B at a concentration of 0.5 μM is often used as an effective inhibitor of Ca^{2+}-ATPases. The Ca^{2+}-ATPases feature a higher affinity to Ca^{2+} (K_M = 0.1–2.0 μM) and a lower capacity, as compared to Ca^{2+}/H^+ antiporters (K_M = 10–15 μM).

Based on the analysis of amino acid sequences, the plant cell calcium pumps are divided into two families (in similarity with the classification of animal Ca^{2+} pumps) [95, 96, 179]: the pumps of IIB type (plasmalemma-type or ACA-type, autoinhibited Ca^{2+}-ATPase) and the pumps of IIA type (endoplasmic reticulum-type

Ca^{2+}-ATPase, ECA). The pumps of these two types differ in sensitivity to CaM and cyclopiazonic acid [180]. The main distinction of IIB-type Ca^{2+}-ATPases is their activation by Ca^{2+}-dependent protein CaM. Unlike animal cells, plant cells contain IIB-type Ca^{2+} pumps not only in the plasma membrane but also in other membranes (tonoplast, endoplasmic reticulum) [95, 96, 181].

Apparently, the Ca^{2+} pumps of IIB-type constitute the basis for precise regulation of calcium level in the cytoplasm. The system of Ca^{2+} homeostasis seems to include CaM and Ca^{2+}-dependent protein kinases (CDPK) that exert regulatory effect on the activities of Ca^{2+} pumps. Hwang *et al.* [182] showed that, at low calcium concentrations in the cytosol, one of CDPK (CPK1) inhibits the activity of Ca^{2+}-ATPase in the endoplasmic reticulum by means of phosphorylation of serine-45. As calcium concentration increases, CaM is activated and binds to the autoinhibitory domain of the Ca^{2+} pump, thereby releasing the inhibition caused by CDPK. Thus, the Ca^{2+} flux from the cytoplasm to the endoplasmic reticulum and, consequently, the level of ionized calcium in the cytosol are determined by the activity ratio of two calcium sensors, i.e., CaM and CDPK having different affinities to Ca^{2+}.

The Ca^{2+} pumps of IIA type are insensitive to CaM but are specifically inhibited by cyclopiazonic acid at concentrations below 0.1 μM. The IIA-type Ca^{2+}-ATPases were detected not only in the endoplasmic reticulum, but also in the tonoplast and plasmalemma [96]. Generally, the structures of Ca^{2+}-ATPases of IIB- and IIA-types are similar. These ATPases are composed of ten transmembrane domains. The molecular–genetic analysis of the peptide sequences showed that domains 4, 5, 6, and 8 are needed for translocation of Ca^{2+} ions across the membrane and that the large central loop contains the sites for ATP binding and phosphorylation. The IIB-type Ca^{2+}-ATPase, unlike the IIA-type Ca^{2+} pump, contains an autoinhibitory domain that mediates interactions with CaM [96].

It should be noted that the autoinhibitory domain in the IIB-type Ca^{2+}-ATPase of plants is located on the N-terminus of the protein molecule, whereas in animals it occupies the C-terminal side. In *Arabidopsis*, there are 14 genes encoding Ca^{2+}-ATPases [178]. Several plant genes are known to encode IIB-type pumps. These are *ACA1* and *ACA2* of *Arabidopsis* [183, 184] and *BCA1* of *Brassica oleracea* [181]. Several genes encoding IIA-type pumps have been cloned, including *LCA1* of tomato [185], *OCA1* of rice [186] and *ECA1p/ACA3p* of *Arabidopsis* [187].

Ca^{2+}/H^+ Exchangers

The Ca^{2+}/H^+ antiporters can drive Ca^{2+} against its concentration gradient at the expense of energy of the electrochemical proton gradient [188]. The Ca^{2+}/H^+ antiporters have comparatively low affinity to Ca^{2+} (K_M = 10–15 μM) and a powerful capacity. The stoichiometry of coupled Ca^{2+}/H^+ transport at the tonoplast is 1 : 3. The Ca^{2+}/H^+ antiporters are usually activated upon drastic increase in the cytosolic calcium content. The proton gradient at the vacuolar membrane originates from the operation of H^+-ATPase or H^+-pyrophosphatase. The coupled Ca^{2+}/H^+ transport can be easily detected, as it is sensitive to a specific inhibitor of vacuolar ATPases bafilomycin and protonophores and insensitive to *ortho*-vanadate, an inhibitor of P-type ion-transporting ATPases [96, 97, 178].

The first cloned gene encoding the Ca^{2+}/H^+ antiporter of plants was *CAX1* (cation exchanger 1) [97, 189]. The gene was identified by its ability to restore growth on high-Ca^{2+} media of a yeast mutant defective in vacuolar Ca^{2+} transport. The CAX1 antiporter appears to transport Ca^{2+} with a low affinity (K_M is about 13 μM), comparable with the kinetics of Ca^{2+}/H^+ antiport activity in the tonoplast of oat root vacuoles [190]. Although these data suggest that CAX1 is localized in the vacuolar membrane, the presence of Ca^{2+}/H^+ antiporters in other locations, such as the plasma membrane, is not excluded [191]. The tonoplast-localized Ca^{2+}/H^+ antiporters have been isolated from *Arabidopsis* [189] and mung bean [192]. Experiments on *Arabidopsis* revealed more than ten genes encoding exchangers homologous to *CAX1* [135]. However, it remains yet unknown whether all proteins encoded by these genes are involved in the transport of Ca^{2+}. For example, CAX2 was found to transport Mn^{2+} in addition to Ca^{2+} [97].

PRINCIPLES OF GENERATION AND PROPAGATION OF CALCIUM SIGNAL

In a resting unexcited cell, Ca^{2+} channels usually reside in closed state. The signals arriving to organisms from the environment and internal media activate Ca^{2+} channels, which initiate calcium flows from the compartments with high electrochemical potential towards low electrochemical potential of Ca^{2+}. As a result, the cytoplasmic regions near the inner gate of Ca^{2+} channels turn to be local sites with elevated concentration of ionized calcium. Such a local signal is an elementary event that underlies the system of calcium signaling [6, 66, 84, 193, 194]. Elementary (local) calcium signals may give rise to responses of two types. First, they may influence cellular processes in the immediate vicinity to activated channels. Second, the elementary signals may affect other types of Ca^{2+} channels, thereby initiating much larger increases in cytoplasmic Ca^{2+} level that appear as Ca^{2+} oscillations and Ca^{2+} waves. These changes may activate global cellular processes.

The most typical example of Ca^{2+} signals generated locally and spreading over the cell is the responses of *Fucus* embryo rhizoids to the action of hypoosmotic shock, reactive oxygen species, and IP_3 [66, 195]. The local increase in Ca^{2+}_{cyt} in response to the above treatments originates near the nucleus region and lasts for

about 15–30 ms. When several single Ca^{2+} signals are combined into a cluster, a spreading Ca^{2+} wave are generated. The Ca^{2+}-induced Ca^{2+} release (CICR) from the organelles and the respective initiation of a Ca^{2+} wave are apparently caused by reactive oxygen species (such as H_2O_2) arising in *Fucus* rhizoid cells in response to osmotic stress [195]. The process of signal propagation over the cytoplasm is ensured by sequential activation/inhibition of Ca^{2+} channels and Ca^{2+} pumps in the endoplasmic reticulum. Thus, the calcium impulse is propagated owing to cyclic increases and decreases of Ca^{2+} concentration in the cytoplasm [7, 193].

The amplification of single Ca^{2+} signal is realized through mobilization of endomembrane Ca^{2+} channels and Ca^{2+} pumps, through activation of other second messengers (IP_3, cADPR), modification of cytoskeleton elements, and, most important, via interaction of calcium with Ca^{2+}-binding sensor proteins (figure). After binding Ca^{2+} to sensor proteins (CaM, Ca^{2+}-dependent protein kinase, calreticulin, etc.), the calcium signal is transmitted (usually by means of reversible phosphorylation) to effector mechanisms (enzymes, cytoskeletal proteins, transcription factors, and ionic channels), which initiates Ca^{2+}-dependent physiological processes. The Ca^{2+} signal is later extinguished when the excess of Ca^{2+} is removed from the cytoplasm by Ca^{2+} pumps or deposited in the organelles. The depletion of cytosolic Ca^{2+} is accompanied by protein dephosphorylation, which returns the cell to the initial state [10–12].

The Coding of Calcium Signals

How can Ca^{2+} convey stimulus specificity during transduction of various signals while being involved in so many signaling pathways? The main system elements for coding and decoding Ca^{2+} signals are different types of Ca^{2+} channels, Ca^{2+}-ATPases, and Ca^{2+}-binding sensor proteins. The basis for specific coding calcium signals is that different environmental stimuli induce specific oscillations of the cytosolic Ca^{2+}, dissimilar in localization, amplitude, and frequency [14, 194, 196, 197]. The critical role belongs to plant cell Ca^{2+} channels and Ca^{2+}-ATPases involved in coding and transduction of Ca^{2+} signals [10–12, 14, 84]. Specific features of Ca^{2+} signal depend not only on Ca^{2+} channels of the plasma membrane, endoplasmic reticulum, and tonoplast, but also on Ca^{2+}-transporting membrane systems located in chloroplasts, the inner mitochondrial membrane, and nuclear membrane [98–101, 198]. The presence of a variety of Ca^{2+} channels implies multiple factors regulating the Ca^{2+} entry into the cytoplasm: membrane potential, IP_3, cyclic nucleotides, and mechano-osmotic treatments. The Ca^{2+} pumps play an important role in generation and coding the Ca^{2+} signals in plant cells. By extruding Ca^{2+} from the cytosol the pumps participate (together with Ca^{2+} channels) in creating specific spatiotemporal patterns of the Ca^{2+} signal.

Unlike other ions, Ca^{2+} cannot readily diffuse within the cytoplasm. The rate of Ca^{2+} diffusion in the cytosol is two orders of magnitude lower than in aqueous solutions; this is because Ca^{2+} is absorbed by organelles and binds to cytoplasmic and cytoskeletal proteins [16, 194]. The slow diffusion rate is one of the factors conferring specificity to Ca^{2+} signals because it allows local Ca^{2+} increases in particular regions of the cytoplasm, in the time range sufficient for signal transduction through specific calcium sensor proteins. The Ca^{2+} inability of rapid diffusion facilitates the formation of sustained Ca^{2+} gradients within the cytoplasm, and these gradients provide the basis for vesicle secretion and tip growth of cells.

The cells comprise numerous compartments containing abundant amounts of calcium. Different signals mobilize Ca^{2+} from different sources to increase its level in the cytoplasm [54, 56, 196]. For example, the chilling-induced rise in cytosolic Ca^{2+} was prevented by inhibitors of the plasma-membrane Ca^{2+} channels but was insensitive to inhibitors of Ca^{2+} channels of organelles. By contrast, the wind-induced Ca^{2+} rise was sensitive to Ca^{2+} channel blockers specific to organelles but not the plasmalemma [199]. When the plant cells were treated with NaCl and mannitol, calcium flowed out of the vacuole [28, 59]. Elicitors induced calcium entry from the apoplast [31, 63]. Anoxia triggered the Ca^{2+} efflux from mitochondria but not from vacuoles [61]. The ABA treatment raised the Ca^{2+} level by mobilizing Ca^{2+} from both intra- and extracellular sources [45, 73, 75].

To emphasize the heterogeneity of calcium signals in their amplitude, frequency, and localization, a term *"calcium signature"* was recently introduced. Some authors [194] draw an analogy of calcium responses to particular signals with fingerprints, because each of these signals features unique spatial structure. The hypothesis of Ca^{2+} signature is often applied to explain the specificity of signaling pathways involving Ca^{2+} as a second messenger. In this case, the specificity of Ca^{2+} signal, coded into its spatial properties, may serve as a selective trigger for cell responses. There are numerous processes to rely on the shape of Ca^{2+} signal as a basically important trait for coding specific information [194, 197]. The brightest examples include pollen tube growth and cell responses to ABA and symbiotic signals (Nod-factors) [25, 26, 45, 47]. However, some facts indicate that the Ca^{2+} signature mechanism is not the universal tool of Ca^{2+}-mediated information transmission. Recent evidence favor an alternative hypothesis, according to which calcium can not provide the signal specificity by itself; the Ca^{2+} signal seems to be only a chemical trigger acting in concert with other signaling pathways [200].

Oscillations of Ca^{2+} concentration in the cytoplasm. The diffusion flows are frequently ineffective for information transmission over the cell. The wave-type information transmission is faster and economically reasonable. In this case the signal encoding can rely both on frequency and amplitude of oscillations of cytosolic Ca^{2+} level. A paradoxical feature of calcium signaling is the following. Although the increase in Ca^{2+} level is indispensable for signal generation, a long-term maintenance of high calcium concentration in the cytoplasm can be lethal for cell, partly because Ca^{2+} binds to phosphates and disturbs the cell energetics [6, 194]. Therefore, the cells are able to transfer Ca^{2+}-encoded information without risk of lethal damage by using either low-amplitude or very short calcium signals. The oscillations of Ca^{2+}_{cyt} during signal transduction appear as individual spikes (single oscillations of cytosolic Ca^{2+}), oscillations (rhythmic changes of cytosolic Ca^{2+}, repetitive spikes), or Ca^{2+} waves spreading over the cell [84, 194, 197]. The calcium waves, oscillations, spikes, and gradients were observed in many cell types and are considered as primary forms of spatiotemporal organization of the Ca^{2+} signal [194].

Ca^{2+} spikes. Individual oscillations (spikes) of ionized calcium level in the cytoplasm arise upon a variety of treatments: heat [58]; chilling [55, 57]; hypoosmotic [64, 65] and hyperosmotic [52] shocks; anoxia [60–62]; oxidative stress [68, 69]; elicitors [28, 30, 31, 63]; illumination with red [20, 40], blue [41], and UV [201] light; and mechanical stimulation [28,67]. It should be noted that Ca^{2+} spikes of variable shape (calcium signatures) are induced not only by different stimuli but also by identical stimuli applied to different cells [56].

Ca^{2+} oscillations. Rhythmic changes of the ionized calcium concentration in the cytosol (Ca^{2+} oscillations) can arise from alternation of Ca^{2+} fluxes directed into the cytoplasm and outward. They result from the cyclicity of Ca^{2+} release and Ca^{2+} uptake (through Ca^{2+} channels and Ca^{2+} pumps) by the cell compartments, such as endoplasmic reticulum, cell wall, and other organelles. The membrane system capable of generating one or several Ca^{2+} spikes by means of inward and outward Ca^{2+} fluxes is sometimes called *Ca^{2+} oscillator* [202]. Such an integrated system includes at least one type of Ca^{2+} channels and one type of Ca^{2+} pumps that are capable of forming specific pattern of Ca^{2+} signal (Ca^{2+} signature) by cyclic operation in a coupled mode.

Felle [77] was the first to reveal Ca^{2+} oscillations in plants: the oscillations were observed on maize coleoptile cells treated with auxin. The use of fluorescent probes revealed repetitive Ca^{2+} spikes in cells of oat coleoptiles irradiated with red and far red light [21], as well as in root cells of *Arabidopsis* exposed to anaerobic conditions [60]. Growing pollen tubes produce a sustained Ca^{2+} gradient increasing towards the apex; this gradient oscillates with a period of about 40 s [47, 203, 204]. In the epidermis and pericycle of *Arabidopsis* roots, oscillations of ionized calcium were induced by mannitol or NaCl treatments [56].

Calcium oscillations can be induced by Nod-factors in the cytoplasm of root hairs of legumes. After the inoculation of *Medicago sativa* with *Rhizobium* (or after the treatment with Nod-factors), rhythmic Ca^{2+} oscillations appeared in the root hairs (with the period of about 90 s and the amplitude of 400–500 nM) and continued until the onset of nodule formation [26]. Similar Ca^{2+} oscillations were also observed in the root hairs of pea and alfalfa *M. truncatula* upon the treatment with the respective Nod-factors [205, 206]. Some mutants of alfalfa (*dmi1* and *dmi2*) and pea (*sym8, sym10*, and *sym 19*) with the poor nodule formation produced no Ca^{2+} oscillations or very weak oscillations in response to Nod-factors. However, other mutants also defective in early nodulation responses (*dmi3* in alfalfa and *sym 2A, sym7, sym9*, and *sym30* in pea) produced Ca^{2+} oscillations that were identical to those in the wild-type plants.

The earliest response of stomatal guard cells to ABA treatment consists in the increase (within 2 s) of Ca^{2+} level in the cytosol due to activation of Ca^{2+} channels in the plasma membrane [44, 45, 75, 157, 207]. The phytohormone is thought to activate the voltage-dependent poorly selective Ca^{2+} channels of the plasmalemma. The synthesis of second messengers (IP_3 and cADPR) is activated almost synchronously and initiates the Ca^{2+} release from the vacuole. The increase in Ca^{2+} concentration in the cytoplasm is accompanied by the activation of anion channels in the plasma membrane and its prolonged depolarization caused by the efflux of anions from the guard cells. The plasma membrane depolarization and the increase in Ca^{2+}_{cyt} lead to closing of inward-rectifying K^+ channels and opening of outward-rectifying K^+ channels responsible for the inward and outward flows of potassium in the guard cells. These events result in the drop of turgor pressure and stomatal closure.

Remarkably, the ABA-induced changes in cytosolic Ca^{2+} level proceed in oscillating regime (with the period of 10–12 min and amplitude of 200–600 nM) and remain evident for almost 60 min after the hormone treatment [74, 207]. The Ca^{2+} oscillations were induced not only by ABA but also by changes in the external Ca^{2+} concentration. The parameters of Ca^{2+} oscillations depended on the extracellular Ca^{2+} concentration, i.e., the higher was the Ca^{2+} level in the external medium, the larger was the amplitude of Ca^{2+} oscillations in the cytoplasm of stomatal guard cells.

The mechanism of osmotic regulation in the stomatal guard cells cannot be comprehended without considering the role of vacuole in this process, because the vacuole occupies up to 90% of cell volume. The movements of stomata are accompanied by translocation of solutes not only across the plasmalemma but also the tonoplast. The main route for the efflux of K^+

from the vacuole is through the vacuolar K^+ channels (VK-channels) that are activated upon the increase in Ca^{2+}_{cyt} level up to 1 μM. When the cytosolic Ca^{2+} concentration decreases, the efflux of K^+ from the vacuole is mediated by fast vacuolar tonoplast channels (FV-channels) [45, 75, 157].

The unique ability of guard cells to integrate information on stimuli causing Ca^{2+} oscillations and "decipher" the parameters of Ca^{2+} signal apparently allows them to adjust the stomatal aperture [74, 75, 84, 207–209]. The number, frequency, and the amplitude of Ca^{2+} oscillations control the maintaining guard cells in a low-turgor condition (closed stomata). Specific Ca^{2+} oscillations resulting in stomatal closure are induced not only by ABA but also by light, CO_2, and drought [75].

Allen and colleagues [209] analyzed Ca^{2+} oscillations and stomatal movements on ABA-insensitive *Arabidopsis* mutant *gca2*. Although ABA induced Ca^{2+} oscillations in this mutant, these oscillations substantially differed from those observed in the guard cells of the wild-type plants. In addition, the ABA treatment did not cause closing of stomata in the *gca2* mutant. In subsequent experiments [209] the authors attempted to restore the ability of stomatal movements in mutant plants by artificial induction of Ca^{2+}_{cyt} oscillations. Using the parameters of Ca^{2+} oscillations observed during ABA-induced closing of stomata in the wild-type plants, the authors successfully induced the stomatal closure in the *gca2* mutant of *Arabidopsis*. These results provide convincing evidence that guard cells are indeed capable of deciphering information coded in the parameters of Ca^{2+}_{cyt} oscillations.

Ca^{2+} waves. The information transmission by means of Ca^{2+} wave propagation is particularly interesting. While spreading over the cell, the Ca^{2+} wave activates global cell processes rather than local events [194, 210]. Upon the interaction of several cells, Ca^{2+} waves can be even transmitted to neighboring cells [211]. Cells with tip growth, such as pollen tubes, provide a good example of Ca^{2+} wave generation [25, 194]. The growing pollen tube maintains a sharp tip-focused oscillating gradient of Ca^{2+} ions [47, 203, 204]. The peak concentration of Ca^{2+} is attributed to a small region in the apical part of pollen tube where the membrane of vegetative cell is continuously renewed owing to incorporation of new vesicles [194]. As soon as the Ca^{2+} wave approaches the apex, the growth of pollen tube ceases and changes its direction. The redirection of pollen tube growth is preceded by the local increase in cytosolic Ca^{2+} level on a cell side to which the pollen tube will bend [194].

Rigorous studies of the above events showed that the apical Ca^{2+} oscillations coincide in frequency with the rhythmic changes in pollen tube growth rate [47, 203]. The Ca^{2+} waves, generated with a period of 20–80 s and the amplitude peaking 1 μM Ca^{2+} in the nucleus region, are propagated toward the apex. The higher was the peak of calcium level changes, the higher was the growth rate of the pollen tube. The question of whether the Ca^{2+}_{cyt} oscillations are the cause or the result of growth oscillations remains open because some data suggest that inward Ca^{2+} currents are unrelated to growth pulsations [204, 212]. It was found that Ca^{2+} oscillations were delayed with respect to growth rate pulsation by 4–11 s [204]. This finding suggests that the Ca^{2+} entry occurs after growth rate pulsation of the pollen tube. It was supposed that Ca^{2+} oscillations are engaged in regulation of periodic secretion of new material required for pollen tube growth.

The waves of ionized calcium were also recorded during fertilization [92, 213]. The penetration of sperm cell into the maize egg cell initiated within 1.8 ± 0.6 s a sharp local increase in Ca^{2+} concentration at the fusion site. Next, this calcium signal was amplified and propagated as a Ca^{2+} wave, spreading all over the cell within 50–60 min at a rate of 1.13 μm/s.

The Decoding of Calcium Signals

Calcium-binding proteins are the main intracellular targets for Ca^{2+} ions. Some of these proteins are engaged in ion transport, and others serve as Ca^{2+} buffer supporting low calcium level in the cytosol [178]. The binding of Ca^{2+} with such proteins does not produce significant changes in their structure. On the other hand, when calcium interacts with proteins exerting regulatory functions, the formation of Ca^{2+}–protein complex leads to substantial structural changes in the protein molecule, and the molecule acquires the ability of further signal transduction. These proteins, called *sensors* are responsible for the decoding of calcium signal (figure). In *Arabidopsis*, there are more than 150 proteins engaged in the Ca^{2+}-mediated signal transduction [15]. Some data suggest that about 2% of *Arabidopsis* genome code for proteins that are involved in the calcium signaling system. The sensor proteins contain three types of Ca^{2+}-binding sites: the EF hand (EF-motif) [214, 215], the annexin folds [216–218], and the C_2-domain [219, 220].

The major part of identified Ca^{2+}-binding proteins contains a unique sequence of 12 amino acid residues. This sequence binds Ca^{2+} with a high affinity [221]. Kretsinger *et al.* [215] were the first to investigate the structure of Ca^{2+}-binding site by using a Ca^{2+}-binding protein parvalbumin and the X-ray structural analysis. The structure of Ca^{2+}-binding sites is often compared with a right hand model. The thumb and the forefinger stretched apart correspond to two α-helical segments of the protein, whereas other clenched fingers represent the Ca^{2+}-binding loop. Since one of α-helices on X-ray diffraction images was designated "E" and the other was termed "F," specific Ca^{2+}-binding sites were named *EF-hand* (or *EF motif*). Calcium is positioned in the center of the octahedron; it is held by coordination

bonds with 6–8 oxygen-containing groups of constituent amino acids of the Ca^{2+}-binding loop.

The Ca^{2+}-binding proteins containing EF-hands respond to Ca^{2+} concentration increase in two ways. The proteins of the first group, e.g., parvalbumin and calbindin do not appreciably change their conformation upon binding Ca^{2+} and serve as Ca^{2+} buffers or Ca^{2+} carriers. The second group of EF-hand proteins includes Ca^{2+} sensors that undergo conformational change upon binding Ca^{2+}, which enables them to interact with target proteins and modulate their functions [221, 222]. After the cytosolic Ca^{2+} concentration decreases to the initial level, Ca^{2+} dissociates from the Ca^{2+}-binding proteins, thus inactivating them and making unable to interact with their target proteins. The proteins of this superfamily differ in the number of EF-hands (from 1 to 6), affinity to Ca^{2+} (K_d ranging from 10^{-5} to 10^{-9} M), selectivity, and affinity to target proteins [223, 224]. The calcium sensors are arbitrarily divided in four types: calmodulins, Ca^{2+}-dependent protein kinases (CDPK), and other proteins either containing or lacking EF-hands.

Calmodulin and Other Proteins Containing EF-Motif

Calmodulin is a highly conserved and unique calcium receptor of eukaryotes [221, 223–225]. A typical CaM consists of 148 amino acid residues and contains 4 EF-hands that bind four Ca^{2+} ions. The CaM content in the cytoplasm ranges from 10^{-5} to 10^{-6} M. CaM was identified in all lower and vascular plants. CaM of plant organisms differs in structural–functional features from animal and yeast CaM. Molecular weight of plant calmodulins is about 16.7–16.8 kD. The amino acid sequences for plant and algal CaM are highly conserved (84–100% homology). Calmodulins of higher plants are characterized (unlike animal CaM) by a large variety of isoforms. The CaM-binding proteins in plants are more numerous than in animals [222–224].

One of surprising features of CaM is its ability to bind specifically to multiple target proteins and modulate their activity. Calmodulin acquires this capacity after binding four Ca^{2+} ions (K_d 10^{-7}–10^{-6} M). In plants CaM-modulated proteins include glutamate dehydrogenase and NAD kinase, DNA-binding proteins, superoxide dismutase, some cytoskeletal proteins, myosins and heat shock proteins, Ca^{2+}-ATPases and ion channels, protein kinases and enzymes of phospholipid metabolism, chaperones and proteins engaged in hormonal signal transduction [15, 222, 224]. The binding of 4 Ca^{2+}–CaM complex to the respective target proteins (affinity of this binding is in the nanomolar range) occurs by virtue of hydrophobic and electrostatic interactions. In most cases the target proteins interact with the activated calcium form of CaM (4Ca-CaM form). However, some proteins are capable of binding to CaM in the absence of calcium [226].

Since CaM has no enzymatic activity of its own, it serves as a link between external signals and cell physiological responses in a variety of signaling pathways. CaM participates in regulation of cell growth and proliferation [225], as well as in plant responses to such stimuli as red and blue light, gravity and mechanical treatments, phytohormones and pathogens, salt and osmotic stresses, anoxia and heavy metals, the cold and osmotic shocks [224]. The genes coding for different CaM isoforms are differentially expressed in response to different stimuli [222–224]. The presence of numerous CaM isoforms in plants is basically important for providing differential sensitivity to elevations of Ca^{2+}_{cyt} in response to various stress factors. For example, one of eight CaM isoforms in potato (PCaM1) is induced by touch [227], and two of five CaM isoforms in soybeans (SCaM4-5) are induced under the action of elicitors or pathogens [228].

Apart from CaM, the EF-hand containing calcium-binding sensor proteins include calcineurin B-like (CBL) proteins [229–231]. In *Arabidopsis* there are six *CBL* genes that code for similar but functionally different Ca^{2+}-binding proteins [230]. The drought, cold, and wind induce the appearance of gene transcripts *AtCBL*1, whereas *AtCBL*2 and *AtCBL*3 are expressed constitutively [230]. One of CBL proteins involved in adaptation to salt stress is SOS3 (abbreviated from salt-overly-sensitive); it contains three EF-hands. The gene *SOS*3 encodes a Ca^{2+} sensor similar to calcineurin. The SOS3 protein is thought to regulate the K^+/Na^+-transporting system in a Ca^{2+}-regulated fashion [229, 232].

In *Arabidopsis* there are also some other CaM-like genes; one of them, *TCH* (abbreviated from "touch") is induced in response to mechanical and chemical stimuli [233]. The TCH protein is composed of 324 amino acids and contains six EF-hands [234]. A CaM-like protein, called EFA27 with a mol wt of 27 kD was isolated from rice seedlings treated with ABA. This protein contains one EF-motif and is induced in response to salinity, dehydration, and ABA treatment. *Arabidopsis* contains several genes homologous to *EFA27* [235]. Another Ca^{2+}-binding protein AtCP1 (calcium binding protein) has three EF-hands [236]. The expression of gene *AtCP1* was induced by treatment with NaCl but not ABA. The infection of *Arabidopsis* plants with bacteria induced the expression of genes of primary response. These genes code for a small (about 20 kD) Ca^{2+}-binding protein centrin that comprises 4 EF-hands [237]. During the hypersensitive response that develops in soybean plants injured by *Pseudomonas syringae*, a gene *PvHra*32 is actively expressed. This gene encodes a Ca^{2+}-binding protein composed of 161 amino acids that produce four EF-hands [238].

Ca^{2+}-Dependent Protein Kinases

After binding Ca^{2+} to sensor proteins, subsequent transduction of the Ca^{2+} signal is realized via phosphorylation of target proteins and modulation of gene expression. Phosphorylation is one of the best-studied

types of post-translation modification of proteins [15, 221, 222, 224]. This process usually involves three proteins and two reactions catalyzed, respectively, by protein kinase and protein phosphatase.

In plant organisms, at least five types of protein kinases controlled by CaM and/or Ca^{2+} are distinguished: (1) Ca^{2+}–CaM-dependent protein kinases, CCaMK; (2) CaM-dependent protein kinases, CaMK; (3) Ca^{2+}-dependent (CaM-independent) protein kinases, CDPK; (4) CDPK-related protein kinases, CRK; (5) SOS3/CBL-interacting protein kinases, SIPK/CIPK [15, 239–241].

Ca^{2+}-dependent (CaM-independent) protein kinases (CDPK) are the most widespread Ca^{2+}-regulated plant proteins. CDPK were observed only in plants and remain undetected in yeast or animal cells [222, 239, 240]. The analysis of *Arabidopsis* genome indicates the presence of 34 genes coding for CDPK [240]. These 34 genes are distributed among all five chromosomes of *Arabidopsis.* Apart from *Arabidopsis*, other plant species, such as soybean, tomato, rice, and maize also contain multigene CDPK families [242].

The CDPK molecule comprises a protein kinase domain and a similar CaM-regulated (autoinhibitory) domain containing four Ca^{2+}-binding EF-hands that inhibits the enzyme activity in the absence of Ca^{2+}. Upon binding Ca^{2+} to CDPK, the inhibition is released, which activates the enzyme [239]. The catalytic domain of CDPK is similar to Ca^{2+}/CaM-dependent protein kinase. Thus, CDPK represents a particular type of Ca^{2+} sensor capable of both binding Ca^{2+} and exhibiting protein kinase activity. The CDPK activity can be regulated not only by Ca^{2+} but also by phosphorylation–dephosphorylation processes, some phospholipases, and 14-3-3 proteins. The attachment of myristic acid (14 : 0 fatty acid) to the N-terminal glycine (with a covalent bond) facilitates the interaction of CDPK with the membranes [239, 240]. Therefore, CDPK can exist both in soluble form in the cytosol and in the bound form associated with membranes, chromatin, or cytoskeleton.

The substrates for CDPK include the enzymes of carbon and nitrogen metabolism, stress proteins, membrane carriers, ion channels, cytoskeletal proteins, and transcription factors. The CDPK activity can be modulated by various stress factors and phytohormones (ABA, IAA, gibberellin, jasmonic acid, and cytokinins). Various forms of CDPK can control growth and development, carbon and nitrogen metabolism, membrane transport, expression of stress-inducible genes, and the system of defense responses against pathogens [239, 240, 243, 244].

Ca^{2+}-Binding Proteins without EF-Motifs

In plant cells, there are several types of Ca^{2+}-binding proteins without EF-hands in their structure. These proteins include annexins [245], C_2-domain-containing proteins [220], calreticulin and calnexin [246], and some others [15].

Annexins represent a multigene family of Ca^{2+}-, phospholipid-, and cytoskeleton-binding proteins. In plants, animals, and fungi annexins may constitute up to 0.1% of cell proteins [217, 218, 245, 247]. Annexins were observed in many plant species and are represented by a small gene family. For example, *Arabidopsis* contains seven identified genes coding for annexins [247]. Annexins are supposed to participate in vesicular secretion and membrane calcium transport [99, 247]. The identity of annexins is due to a conserved C-terminal domain called an "annexin core." This domain, containing up to 70 amino acid residues, allows annexins to interact with membranes in a Ca^{2+}-dependent manner and/or form voltage-dependent Ca^{2+} channels [248]. The annexin core is composed of four tandem-type repeats with similar amino acid sequences. Each repeat consists of five tightly packed α-helices and contain a region (termed "endonexin fold") with a specific amino acid motif (G-X-G-T-D/E) capable of Ca^{2+} binding [217, 218]. The cation permeability of some annexins ensures a sufficient Ca^{2+} conductance and is inhibited by La^{3+}, Gd^{3+}, and nifedipine. The ion conductance resides in the ability of α-helical domains to form a pore lined up with hydrophilic amino acid residues that constitute an ion channel.

The best-studied example of *C_2-domain-containing proteins* is protein kinase C (PKC) of animals. The composition of various protein kinases C includes a catalytic site, regulatory modules, and three (C_1–C_3) or four (C_1–C_4) conserved domains. The first, third, and fourth conserved domains are present in all isoforms of protein kinase C, whereas the second (C_2) domain is only characteristic of Ca^{2+}dependent types of the enzyme, i.e., PKCα, PKCβ, and PKCγ. The ability of Ca^{2+} binding is specifically attributed to the C_2-domain of the protein molecule [220]. In animals, C_2 domains are found in many proteins. In plants, these domains have been only revealed as a component of phosphatidylinositol-4,5-diphosphate (PIP_2)-specific phospholipase C (PI–PLC) and phospholipase D (PLD); these phospholipases represent Ca^{2+}-binding proteins involved in lipid metabolism. Therefore, these enzymes are presumably able to translate the Ca^{2+} signal to membrane lipids [220]. The plant PI-PLC contain one C_2 domain at the C-terminus of protein molecule and are activated by calcium at nanomolar concentrations [220, 249]. In plants, several isoforms of phospholipases D were identified (PLDα, PLDβ, PLDγ, and PLDδ) that showed different sensitivity to Ca^{2+} and PIP_2. For example, PLDα is activated by Ca^{2+} in the millimolar concentration range and is independent of PIP_2, whereas the activity of PLDβ depends on PIP_2 and is stimulated by micromolar Ca^{2+} concentrations [250, 251].

The Ca^{2+}-binding sensor proteins, *calreticulin* and *calnexin* belong to the family of reticuloplasmins and

are localized in the lumen of endoplasmic reticulum [246]. Their attribution to proteins of endoplasmic reticulum is evidenced by the presence of the HDEL signaling sequence at the C-terminal part of the molecule. Calreticulin and calnexin were shown to serve as molecular chaperones.

CONCLUSION

A distinguished expert in calcium signaling, Berridge entitled one of his works "Calcium—a life and death signal" [6]. This definition is fully valid for plant physiology. The Ca^{2+} ions are involved in the control of pollen tube growth, recognition in the pollen–pistil system and fertilization, cell development and differentiation, hormonal signal transduction and adaptation to stress factors, programmed cell death and tropisms, interactions of plants with phytopathogens and root-nodule bacteria [9–12, 14, 18, 19, 25, 84]. The Ca^{2+}-binding sensor proteins control the enzyme activities of the carbon and nitrogen metabolism, membrane carriers and ion channels, cytoskeletal proteins, and transcription factors [15, 221–225, 239, 240]. The cytosolic calcium may link many signaling pathways, thus promoting the formation of signaling network in the plant cell. This network combines different signaling systems and ensures adequate cell responses to changes in the environmental conditions [10, 16, 17]. However, despite a considerable progress in understanding the signaling role of Ca^{2+} in plants, many questions remain open.

To date, many proteins engaged in membrane transport and Ca^{2+} binding are not yet identified. The pathways by which the environmental signals are conveyed to the system of Ca^{2+} channels are still unknown. The mechanisms responsible for Ca^{2+} homeostasis in plant cells and the origin of specificity of Ca^{2+} signature are not yet understood. The mechanisms of generation of Ca^{2+} oscillations and Ca^{2+} wave propagation in plant cells remain largely obscure. It is not yet known how the amplitude and frequency characteristics of Ca^{2+} signal are translated to the sensor protein system. The principles of transmitting the Ca^{2+} signal to the genome level and of its interaction with other signaling systems are not yet investigated. The question of specificity in generation of Ca^{2+} signal and of its interaction with target proteins still remains open. Finally, a highly important question is whether calcium can ensure the specificity of the signal transmitted or whether Ca^{2+} acts a chemical trigger enabling the operation of other signaling pathways. It should be borne in mind that oscillations in Ca^{2+}_{cyt} may be unrelated to any signaling functions but arise as a side effect being a consequence (rather than a cause) of other processes.

ACKNOWLEDGMENTS

I am grateful to Dr. E.I. Sharova, Dr. A.Yu. Batov, and Dr. O.V. Tankelyun for reading the manuscript and their comments.

The work was supported by Russian Foundation of Basic Research (project no. 02-04-49171) and INTAS (project no. 01-0602).

REFERENCES

1. Ringer, S., A Further Contribution Regarding the Influence of the Different Constituents of the Blood on the Contraction of the Heart, *J. Physiol.*, 1883, vol. 4, pp. 29–42.
2. Rasmussen, H., *Calcium and cAMP as a Synarchic Messengers*, New York: Wiley, 1981.
3. Rasmussen, H., Barrett, P., and Smallwood, J., Calcium Ion as Intracellular Messenger and Cellular Messenger and Cellular Toxin, *Environ. Helth. Perspect.*, 1990, vol. 84, pp. 17–25.
4. Avdonin, P.V. and Tkachuk, V.A., *Retseptory i vnutrikletochnyi kal'tsii* (Receptors and Intracellular Calcium). Moscow: Nauka, 1994.
5. Berridge, M.J., The AM and FM of Calcium Signalling, *Nature*, 1997, vol. 386, pp. 759–760.
6. Berridge, M.J., Bootman, M.D., and Lipp, P., Calcium – a Life and Death Signal, *Nature*, 1998, vol. 395, pp. 645–648.
7. Bootman, M.D., Berridge, M.J., and Roderick, H.L., Calcium Signalling: More Messengers, More Channels, More Complexity, *Curr. Biol.*, 2002, vol. 12, pp. R563–R565.
8. Krutetskaya, Z.I., Lebedev, O.E., and Kurilova, L.S., *Mekhanizmy vnutrikletochnoi signalizatsii* (Mechanisms of Intracellular Signaling), St. Petersburg: St. Petersburg Gos. Univ., 2003.
9. Bush, D.S., Calcium Regulation in Plant Cells and Its Role in Signalling, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1995, vol. 46, pp. 95–122.
10. Trewavas, A.J. and Malho, R., Ca^{2+} Signalling in Plant Cells: The Big Network! *Curr. Opin. Plant Biol.*, 1998, vol. 1, pp. 428–433.
11. Sanders, D., Brownlee, C., and Harper, J.F., Communication with Calcium, *Plant Cell*, 1999, vol. 11, pp. 691–706.
12. Sanders, D., Pelloux, J., Brownlee, C., and Harper, J.F., Calcium at the Crossroads of Signalling, *Plant Cell*, 2002, vol. 14, pp. 401–417.
13. Roos, W., Ion Mapping in Plant Cells – Methods and Applications in Signal Transduction Research, *Planta*, 2000, vol. 210, pp. 347–370.
14. Rudd, J.J. and Franklin-Tong, V.E., Unravelling Response-Specificity in Ca^{2+} Signalling Pathways in Plant Cells, *New Phytol.*, 2001, vol. 151, pp. 7–33.
15. Reddy, A.S.N., Calcium: Silver Bullet in Signalling, *Plant Sci.*, 2001, vol. 160, pp. 381–404.
16. Trewavas, A.J., Signal Perception and Transduction, *Biochemistry and Molecular Biology of Plants*, Buchanan, B., Gruissem, W., and Jones, R., Ed., Rockville: Am. Soc. Plant Physiol., 2000, pp. 930–987.

17. Tarchevsky, I.A., *Signal'nye sistemy kletok rastenii* (Signal Transduction Pathways of Plant Cells), Moscow: Nauka, 2002.
18. Hepler, P.K. and Wayne, R.O., Calcium and Plant Development, *Annu. Rev. Plant Physiol.*, 1985, vol. 36, pp. 397–439.
19. Medvedev, S.S., *Fiziologicheskie osnovy polyarnosti rastenii* (Physiological Basics of Plant Polarity), St. Petersburg: Kol'na, 1996.
20. Shacklock, P.S., Read, N.D., and Trewavas, A.J., Cytosolic Free Calcium Mediates Red Light Induced Photomorphogenesis, *Nature*, 1992, vol. 358, pp. 153–155.
21. Volotovski, I.D., Sokolovski, S.G., Nikiforov, E.L., and Zinchenko, V.P., Calcium Oscillations in Plant-Cell Cytoplasm Induced by Red and Far-Red Light Irradiation, *J. Photochem. Photobiol.*, 1993, vol. 20, pp. 95–100.
22. Bowler, C., Neuhaus, G., Yamagata, H., and Chua, N.-H., Cyclic GMP and Calcium Mediate Phytochrome Phototransduction, *Cell*, 1994, vol. 77, pp. 73–81.
23. Medvedev, S.S., Polarity and Embryogeny in Plants, *Embriologiya tsvetkovykh rastenii*, (Embryology of Flowering Plants), vol. 2, Batygina, T.B., Ed., St. Petersburg: Mir i sem'ya, 1997, pp. 594–601.
24. Franklin-Tong, V.E., Hackett, G., and Hepler, P.K., Ratio-Imaging of Ca^{2+} in the Self-Incompatibility Response in Pollen Tubes of *Papaver rhoeas, Plant J.*, 1997, vol. 12, pp. 1375–1386.
25. Franklin-Tong, V.E., Signaling and the Modulation of Pollen Tube Growth, *Plant Cell*, 1999, vol. 11, pp. 727–738.
26. Ehrhard, D.W., Wais, R., and Long, S.R., Calcium Spiking in Plant Root Hairs Responding to *Rhizobium* Nodulation Signals, *Cell*, 1996, vol. 85, pp. 673–681.
27. Lhuissier, F.G.P., de Ruijter, N.C.A., Sieberer, B.J., Esseling, J.J., and Emons, A.M.C., The Course of Cell Biological Events Evoked in Legume Root Hairs by *Rhizobium* Nod Factors: State of the Art, *Ann. Bot.* (London), 2001, vol. 87, pp. 289–302.
28. Knigth, M.R., Campbell, A.K., Smith, S.M., and Trewavas, A.J., Transgenic Plant Aequorin Reports the Effects of Touch, Cold Shock, and Elicitors on Cytoplasmic Calcium, *Nature*, 1991, vol. 352, pp. 524–526.
29. Bach, M., Schnitzler, J.-P., and Seitz, H.U., Elicitor-Induced Changes in Ca^{2+} Influx, K^+ Efflux, and 4-Hydroxybenzoic Acid Synthesis in Protoplasts of *Daucus carota* L., *Plant Physiol.*, 1993, vol. 103, pp. 407–412.
30. Mithöfer, A., Ebel, J., Bhagwat, A.A., Boller, T., and Neuhaus, G., Transgenic Aequorin Monitors Cytosolic Calcium Transients in Soybean Cells Challenged with β-Glucan or Chitin Elicitors, *Planta*, 1999, vol. 207, pp. 566–574.
31. Blume, B., Nürnberger, T., Nass, N., and Scheel, D., Receptor-Mediated Increase in Cytoplasmic Free Calcium Required for Activation of Pathogen Defense in Parsley, *Plant Cell*, 2000, vol. 12, pp. 1425–1440.
32. Grant, M., Brown, I., Adams, S., Knight, M., Ainslie, A., and Mansfield, J., The *RPM1* Plant Disease Resistance Gene Facilitates a Rapid and Sustained Increase in Cytosolic Calcium That Is Necessary for the Oxidative Burst and Hypersensitive Cell Death, *Plant J.*, 2000, vol. 23, pp. 1–11.
33. Poovaiah, B.W., McFadden, J.J., and Reddy, A.S.N., The Role of Calcium Ions in Gravity Signal Perception and Transduction, *Physiol. Plant.*, 1987, vol. 71, pp. 401–407.
34. Medvedev, S.S. and Shtonda, I.A., The Role of Calcium Ions in Gravitropism, *Biol. Nauki*, 1989, no. 6, pp. 94–97.
35. Medvedev, S.S., Maksimov, G.B., and Markova, I.V., The Role of Calcium Ions in the Regulation of Gravitropism, *Exp. Biol.* (Vilnius), 1991, no. 4, pp. 71–92.
36. Sinclair, W. and Trewavas, A.J., Calcium in Gravitropism: Re-Examination, *Planta*, 1997, vol. 203, pp. 585–590.
37. Plieth, C. and Trewavas, A.J., Reorientation of Seedlings in the Earth's Gravitational Field Induces Cytosolic Calcium Transients, *Plant Physiol.*, 2002, vol. 129, pp. 786–796.
38. Gehring, C.A., Williams, D.A., Cody, S.H., and Parish, R.W., Phototropism and Geotropism in Maize Coleoptiles Are Spatially Correlated with Increases in Cytosolic Free Calcium, *Nature*, 1990, vol. 345, pp. 528–530.
39. Medvedev, S.S. and Markova, I.V., The Cytoskeleton and Plant Polarity, *Fiziol. Rast.* (Moscow), 1998, vol. 45, pp. 185–197 (*Russ. J. Plant Physiol.*, Engl. Transl.).
40. Neuhaus, G., Bowler, C., Hiratsuka, K., Yamagata, H., and Chua, N.-H., Phytochrome-Regulated Repression of Gene Expression Requires Calcium and cGMP, *EMBO J.*, 1997, vol. 16, pp. 2554–2564.
41. Baum, G., Long, J.C., Jenkins, G.I., and Trewavas, A.J., Stimulation of the Blue Light Receptor NPH1 Causes a Transient Increase in Cytosolic Ca^{2+}, *Proc. Natl. Acad. Sci. USA*, 1999, vol. 96, pp. 13 554–13 559.
42. Gilroy, S., Read, N., and Trewavas, A., Elevation of Cytoplasmic Ca^{2+} by Caged Calcium or Caged Inositol Triphosphate Initiates Stomatal Closure, *Nature*, 1990, vol. 346, pp. 769–771.
43. Irving, H.R., Gehring, C.A., and Parish, R.W., Changes in Cytosolic pH and Calcium of Guard Cells Precede Stomatal Movement, *Proc. Natl. Acad. Sci. USA*, 1992, vol. 89, pp. 1790–1794.
44. McAinsh, M.R., Browlee, C., and Hetherington, A.M., Calcium Ions as Second Messengers in Guard Cell Signalling, *Physiol. Plant.*, 1997, vol. 100, pp. 16–29.
45. Ng, C.K.-Y., McAinsh, M.R., Gray, J.E., Hunt, L., Leckie, C.P., Mills, L., and Hetherington, A.M., Calcium-Based Signalling Systems in Guard Cells, *New Phytol.*, 2001, vol. 151, pp. 109–120.
46. Pierson, E.S., Miller, D.D., Callaham, D.A., Shipley, A.M., and Rivers, B.A., Pollen Tube Growth Is Coupled to the Extracellular Calcium Ion Flux and the Intracellular Gradient: Effect of BAPTA-Type Buffers and Hypertonic Media, *Plant Cell*, 1994, vol. 6, pp. 1815–1828.
47. Pierson, E.S., Miller, D.D., Callaham, D.A., van Aken, J., Hackett, G., and Hepler, P.K., Tip-Localized Calcium Entry Fluctuates during Pollen Tube Growth, *Dev. Biol.*, 1996, vol. 174, pp. 160–173.
48. Felle, H.H. and Hepler, P.K., The Cytosolic Ca^{2+} Concentration Gradient of *Sinapsis alba* Root Hairs as Revealed by Ca-Selective Microelectrodes Tests and Fura–Dextran Ratio Imaging, *Plant Physiol.*, 1997, vol. 114, pp. 39–45.
49. Wymer, C.L., Bibikova, T.N., and Gilroy, S., Cytoplasmic Free Calcium Distribution during the Development

of Root Hairs of *Arabidopsis thaliana, Plant J.*, 1997, vol. 12, pp. 427–439.

50. Bibikova, T.N., Zhigilei, A., and Gilroy, S., Root Hair Growth in *Arabidopsis thaliana* Is Directed by Calcium and an Endogenous Polarity, *Planta*, 1997, vol. 203, pp. 495–505.
51. Brownlee, C. and Wood, J.W., A Gradient of Cytoplasmic Free Calcium in Growing Rhizoid Cells of *Fucus serratus, Nature*, 1986, vol. 320, pp. 624–626.
52. Taylor, A.R., Manison, N.F.H., Fernandez, C., Wood, J.W., and Brownlee, C., Spatial Organization of Calcium Signalling Involved in Cell Volume Control in the *Fucus* Rhizoid, *Plant Cell*, 1996, vol. 8, pp. 2015–2031.
53. Kropf, D.L., Induction of Polarity in Fucoid Zygotes, *Plant Cell*, 1997, vol. 9, pp. 1011–1020.
54. Knight, H., Calcium Signalling during Abiotic Stress in Plants, *Int. Rev. Cytol.*, 2000, vol. 195, pp. 1011–1020.
55. Knight, H., Trewavas, A.J., and Knight, M.R., Cold Calcium Signalling in *Arabidopsis* Involves Two Cellular Pools and Change in Calcium Signature after Acclimation, *Plant Cell*, 1996, vol. 8, pp. 489–503.
56. Kiegle, E., Moore, K., Haseloff, J., Tester, M.A., and Knight, M.R., Cell-Type-Specific Calcium Responses to Drought, Salt, and Cold in the *Arabidopsis* Root, *Plant J.*, 2000, vol. 23, pp. 267–278.
57. Zhu, J.-K., Cell Signalling under Salt, Water, and Cold Stresses, *Curr. Opin. Plant Biol.*, 2001, vol. 4, pp. 401–406.
58. Gong, M., van de Luit, A.H., Knigth, M.R., and Trewavas, A.J., Heat-Shock-Induced Changes in Intracellular Ca^{2+} Level in Tobacco Seedlings in Relations to Thermotolerance, *Plant Physiol.*, 1998, vol. 116, pp. 429–437.
59. Knight, H., Trewavas, A.J., and Knight, M.R., Calcium Signalling in *Arabidopsis thaliana* Responding to Drought and Salinity, *Plant J.*, 1997, vol. 12, pp. 1067–1078.
60. Subbaiah, C.C., Bush, D.S., and Sachs, M.M., Elevation of Cytosolic Calcium Precedes Anoxic Gene Expression in Maize Suspension-Cultured Cells, *Plant Cell*, 1994, vol. 6, pp. 1747–1762.
61. Subbaiah, C.C., Bush, D.S., and Sachs, M.M., Mitochondrial Contribution to the Anoxic Ca^{2+} Signal in Maize Suspension-Cultured Cells, *Plant Physiol.*, 1998, vol. 118, pp. 759–771.
62. Sedbrook, J.C., Kronebusch, P.J., Borisy, G.G., Trewavas, A.J., and Masson, P.H., Transgenic Aequorin Reveals Organ-Specific Cytosolic Ca^{2+} Responses to Anoxia in *Arabidopsis thaliana* Seedlings, *Plant Physiol.*, 1996, vol. 111, pp. 243–257.
63. Fellbrich, G., Blume, B., Brunner, F., Hirt, H., Kroj, T., Ligterink, W., Romanski, A., and Nurnberger, T., *Phytophthora parasitica* Elicitor-Induced Reactions in Cells of *Petroselinum crispum, Plant Cell Physiol.*, 2000, vol. 41, pp. 692–701.
64. Takahashi, K., Isobe, M., Knight, M.R., Trewavas, A.J., and Muto, S., Hypo-Osmotic Shock Induces Increases in Cytosolic Ca^{2+} in Tobacco Suspension Culture Cells, *Plant Physiol.*, 1997, vol. 113, pp. 587–594.
65. Cessna, S.G., Chandra, S., and Low, P.S., Hypo-Osmotic Shock of Tobacco Cells Stimulates Ca^{2+} Fluxes Deriving First from External and Then Internal Ca^{2+} Stores, *J. Biol. Chem.*, 1998, vol. 273, pp. 27286–27291.
66. Goddard, H., Manison, N.F.H., Tomos, D., and Brownlee, C., Elemental Propagation of Calcium Signals in Response-Specific Patterns Determined by Environmental Stimulus Strength, *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, pp. 1932–1937.
67. Haley, A., Russell, A.J., Wood, N., Allan, A.C., Knigth, M., Campbell, A.K., and Trewavas, A.J., Effects of Mechanical Signalling on Plant Cell Cytosolic Calcium, *Proc. Natl. Acad. Sci. USA*, 1995, vol. 92, pp. 4124–4128.
68. Price, A.H., Taylor, A., Ripley, S.J., Griffiths, A., and Trewavas, A.J., Oxidative Signals in Tobacco Increase Cytosolic Calcium, *Plant Cell*, 1994, vol. 6, pp. 1301–1310.
69. Pei, Z.M., Murata, Y., Benning, G., Thomine, S., Klusener, B., Allen, G.J., Grill, E., and Schroeder, J.I., Calcium Channels Activated by Hydrogen Peroxide Mediate Abscisic Acid Signalling in Guard Cells, *Nature*, 2000, vol. 406, pp. 731–734.
70. Gilroy, S.G. and Jones, R.L., Gibberellic Acid and Abscisic Acid Coordinately Regulate Cytoplasmic Calcium and Secretory Activity in Barley Aleurone Protoplasts, *Proc. Natl. Acad. Sci. USA*, 1992, vol. 89, pp. 3591–3595.
71. Gilroy, S., Signal Transduction in Barley Aleurone Protoplasts Is Calcium Dependent and Independent, *Plant Cell*, 1996, vol. 8, pp. 2193–2209.
72. Bethke, P.C., Schuurink, R.C., and Jones, R.L., Hormonal Signalling in Cereal Aleurone, *J. Exp. Bot.*, 1997, vol. 48, pp. 1337–1356.
73. McAinsh, M.R., Brownlee, C., and Hetherington, A.M., Visualizing Changes in Cytosolic-Free Ca^{2+} during the Response of Stomatal Guard Cells to Abscisic Acid, *Plant Cell*, 1992, vol. 4, pp. 1113–1122.
74. Staxen, I., Pical, C., Montgomery, L.T., Gray, J.E., Hetherington, A.M., and McAinsh, M.R., Abscisic Acid Induces Oscillation in Guard-Cell Cytosolic Free Calcium That Involve Phosphoinositide-Specific Phospholipase C, *Proc. Natl. Acad. Sci. USA*, 1999, vol. 96, pp. 1779–1784.
75. Schroeder, J.I., Allen, G.J., Hugovieux, V., Kwak, J.M., and Warner, D., Guard Cell Signal Transduction, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 2001, vol. 52, pp. 627–658.
76. Polevoi, V.V., *Rol' auksina v sistemakh regulyatsii u rastenii. 44-e Timiryazevskoe chtenie* (Role of Auxin in the Systems of Plant Regulation, the 44th Timiryazev Lecture), Leningrad: Nauka, 1986.
77. Felle, H., Auxin Causes Oscillation of Cytosolic Free Calcium and pH in *Zea mays* Coleoptiles, *Planta*, 1988, vol. 174, pp. 495–499.
78. Medvedev, S.S., Markova, I.V., Batov, A.Y., and Moshkov, A.V., Membrane Mechanism of IAA Action, *Biologia*, (Vilnius), 1998, no. 3, pp. 31–34.
79. Medvedev, S.S., Batov, A.Yu., Moshkov, A.V., and Markova, I.V., The Role of Ion Channels in Transduction of the Auxin Signal, *Fiziol. Rast.* (Moscow), 1999, vol. 46, pp. 620–625 (*Russ. J. Plant Physiol.*, Engl. Transl.).
80. Medvedev, S.S and Markova, I.V., Role of Calcium Ions in Plant Growth and Mechanism of IAA Action, *Phytohormones in Plant Biotechnology and Agriculture*, Macháčková, I. and Romanov, G.A., Eds., Dordrecht: Kluwer, 2003, pp. 157–169.

81. Romanov, G.A., Kieber, J.J., and Schmülling, T., A Rapid Cytokinin Response Assay in Rapid *Amaranthus* Seedlings Test, *Plant Growth Regul.*, 2000, vol. 32, pp. 337–344.

82. Romanov, G.A., Getman, I.A., and Schmülling, T., Investigation of Early Cytokinin Effects in a Rapid *Arabidopsis* Test Indicates a Role for Phospholipase D in Cytokinin Signalling, *FEBS Lett.*, 2002, vol. 515, pp. 39–43.

83. Markova, I.V., Rumyantseva, E.A., Getman, I.A., Romanov, G.A., and Medvedev, S.S., Investigation of the Signal Role of Calcium Ions in Cytokinin-Dependent Responses in *Amaranthus caudatus* L., *Vestn. St. Petersburg. Gos.Univ.*, Ser. 3, 2004, no. 19, pp. 47–55.

84. Ng, C.K.-Y. and McAinsh, M.R., Encoding Specificity in Plant Calcium Signalling: Hot-Spotting the Ups and Downs and Waves, *Ann. Bot. (London)*, 2003, vol. 92, pp. 477–485.

85. Levitskii, D.O., *Kal'tsii i biologicheskie membrany* (Calcium and Biological Membranes), Moscow: Vysshaya Shkola, 1990.

86. Miedema, H., Bothwell, J.H.F., Brownlee, C., and Davies, J., Calcium Uptake by Plant Cells – Channels and Pumps Acting in Concert, *Trends Plant Sci.*, 2001, vol. 6, pp. 514–519.

87. Tsien, R.Y., A Non-Disruptive Technique for Loading Calcium Buffers and Indicators into Cells, *Nature*, 1981, vol. 290, pp. 527–528.

88. Miyawaki, A., Llopis, J., Heim, R., McCaffery, J.M., Adams, J.A., Ikura, M., and Tsien, R.Y., Fluorescent Indicators for Ca^{2+} Based on Green Fluorescent Proteins and Calmodulin, *Nature*, 1997, vol. 388, pp. 882–887.

89. Brownlee, C., Cellular Calcium Imaging: So, What's New? *Trends Cell Biol.*, 2000, vol. 10, pp. 451–457.

90. *Calcium Signalling: Practical Approach*, Tepikin, A.V., Ed., Oxford: Oxford Univ. Press, 2001.

91. Takahashi, A., Camacho, P., Lechleitern, J., and Herman, B., Measurement of Intracellular Calcium, *Physiol. Rev.*, 1999, vol. 79, pp. 1089–1125.

92. Antoine, A.F., Faure, J.-E., Cordeiro, S., Dumas, C., Rougier, M., and Feijo, J.A., A Calcium Influx Is Triggered and Propagates in the Zygote as Wavefront during *In Vitro* Fertilization of Flowering Plants, *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, pp. 10 643–10 648.

93. Neher, E. and Sakmann, B., Single-Channel Currents Recorded from Membranes of Denervated Frog Muscle Fibres, *Nature*, 1976, vol. 260, pp. 779–802.

94. Pauly, N., Knight, M.R., Thuleau, P., van der Luit, A.H., Moreau, M., Trewavas, A.J., Ranjeva, R., and Mazars, C., Cell Signalling: Control of Free Calcium in Plant Cell Nuclei, *Nature*, 2000, vol. 405, pp. 754–755.

95. Geisler, M., Axelsen, K.B., Harper, J.F., and Palmdren, M.G., Molecular Aspects of Higher Plant P-Type Ca^{2+}-ATPases, *Biochim. Biophys. Acta*, 2000, vol. 1465, pp. 52–78.

96. Sze, H., Liang, F., Hwang, I., Curran, A.C., and Harper, J.F., Diversity and Regulation of Plant Ca^{2+} Pumps: Insights from Expression in Yeast, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 2000, vol. 51, pp. 433–462.

97. Hirschi, K., Vacuolar H^+/Ca^{2+} Transport: Who Is Directing the Traffic? *Trends Plant Sci.*, 2001, vol. 6, pp. 100–104.

98. White, P.J., Calcium Channels in Higher Plants, *Biochim. Biophys. Acta*, 2000, vol. 1465, pp. 171–189.

99. White, P.J., Bowen, H.C., Demidchik, V., Nichols, C., and Davies, J.M., Genes for Calcium-Permeable Channels in the Plasma Membrane of Plant Root Cells, *Biochim. Biophys. Acta*, 2002, vol. 1564, pp. 299–309.

100. Demidchik, V., Davenport, R.J., and Tester, M., Nonselective Cation Channels in Plants, *Annu. Rev. Plant Phys. Plant Mol. Biol.*, 2002, vol. 53, pp. 67–107.

101. Véry, A.-A. and Sentenac, H., Cation Channels in the *Arabidopsis* Plasma Membrane, *Trends Plant Sci.*, 2002, vol. 7, pp. 168–175.

102. White, P.J., Characterization of High-Conductance Voltage-Dependent Cation Channels from the Plasma Membrane of Rye Roots in Planar Lipid Bilayers, *Planta*, 1993, vol. 191, pp. 541–551.

103. Sokolik, A.I., Non-Selective Ion Conductance across the Plasmalemma – Important Component of Membrane Ion Transport in Plants, *Dokl. Belor. AN*, 1999, vol. 43, pp. 77–80.

104. White, P.J. and Ridout, M.S., An Energy-Barrier Model Describing the Permeation of Monovalent and Divalent Cations through the Maxi Cation-Channel in the Plasma Membrane of Rye Roots, *J. Membr. Biol.*, 1999, vol. 168, pp. 63–75.

105. Krol, E. and Trebacz, K., Ways of Ion Channel Gating in Plant Cells, *Ann. Bot.* (London), 2000, vol. 86, pp. 449–469.

106. Markova, I.V., Batov, A.Yu., Moshkov, A.V., Maksimov, G.B., and Medvedev, S.S., Calcium-Transporting Systems in the Plasmalemma of Maize Coleoptiles, *Fiziol. Rast.* (Moscow), 1995, vol. 42, pp. 262–267 (*Russ. J. Plant Physiol.*, Engl. Trasl.).

107. Thuleau, P., Ward, J.M., Ranjeva, R., and Shroeder, J.I., Voltage-Dependent Calcium Permeable Channels in the Plasma Membrane of Higher Plant Cell, *EMBO J.*, 1994, vol. 13, pp. 2970–2975.

108. Thion, L., Mazars, C., Thuleau, P., Graziana, A., Rossignol, M., Moreau, M., and Ranjeva, R., Activation of Plasma Membrane Voltage-Dependent Calcium-Permeable Channels by Disruption of Microtubules in Carrot Cells, *FEBS Lett.*, 1996, vol. 340, pp. 45–50.

109. Thion, L., Mazars, C., Nacry, P., Bouchez, D., Moreau, M., Ranjeva, R., and Thuleau, P., Plasma Membrane Depolarization-Activated Calcium Channels, Stimulated by Microtubule-Depolymerizing Drugs in Wild-Type *Arabidopsis thaliana* Protoplasts, Display Constitutively Large Activities and a Longer Half-Life in *ton2* Mutant Cells Affected in the Organization of Cortical Microtubules, *Plant J.*, 1998, vol. 13, pp. 603–610.

110. Piňeros, M. and Tester, M., Characterization of a Voltage-Dependent Ca^{2+}-Selective Channel from Wheat Roots, *Planta*, 1995, vol. 195, pp. 478–488.

111. Piňeros, M. and Tester, M., Calcium Channels in Higher Plant Cells: Selectivity, Regulation and Pharmacology, *J. Exp. Bot.*, 1997, vol. 48, pp. 551–557.

112. White, P.J., Piňeros, M., Tester, M., and Ridout, M.S., Cation Permeability and Selectivity of a Root Plasma

Membrane Calcium Channel, *J. Membr. Biol.*, 2000, vol. 174, pp. 71–83.

113. White, P.J., Specificity of Ion Channel Inhibitors for the Maxi Cation Channel in Rye Root Plasma Membranes, *J. Exp. Bot.*, 1996, vol. 47, pp. 713–716.
114. White, P.J., Calcium Channels in the Plasma Membrane of Root Cells, *Ann. Bot.* (London), 1998, vol. 81, pp. 173–183.
115. Kiegle, E., Gilliham, M., Haseloff, J., and Tester, M.A., Hyperpolarization-Activated Calcium Currents Found Only in Cells from the Elongation Zone of *Arabidopsis thaliana* Roots, *Plant J.*, 2000, vol. 21, pp. 225–229.
116. Véry, A.-A. and Davies, J.M., Hyperpolarization-Activated Calcium Channels at the Tip of *Arabidopsis* Root Hairs, *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, pp. 9801–9806.
117. Schroeder, J.I. and Hagiwara, S., Repetitive Increases in Cytosolic Ca^{2+} of Guard Cells by Abscisic Acid Activation of Nonselective Ca^{2+} Permeable Channels, *Proc. Natl. Acad. Sci. USA*, 1990, vol. 87, pp. 9305–9309.
118. Hamilton, D.W.A., Hills, A., Kohler, B., and Blatt, M.R., Ca^{2+} Channels at the Plasma Membrane of Stomatal Guard Cells Are Activated by Hyperpolarization and Abscisic Acid, *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, pp. 4967–4972.
119. Hutcheson, S.W., Current Concepts of Active Defense in Plants, *Annu. Rev. Phytopathol.*, 1998, vol. 36, pp. 59–90.
120. Gelli, A. and Blumwald, E., Hyperpolarization-Activated Ca^{2+}-Permeable Channels in the Plasma Membrane of Tomato Cells, *J. Membr. Biol.*, 1997, vol. 155, pp. 35–45.
121. Gelli, A., Higgins, V.J., and Blumwald, E., Activation of Plant Plasma Membrane Ca^{2+}-Permeable Channels by Race-Specific Fungal Elicitors, *Plant Physiol.*, 1997, vol. 113, pp. 269–279.
122. McAinsh, M.R., Brownlee, C., and Hetherington, A.M., Abscisic Acid-Induced Elevation of Guard Cell Cytosolic Ca^{2+} Precedes Stomatal Closure, *Nature*, 1990, vol. 343, pp. 186–188.
123. Ward, J.M., Pei, Z.-M., and Schroeder, J.I., Roles of Ion Channels in Initiation of Signal Transduction in Higher Plants, *Plant Cell*, 1995, vol. 7, pp. 833–844.
124. Shishova, M.F., Lindberg, S., and Polevoi, V.V., Auxin Activation of Ca^{2+} Transport across the Plasmalemma of Plant Cells, *Fiziol. Rast.* (Moscow), 1999, vol. 46, pp. 1–9 (*Russ. J. Plant Physiol.*, Engl. Transl.).
125. Zimmermann, S., Thomine, S., Guern, J., and Barbier-Brygoo, H., An Anion Current at the Plasma Membrane of Tobacco Protoplasts Shows ATP-Dependent Voltage Regulation and Is Modulated by Auxin, *Plant J.*, 1994, vol. 6, pp. 707–716.
126. Marten, I., Lohse, G., and Hedrich, R., Plant Growth Hormones Control Voltage-Dependent Activity of Anion Channels in Plasma Membrane of Guard Cells, *Nature*, 1991, vol. 353, pp. 758–762.
127. Zimmermann, S., Nurnberger, T., Frachisse, J.M., Wirtz, W., Guern, J., Hedrich, R., and Scheel, D., Receptor-Mediated Activation of a Plant Ca^{2+}-Permeable Ion Channel Involved in Pathogen Defense, *Plant Biol.*, 1997, vol. 94, pp. 2751–2755.
128. Pickard, B.G. and Ding, J.P., The Mechanosensory Calcium-Selective Ion Channel: Key Component of a Plasmalemma Control Center? *Aust. J. Plant Physiol.*, 1993, vol. 20, pp. 555–570.
129. Marshall, J., Corzo, A., Leigh, R.A., and Sanders, D., Membrane Potential-Dependent Calcium Transport in Right-Side-Out Plasma Membrane Vesicles from *Zea mays* L. Roots, *Plant J.*, 1994, vol. 5, pp. 683–694.
130. Klüsener, B., Boheim, G., Lib, H., Engelberth, J., and Weiler, E.W., Gadolinium-Sensitive, Voltage-Dependent Calcium Release Channels in the Endoplasmic Reticulum of a Higher Plant Mechanoreceptor Organ, *EMBO J.*, 1995, vol. 14, pp. 2708–2714.
131. Ding, J.P. and Pickard, B.G., Mechanosensory Calcium-Selective Cation Channels in Epidermal Cells, *Plant J.*, 1993, vol. 3, pp. 83–110.
132. Furuichi, T., Cunningham, K.W., and Muto, S., A Putative Two-Pore Channel AtTPC1 Mediates Ca^{2+} Flux in *Arabidopsis* Leaf Cells, *Plant Cell Physiol.*, 2001, vol. 42, pp. 900–905.
133. Schachtman, D.P., Kumar, R., Schroeder, J.I., and Marsh, E.L., Molecular and Functional Characterization of a Novel Low-Affinity Cation Transporter (LCT1) in Higher Plants, *Proc. Natl. Acad. Sci. USA*, 1997, vol. 94, pp. 11 079–11 084.
134. Clemens, S., Antosiewicz, D.M., Ward, J.M., Schachtman, D.P., and Schroeder, J.I., The Plant cDNA *LCT1* Mediates the Uptake of Calcium and Cadmium in Yeast, *Proc. Natl. Acad. Sci. USA*, 1998, vol. 95, pp. 12 043–12 048.
135. Mäser, P., Thomine, S., Schroeder, J.I., Ward, J.M., Hirschi, K., Sze, H., Talke, I.N., Amtmann, A., Maathuis, F.J.M., Sanders, D., Harper, J.F., Tchieu, J., Gribskov, M., Persans, M.W., Salt, D.E., Kim, S.A., and Guerinot, M.L., Phylogenetic Relationships within Cation Transporter Families of *Arabidopsis, Plant Physiol.*, 2001, vol. 126, pp. 1646–1667.
136. Lacombe, B., Becker, D., Hedrich, R., Desalle, R., Hollman, M., Kwak, J.M., Schroeder, J.I., le Novère, N., Nam, H.G., Spalding, E.P., Tester, M., Turano, E.J., Chiu, J., and Coruzzi, G., On the Identity of Plant Glutamate Receptors, *Science*, 2001, vol. 292, pp. 1486–1487.
137. Hille, B., *Ionic Channels of Excitable Membranes*, Massachusetts: Sinauer Associates, 2001.
138. Kohler, C., Merkle, T., and Neuhaus, G., Characterization of a Novel Gene Family of Putative Cyclic Nucleotide- and Calmodulin-Regulated Ion Channels in *Arabidopsis thaliana, Plant J.*, 1999, vol. 18, pp. 97–104.
139. Schuurink, R.C., Shatzer, S.F., Fath, A., and Jones, R.L., Characterization of a Calmodulin-Binding Transporter from the Plasma Membrane of Barley Aleurone, *Proc. Natl. Acad. Sci. USA*, 1998, vol. 95, pp. 1944–1949.
140. Arazi, T., Sunkar, R., Kaplan, B., and Fromm, H., A Tobacco Plasma Membrane Calmodulin-Binding Transporter Confers Ni^{2+} Tolerance and Pb^{2+} Hypersensitivity in Transgenic Plants, *Plant J.*, 1999, vol. 20, pp. 171–182.
141. Zagotta, W.N., Molecular Mechanism of Cyclic Nucleotide-Gate Channels, *J. Bioenerg. Biomembr.*, 1996, vol. 28, pp. 269–278.

142. Arazi, T., Kaplan, B., and Fromm, H., A High-Affinity Calmodulin-Binding Site in a Tobacco Plasma-Membrane Channel Protein Coincides with Characteristic Element of Cyclic Nucleotide-Binding Domains, *Plant. Mol. Biol.*, 2000, vol. 42, pp. 591–601.

143. Kohler, C. and Neuhaus, G., Characterization of Calmodulin Binding to Cyclic Nucleotide-Gated Ion Channels from *Arabidopsis thaliana, FEBS Lett.*, 2000, vol. 471, pp. 133–136.

144. Kohler, C., Merkle, T., Roby, D., and Neuhaus, G., Developmentally Regulated Expression of a Cyclic Nucleotide-Gated Ion Channel from *Arabidopsis* Indicates Its Involvement in Programmed Cell Death, *Planta*, 2001, vol. 213, pp. 327–332.

145. Dingledine, R., Borges, K., Bowie, D., and Traynelis, S.F., The Glutamate Receptor Ion Channels, *Pharm. Rev.*, 1999, vol. 51, pp. 7–61.

146. Dennison, K.L. and Spalding, E.P., Glutamate-Gated Calcium Fluxes in *Arabidopsis, Plant Physiol.*, 2000, vol. 124, pp. 1511–1514.

147. Lam, H.-M., Chiu, J., Hsieh, M.-H., Meisel, L., and Oliviera, I.C., Glutamate Receptor Genes in Plants, *Nature*, 1998, vol. 396, pp. 125–126.

148. Chiu, J., Desalle, R., Lam, H.-M., Maisel, L., and Coruzzi, G., Molecular Evolution of Glutamate Receptor: A Primitive Signalling Mechanism That Existed before Plants and Animals Diverged, *Mol. Biol. Evol.*, 1999, vol. 16, pp. 826–838.

149. Allen, G.J. and Sanders, D., Vacuolar Ion Channels of Higher Plants, *Adv. Bot. Res.*, 1997, vol. 25, pp. 218–252.

150. Johannes, E., Brosnan, J.M., and Sanders, D., Calcium Channels in the Vacuolar Membrane of Plants: Multiple Pathways for Intracellular Calcium Mobilization, *Phil. Trans. R. Soc., London, Ser. B*, 1992, vol. 338, pp. 105–112.

151. Gelli, A. and Blumwald, E., Calcium Retrieval from Vacuolar Pools, *Plant Physiol.*, 1993, vol. 102, pp. 1139–1146.

152. Allen, G.J. and Sanders, D., Two Voltage-Gate Calcium Channels Coreside in the Vacuolar Membrane of Guard Cells, *Plant Cell*, 1994, vol. 6, pp. 685–694.

153. Ward, J.M. and Schroeder, J.I., Calcium Activated K^+-Channels and Calcium-Induced Calcium Release by Slow Vacuolar Ion Channels in Guard Cell Vacuoles Implicated in the Control of Stomatal Closure, *Plant Cell*, 1994, vol. 6, pp. 669–683.

154. Schultz-Lessdorf, B. and Hedrich, R., Protons and Calcium Modulate SV-Type Channels in the Vacuolar-Lysosomal Compartment-Channel Interaction with Calmodulin Inhibitors, *Planta*, 1995, vol. 197, pp. 655–671.

155. Allen, G.J. and Sanders, D., Control of Ionic Currents in Guard Cell Vacuoles by Cytosolic and Lumenal Calcium, *Plant J.*, 1996, vol. 10, pp. 1055–1067.

156. Hedrich, R. and Neher, E., Cytoplasmic Calcium Regulates Voltage Dependent Ion Channels in Plant Vacuoles, *Nature*, 1987, vol. 329, pp. 833–836.

157. Ward, J.M., Pei, Z.M., and Schroeder, J.I., Roles of Ion Channels in Initiation of Signal Transduction in Higher Plants, *Plant Cell*, 1995, vol. 7, pp. 833–844.

158. Bewell, M.A., Maathuis, F.J.M., Allen, G.J., and Sanders, D., Calcium-Induced Calcium Release Mediated by a Voltage-Activated Cation Channel in Vacuolar Vesicles from Red Beet, *FEBS Lett.*, 1999, vol. 458, pp. 41–44.

159. Pottosin, I.I., Tikhonova, L.I., Hedrich, R., and Schonknecht, G., Slowly Activating Vacuolar Channels Cannot Mediate Ca^{2+}-Induced Ca^{2+} Release, *Plant J.*, 1997, vol. 12, pp. 1387–1398.

160. Gambale, F., Bergante, M., Stragepede, F., and Cantu, A.M., Ionic Channels of the Sugar Beet Tonoplast Are Regulated by Multi-Ion Single-File Permeation, *J. Membr. Biol.*, 1996, vol. 154, pp. 69–79.

161. Johannes, E. and Sanders, D., Luminal Calcium Modulates Unitary Conductance and Gating of an Endomembrane Calcium Release Channel, *J. Membr. Biol.*, 1995, vol. 146, pp. 211–224.

162. Schumaker, K.S. and Sze, H., Inositol 1,4,5-*Tris*phosphate Releases Ca^{2+} from Vacuolar Membrane Vesicles of Oat Roots, *J. Biol. Chem.*, 1987, vol. 262, pp. 3944–3946.

163. Alexandre, J., Lassalles, J.P., and Kado, R.T., Opening of Ca^{2+} Channels in Isolated Red Beet Root Vacuole Membrane by Inositol 1,4,5-*Tris*phosphate, *Nature*, 1990, vol. 343, pp. 567–570.

164. Allen, G.J., Muir, S.R., and Sanders, D., Release of Ca^{2+} from Individual Plant Vacuoles by Both $InsP_3$ and Cyclic ADP-Ribose, *Science*, 1995, vol. 268, pp. 735–737.

165. Muir, S.R., Bewell, M.A., Sanders, D., and Allen, G.J., Ligand-Gated Ca^{2+} Channels and Signalling in Higher Plants, *J. Exp. Bot.*, 1997, vol. 48, pp. 589–597.

166. Leckie, C.P., McAinsh, M.R., Allen, G.J., Sanders, D., and Hetherington, A.M., Abscisic Acid-Induced Stomatal Closure Mediated by Cyclic ADP-Ribose, *Proc. Natl. Acad. Sci. USA*, 1998, vol. 95, pp. 15 837–15 842.

167. Allen, G.J. and Sanders, D., Osmotic Stress Enhances the Competence of *Beta vulgaris* Vacuoles to Respond to Inositol 1,4,5-*Tris*phosphate, *Plant J.*, 1994, vol. 6, pp. 687–695.

168. Bezprozvanny, I., Watras, J., and Ehrlich, B.E., Bell-Shaped Calcium Response Curves of $Ins(1,4,5)P_3$- and Calcium-Gated Channels from Endoplasmic Reticulum of *Cerebellum, Nature*, 1991, vol. 351, pp. 751–754.

169. Taylor, C.W. and Traynor, D., Calcium and Inositol *Tris*phosphate Receptors, *J. Membr. Biol.*, 1995, vol. 145, pp. 109–118.

170. Klüsener, B., Boheim, G., and Weiler, E.W., Modulation of the ER Ca^{2+}-Channel BCC1 from Tendrils of *Bryonia dioica* by Divalent Cations, Protons and H_2O_2, *FEBS Lett.*, 1997, vol. 407, pp. 230–234.

171. Klüsener, B. and Weiler, E.W., A Calcium-Selective Channels from Root Tip Endomembranes of Cress, *Plant Physiol.*, 1999, vol. 119, pp. 1399–1405.

172. Muir, S.R. and Sanders, D., Inositol 1,4,5-*Tris*phosphate-Sensitive Ca^{2+} Release across Non-Vacuolar Membranes in Cauliflower, *Plant Physiol.*, 1997, vol. 114, pp. 1511–1521.

173. Navazio, L., Mariani, P., and Sanders, D., Mobilization of Ca^{2+} by Cyclic ADP-Ribose from the Endoplasmic Reticulum of Cauliflower Florets, *Plant Physiol.*, 2001, vol. 125, pp. 2129–2138.

174. Navazio, L., Bewell, M.A., Siddiqua, A., Dickinson, G.D., Galione, A., and Sanders, D., Calcium Release from the Endoplasmic Reticulum of Higher Plants Elicited by the NADP Metabolite Nicotinic Acid Adenine Dinucle-

otide Phosphate, *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, pp. 8693–8698.

175. Martineč, J., Feltl, T., Scanlon, C.H., Lumsden, P.J., and Macháčková, I., Subcellular Localization of a High Affinity Binding Site for D-Myo-Inositol 1,4,5-*Tris*-phosphate from *Chenopodium rubrum, Plant Physiol.*, 2000, vol. 124, pp. 475–483.
176. Pottosin, I.I. and Schönknecht, G., Ion Channel Permeable for Divalent and Monovalent Cations in Native Spinach Thylakoid Membranes, *J. Membr. Biol.*, 1996, vol. 152, pp. 223–233.
177. Grygorczyk, C. and Grygorczyk, R.A., Ca^{2+} and Voltage-Dependent Cation Channel in the Nuclear Envelope of Red Beet, *Biochim. Biophys. Acta*, 1998, vol. 1375, pp. 117–130.
178. Pittman, J.K. and Hirshi, K.D., Don't Shoot the (Second) Messenger: Endomembrane Transporters and Binding Proteins Modulate Cytosolic Ca^{2+} Levels, *Curr. Opin. Plant Biol.*, 2003, vol. 6, pp. 257–262.
179. Axelsen, K.V. and Palmgren, M.G., Evolution of Substrate Specificities in the P-Type ATPase Superfamily, *J. Mol. Evol.*, 1998, vol. 46, pp. 84–101.
180. Evans, D.E. and Williams, L.E., P-Type Calcium ATPases in Higher Plants – Biochemical, Molecular and Functional Properties, *Biochim. Biophys. Acta*, 1998, vol. 1376, pp. 1–25.
181. Malmstrom, S., Askerlund, P., and Palmgren, M.G., A Calmodulin-Stimulated Ca^{2+}-ATPase from Plant Vacuolar Membranes with Putative Regulatory Domain at Its N-Terminus, *FEBS Lett.*, 1997, vol. 400, pp. 324–328.
182. Hwang, I., Sze, H., and Harper, J.F., A Calcium-Dependent Protein Kinase Can Inhibit a Calmodulin-Stimulated Ca^{2+} Pump (ACA2) Located in the Endoplasmic Reticulum of *Arabidopsis, Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, pp. 6224–6229.
183. Huang, L., Berkelman, T., Franklin, A.E., and Hoffman, N.E., Characterization of a Gene Encoding a Ca^{2+}-ATPase-Like Protein in Plastid Envelope, *Proc. Natl. Acad. Sci. USA*, 1993, vol. 90, pp. 10066–10070.
184. Harper, J.F., Hong, B., Hwang, I., Guo, H.Q., Stoddard, R., Huang, L., Palmgren, M.G., and Sze, H., A Novel Calmodulin-Regulated Ca^{2+}-ATPase (ACA2) from *Arabidopsis* with an N-Terminal Autoinhibitory Domain, *J. Biol. Chem.*, 1998, vol. 273, pp. 1099–1106.
185. Wimmers, L.E., Ewing, N.N., and Bennett, A.B., Higher Plant Ca^{2+}-ATPase: Primary Structure and Regulation of mRNA Abundance by Salt, *Proc. Natl. Acad. Sci. USA*, 1992, vol. 89, pp. 9205–9209.
186. Chen, X., Chang, M., Wang, B., and Wu, B., Cloning of a Ca^{2+}-ATPase Gene and the Role of Cytosolic Ca^{2+} in the Gibberellin-Dependent Signaling Pathway in Aleurone Cells, *Plant J.*, 1997, vol. 11, pp. 363–371.
187. Liang, F., Cunningham, K.W., Harper, J.F., and Sze, H., ECA1 Complements Yeast Mutant Defective in Ca^{2+} Pumps and Encodes an Endoplasmic Reticulum-Type Ca^{2+}-ATPase in *Arabidopsis thaliana, Proc. Natl. Acad. Sci. USA*, 1997, vol. 94, pp. 8579–8584.
188. Blackford, S., Rea, P.A., and Sanders, D., Voltage Sensitivity of H^+/Ca^{2+} Antiport in Higher Plant Tonoplast Suggests a Role in Vacuolar Calcium Accumulation, *J. Biol. Chem.*, 1990, vol. 265, pp. 9617–9620.
189. Hirschi, K., Zhen, R.G., Cunningham, K.W., Rea, P.A., and Fink, G.R., CAX1: An H^+/Ca^{2+} Antiporter from *Arabidopsis, Proc. Natl. Acad. Sci. USA*, 1996, vol. 93, pp. 8782–8786.
190. Schumaker, K.S. and Sze, H., Calcium Transport into the Vacuole of Oat Root: Characterization of H^+/Ca^{2+} Exchange Activity, *J. Biol. Chem.*, 1986, vol. 261, pp. 12172–12178.
191. Kasai, N. and Muto, S., Ca^{2+} Pump and Ca^{2+}/H^+ Antiporter in Plasma Membrane Vesicles Isolated by Aqueous Two-Phase Partitioning from Maize Leaves, *J. Membr. Biol.*, 1990, vol. 114, pp. 133–142.
192. Ueoka-Nakanishi, H., Nakanishi, Y., Tanaka, Y., and Maeshima, M., Properties and Molecular Cloning of a Ca^{2+}/H^+ Antiporter in the Vacuolar Membrane of Mung Bean, *Eur. J. Biochem.*, 1999, vol. 262, pp. 417–425.
193. Dawson, A.P., Calcium Signalling: How Do IP_3 Receptor Work? *Curr. Biol.*, 1997, vol. 7, pp. R544–R547.
194. Malho, R., Moutinho, A., van der Luit, A., and Trewavas, A.J., Spatial Characteristics of Calcium Signalling: The Calcium Wave as a Basic Unit in Plant Cell Calcium Signalling, *Phil. Trans. R. Soc., London, Ser. B*, 1998, vol. 353, pp. 1463–1473.
195. Coelho, S.M., Taylor, A.R., Ryan, K.R., Sousa-Pinto, I., Brown, M.T., and Brownlee, C., Spatiotemporal Pattering of Reactive Oxygen Production and Ca^{2+} Wave Propagation in *Fucus* Rhizoid Cells, *Plant Cell*, 2002, vol. 14, pp. 2369–2381.
196. Wood, N.T., Allan, A.C., and Haley, A., Viry-Moussaïd, M., and Trewavas, A., The Characterization of Differential Calcium Signalling in Tobacco Guard Cells, *Plant J.*, 2000, vol. 24, pp. 335–344.
197. Evans, N.H., McAinsh, M.R., and Hetherington, A.M., Calcium Oscillations in Higher Plants, *Curr. Opin. Plant Biol.*, 2001, vol. 4, pp. 415–420.
198. Logan, D.C. and Knigth, M.R., Mitochondrial and Cytosolic Calcium Dynamics Are Differentially Regulated in Plants, *Plant Physiol.*, 2003, vol. 133, pp. 21–24.
199. Knigth, M.R., Smith, S.M., and Trewavas, A.J., Wind-Induced Plant Motion Immediately Increases Cytosolic Calcium, *Proc. Natl. Acad. Sci. USA*, 1992, vol. 89, pp. 4967–4971.
200. Scrase-Field, S. and Knight, M.R., Calcium: Just a Chemical Switch? *Curr. Opin. Plant Biol.*, 2003, vol. 6, pp. 500–506.
201. Frohnmeyer, H., Loyall, L., Blatt, M.R., and Grabov, A., A Millisecond UV-B Irradiation Evokes Prolonged Elevation of Cytosolic Free Ca^{2+} and Stimulates Gene Expression in Transgenic Parsley Cell Culture, *Plant J.*, 1999, vol. 20, pp. 109–117.
202. Harper, J.F., Dissecting Calcium Oscillators in Plant Cells, *Trends Plant Sci.*, 2001, vol. 6, pp. 395–397.
203. Messerli, M. and Robinson, K.R., Tip Localized Ca^{2+} Pulses Coincident with Peak Pulsate Growth Rates in Pollen Tubes of *Lilium longiflorum, J. Cell Sci.*, 1997, vol. 110, pp. 1269–1278.
204. Holdaway-Clarke, T.L., Feijo, J.A., Hackett, G.R., Kunkel, J.G., and Hepler, P.K., Pollen Tube Growth and the Intracellular Cytosolic Calcium Gradient Oscillate in Phase While Extracellular Calcium Influx Is Delayed, *Plant Cell*, 1997, vol. 9, pp. 1999–2010.

205. Walker, S.A., Viprey, V., and Downie, J.A., Dissection of Nodulation Signalling Using Pea Mutants Defective for Calcium Spiking Induced Nod Factors and Chitin Oligomers, *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, pp. 13413–13418.

206. Wais, R.J., Galera, C., Oldroyd, G., Catoira, R., Penmetsa, R.V., Cook, D., Gough, C., Denarie, J., and Long, S.R., Genetic Analysis of Calcium Spiking Response in Nodulation Mutants of *Medicago truncatula, Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, pp. 13407–13412.

207. McAinsh, M.R., Webb, A.A.R., Taylor, J.E., and Hetherington, A.M., Stimulus-Induced Oscillations in Guard Cell Cytosolic Free Calcium, *Plant Cell*, 1995, vol. 7, pp. 1207–1219.

208. Hetherington, A.M., Gray, J.E., Leckie, C.P., McAinsh, M.R., Ng, C., Pical, C., Priestley, A.J., Staxen, I., and Webb, A.A.R., The Control of Specificity in Guard Cell Signal Transduction, *Phil. Trans. R. Soc., London, Ser. B*, 1998, vol. 353, pp. 1489–1494.

209. Allen, G.J., Chu, S.P., Harrington, C.L., Schumacher, K., Hoffmann, T., Tang, Y.Y., Grill, E., and Schroeder, J.I., A Defined Range of Guard Cell Calcium Oscillation Parameters Encodes Stomatal Movements, *Nature*, 2001, vol. 411, pp. 1053–1057.

210. Trewavas, A.J. and Malho, R., Signal Perception and Transduction: The Origin of the Phenotype, *Plant Cell*, 1997, vol. 7, pp. 1181–1195.

211. Tuckner, E.B. and Boss, W.F., Mastoparan-Induced Intracellular Ca^{2+} Fluxes May Regulate Cell-to-Cell Communications in Plants, *Plant Physiol.*, 1996, vol. 111, pp. 459–467.

212. Messerli, M.A., Creton, R.C., Jaffe, L.F., and Robinson, K.R., Periodic Increases in Elongation Rate Precede Increases in Cytosolic Ca^{2+} during Pollen Tube Growth, *Dev. Biol.*, 2000, vol. 222, pp. 84–98.

213. Digonnet, C., Aldon, D., Leduc, N., Dumas, C., and Rougier, M., First Evidence of Calcium Transient in Flowering Plants at Fertilization, *Development*, 1997, vol. 124, pp. 2867–2874.

214. Falke, J.J., Drake, S.K., Hazard, A.L., and Peersen, O.B., Molecular Tuning of Ion Binding to Calcium Signalling Proteins, *Q. Rev. Biophys.*, 1994, vol. 27, pp. 219–290.

215. Kretsinger, R.H., Rudnick, S.E., and Weisman, L.J., Crystal Stucture of Calmodulin, *J. Inorg. Biochem.*, 1986, vol. 28, pp. 289–302.

216. Concha, N.O., Head, J.F., Kaetzel, M.A., Dedman, J.R., and Seaton, B.A., Rat Annexin V Crystal Structure: Ca^{2+} Induced Conformational Changes, *Science*, 1993, vol. 261, pp. 1321–1324.

217. Kourie, J.I. and Wood, H.B., Biophysical and Molecular Properties of Annexin-Formed Channels, *Prog. Biophys. Mol. Biol.*, 2000, vol. 73, pp. 91–134.

218. Gerke, V. and Moss, S.E., Annexins: From Structure to Function, *Physiol. Rev.*, 2002, vol. 82, pp. 331–371.

219. Essen, L.O., Perisic, O., Cheung, R., Katan, M., and Williams, R.L., Crystal Structure of Mammalian Phosphoinositide-Specific Phospholipase Cδ, *Nature*, 1996, vol. 380, pp. 595–602.

220. Kopka, J., Pical, C., Hetherington, A.M., and Müller-Röber, B., Ca^{2+}/Phospholipid-Binding (C_2)Domain in Multiple Plant Proteins: Novel Components of the Calcium-Sensing Apparatus, *Plant. Mol. Biol.*, 1998, vol. 36, pp. 627–637.

221. Roberts, D.M. and Harmon, A.C., Calcium-Modulated Proteins: Targets of Intracellular Calcium Signals in Higher Plants, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1992, vol. 43, pp. 375–414.

222. Zielinski, R.E., Calmodulin and Calmodulin-Binding Protein in Plants, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1998, vol. 49, pp. 697–725.

223. Snedden, W.A. and Fromm, H., Calmodulin, Calmodulin-Related Proteins and Responses to the Environment, *Trends Plant Sci.*, 1998, vol. 3, pp. 299–304.

224. Snedden, W.A. and Fromm, H., Calmodulin as a Versatile Calcium Signal Transducer in Plants, *New Phytol.*, 2001, vol. 151, pp. 35–66.

225. Chin, D. and Means, A.R., Calmodulin: A Prototypical Calcium Sensor, *Trends Cell Biol.*, 2000, vol. 10, pp. 322–327.

226. Rhoads, A.R. and Friedberg, F., Sequence Motifs for Calmodulin Recognition, *FASEB J.*, 1997, vol. 11, pp. 331–340.

227. Takezawa, D., Liu, Z.H., An, G., and Poovaiah, B.W., Calmodulin Gene Family in Potato: Developmental and Touch-Induced Expression of the mRNA Encoding a Novel Isoform, *Plant. Mol. Biol.*, 1995, vol. 27, pp. 693–703.

228. Heo, W.D., Lee, S.H., Kim, M.C., Kim, J.C., Chung, W.C., Chun, H.J., Lee, K.J., Park, C.Y., Park, H.C., Choi, J.Y., and Cho, M.J., Involvement of Specific Calmodulin Isoforms in Salicylic Acid-Independent Activation of Plant Disease Resistance Responses, *Proc. Natl. Acad. Sci. USA*, 1999, vol. 19, pp. 766–771.

229. Liu, J. and Zhu, J.K., A Calcium Sensor Homolog Required for Plant Salt Tolerance, *Science*, 1998, vol. 280, pp. 1943–1945.

230. Kudla, J., Xu, Q., Harter, K., Gruissem, W., and Luan, S., Genes for Calcineurin B-Like Proteins in *Arabidopsis* Are Differentially Regulated by Stress Signals, *Proc. Natl. Acad. Sci. USA*, 1999, vol. 96, pp. 4718–4723.

231. Shi, J., Kim, K.N., Ritz, O., Albrecht, V., Gupta, R., Harter, K., Luan, S., and Kudla, J., Novel Protein Kinases Associated with Calcineurin B-Like Calcium Sensors in *Arabidopsis, Plant Cell*, 1999, vol. 11, pp. 2393–2406.

232. Ward, J.M., Hirschi, K.D., and Sze, H., Plants Pass the Salt, *Trends Plant Sci.*, 2003, vol. 8, pp. 200–201.

233. Braam, J., Sistrunk, M.L., Polisensky, D.H., Xu, W., Purugganan, M.M., Antosiewicz, D.M., Campbell, P., and Johnson, K.A., Plant Responses to Environmental Stress: Regulation and Function of the *Arabidopsis TCH* Genes, *Planta*, 1997, vol. 203, pp. 35–41.

234. Sistrunk, M.L., Antosiewicz, D.M., Purugganan, M.M., and Braam, J., *Arabidopsis TCH3* Encodes a Novel Calcium-Binding Protein and Shows Environmentally Induced and Tissue Specific Regulation, *Plant Cell*, 1994, vol. 6, pp. 1553–1565.

235. Frandsen, G., Müller-Uri, F., Nielsen, M., Mundy, J., and Skriver, K., Novel Plant Ca^{2+}-Binding Protein Expressed in Response to Abscisic Acid and Osmotic Stress, *J. Biol. Chem.*, 1996, vol. 271, pp. 343–348.

236. Jang, H.J., Pih, K.T., Kang, S.G., Lim, J.H., Jin, J.B., Piao, H.L., and Hwang, I., Molecular Cloning of a Novel Ca^{2+}-Binding Protein That Is Induced by NaCl Stress, *Plant. Mol. Biol.*, 1998, vol. 37, pp. 839–847.

237. Cordeiro, M.C., Piqueras, R., de Oliveira, D.E., and Castresana, C., Characterization of Early Induced Genes in *Arabidopsis thaliana* Responding to Bacterial Inoculation: Identification of Centrin and of a Novel Protein with Two Regions Related to Kinase Domains, *FEBS Lett.*, 1998, vol. 434, pp. 387–393.

238. Jakobek, J.L., Smith-Becker, J.A., and Lindgren, P.B., A Bean cDNA Expressed during a Hypersensitive Reaction Encodes a Putative Calcium-Binding Protein, *Mol. Plant–Microbe Interact.*, 1999, vol. 12, pp. 712–719.

239. Harmon, A.C., Gribskov, M., and Harper, J.F., CDPKs – a Kinase for Every Ca^{2+} Signal? *Trends Plant Sci.*, 2000, vol. 5, pp. 154–159.

240. Cheng, S.-H., Willmann, M.R., Chen, H.C., and Sheen, J., Calcium Signalling through Protein Kinases: The *Arabidopsis* Calcium-Dependent Protein Kinase Gene Family, *Plant Physiol.*, 2002, vol. 129, pp. 469–485.

241. Zhang, L. and Lu, Y.-T., Calmodulin-Binding Protein Kinases in Plants, *Trends Plant Sci.*, 2003, vol. 8, pp. 123–127.

242. Harmon, A.C., Gribskov, M., Gubrium, E., and Harper, J.F., The CDPK Superfamily of Protein Kinase, *New Phytol.*, 2001, vol. 151, pp. 175–183.

243. Sheen, J., Ca^{2+}-Dependent Protein Kinases and Stress Signal Transduction in Plants, *Science*, 1996, vol. 274, pp. 1900–1902.

244. Lee, J. and Rudd, J.J., Calcium-Dependent Protein Kinases: Versatile Plant Signalling Components Necessary for Pathogen Defense, *Trends Plant Sci.*, 2002, vol. 7, pp. 97–98.

245. Clark, G.B. and Roux, S.J., Annexins of Plant Cells, *Plant Physiol.*, 1995, vol. 109, pp. 1133–1139.

246. Crofts, A.J. and Denecke, J., Calreticulin and Calnexin in Plants, *Trends Plant Sci.*, 1998, vol. 3, pp. 396–399.

247. Clark, G.B., Sessions, A., Eastburn, D.J., and Roux, S.J., Differential Expression of Members of the Annexin Multigene Family in *Arabidopsis, Plant Physiol.*, 2001, vol. 126, pp. 1072–1084.

248. Pollard, H.B., Burns, A.L., and Rojas, E., Synexin (Annexin YII), a Cytosolic Calcium-Binding Protein, Which Promotes Membrane Fusion and Forms Calcium Channels in Artificial Bilayer and Natural Membranes, *J. Membr. Biol.*, 1990, vol. 117, pp. 101–112.

249. Shi, J., Gonzales, R.A., and Bhattacharyya, M.K., Characterization of a Plasma Membrane-Associated Phosphoinositide-Specific Phospholipase C from Soybean, *Plant J.*, 1995, vol. 8, pp. 381–390.

250. Pappan, K., Zheng, L., and Wang, X., Identification and Characterization of a Novel Plant Phospholipase D That Requires Polyphosphoinositides and Submicromolar Calcium for Activity in *Arabidopsis, J. Biol. Chem.*, 1997, vol. 272, pp. 7048–7054.

251. Pappan, K., Qin, W., Dyer, J.H., Zheng, L., and Wang, X., Molecular Cloning and Functional Analysis of Polyphosphoinositide-Dependent Phospholipase D, PLDβ, from *Arabidopsis, J. Biol. Chem.*, 1997, vol. 272, pp. 7055–7061.