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Topical Review

NAADP: An Emerging Calcium Signaling Molecule

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Abstract. Cells possess multiple Ca^{2+} stores and multiple messengers for mobilizing them. In addition to inositol trisphosphate and cyclic ADP-ribose, nicotinic acid adenine dinucleotide phosphate (NAADP), a metabolite of NADP, is shown to be a potent Ca^{2+} signaling molecule in both invertebrate and mammalian cells. This article summarizes the recent results of this newly discovered Ca^{2+} signaling mechanism and explores the implications of the apparent proliferation of Ca^{2+} messengers.

Key words: NAADP — Cyclic ADP-ribose — Calcium stores — Calcium signaling — ADP-ribosyl cyclase — CD38

Introduction

The advent of fluorometric Ca^{2+} imaging techniques (Tsien, 1981, 1989) has dramatically revealed the complexity of Ca^{2+} signaling in cells. Temporally, the cellular Ca^{2+} change can occur in the form of a transient Ca^{2+} wave or as repetitive oscillations. Growing evidence suggests that both the magnitude and the frequency of the Ca^{2+} changes can encode information (Dolmetsch et al., 1997; De Koninck & Schulman, 1998; Dolmetsch, Xu & Lewis, 1998) and reviewed in Berridge (1997). Spatially, Ca^{2+} changes can be highly localized and are not necessarily occurring uniformly throughout the cell. This is the case especially when mobilization of intracellular Ca^{2+} stores is involved. Ca^{2+} sparks observed in muscle cells are examples of such localized changes (Cheng et al., 1993). They represent elemental release of Ca^{2+} from sarcoplasmic reticulum via the ryanodine receptors. It has been proposed that the resulting high Ca^{2+} concentration produced in these localized regions can potentially modulate the function of other Ca^{2+} stores nearby (Rizzuto et al., 1993).

That cells possess multiple Ca^{2+} stores is generally accepted. In addition to the sarcoplasmic (endoplasmic) reticulum and the mitochondria, other organelles, such as the nuclear envelope, Golgi vesicles and secretory vesicles, are also believed to be functional Ca^{2+} stores (reviewed in Pozzan et al., 1994). Several messengers for mobilizing intracellular Ca^{2+} stores have been identified. Both inositol trisphosphate (IP_3) (Berridge, 1993) and cyclic ADP-ribose (cADPR) (Lee et al., 1989; Lee et al., 1994*b*) specifically activate Ca^{2+} release from the endoplasmic reticulum. Accumulating evidence indicates cADPR is an endogenous regulator of the Ca^{2+}-induced Ca^{2+} release mechanism in cells (Galione et al., 1991; Lee, 1993 and reviewed in Lee (1996, 1997)). The most recent addition to this proliferating family of Ca^{2+} signaling molecules is nicotinic acid adenine dinucleotide phosphate (NAADP), a metabolite of NADP. Both the Ca^{2+} stores and the Ca^{2+} release mechanism that NAADP activates have been shown to be independent of those of cADPR and IP_3 (Lee & Aarhus, 1995). This article summarizes the recent results on this newly discovered Ca^{2+} signaling mechanism and explores the implications of the apparent proliferation of Ca^{2+} messengers.

Structure of NAADP

NAADP is a metabolite of NADP and its structure is shown in Fig. 1. The only difference between NAADP

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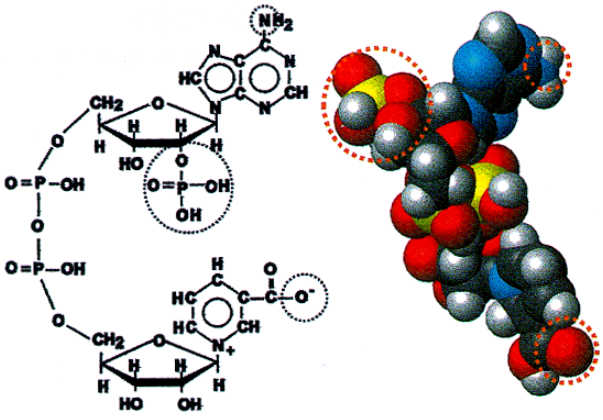

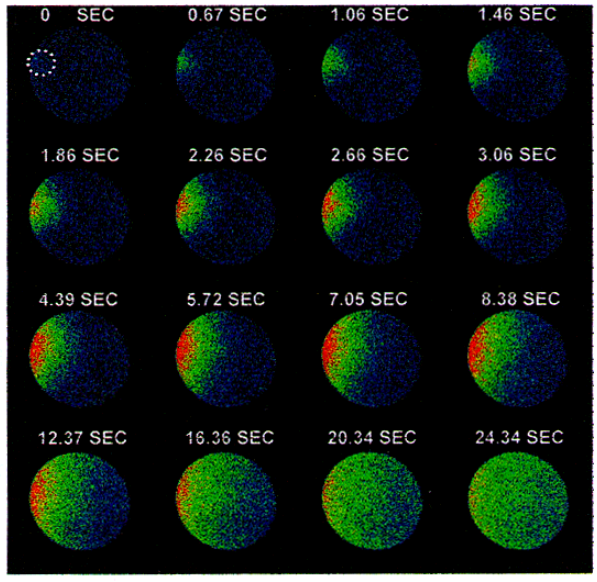

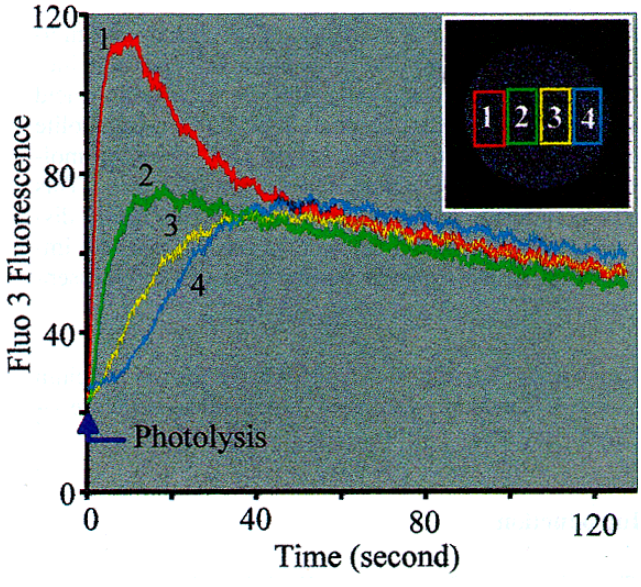

Fig. 1. The structure of NAADP. The structural determinants important to the Ca^{2+} mobilizing function of NAADP are indicated by dotted circles. The space-filling model of the molecule displayed using the program MOLMOL (Koradi et al., 1996) is based on the coordinates of NADP bound to isocitrate dehydrogenase. The amide group of NADP was changed to a carboxyl group and the energy of the resulting structure was minimized using the commercial program ChemBuilder3D. Carbon, black; oxygen, red; phosphate, yellow; nitrogen, blue; hydrogen, white.

Fig. 2. A Ca^{2+} wave produced by localized photo-release of NAADP in a sea urchin egg. The egg was preloaded with caged NAADP (~1μM intracellular concentration) and fluo 3. A nitrogen laser was used to locally photolyze caged NAADP and release NAADP in a spot of about 5–10 μm in diameter (dotted circle, upper panel). The diameter of sea urchin eggs is about 100 μm. The duration of the laser pulse was about 1 msec and the subsequent Ca^{2+} change was monitored by measuring the fluo-3 fluorescence. The upper panel shows a series of fluorescence images of the egg taken at various indicated times after localized photolysis. The lower panel shows the time courses of the change in the averaged fluo 3 fluorescence intensity within the four areas indicated in the inset of the figure. The fluorescence change in area 4 (right side) was delayed by about 20 sec as compared to area 1 (left side), which was very similar to that normally observed during fertilization. Results shown are representative of ten similar measurements.

and its precursor is the replacement of the nicotinamide group in NADP with a nicotinic acid group. The proton NMR spectrum of NAADP is, in fact, identical to NADP (Lee & Aarhus, 1995). This is because the protons that are different in the two molecules, the amide protons and the carboxyl proton, are exchangable and do not show up in the NMR spectra obtained normally in D_2O. However, the mass of the $-NH_2$ group of the amide in NADP is one unit lower than the -OH group of the carboxyl in NAADP and mass spectrometry shows that NAADP is indeed exactly one mass unit larger than its precursor NADP (Lee & Aarhus, 1995). This seemingly minor structural change, however, makes NAADP one of the most effective Ca^{2+} releasing agents ever described. In both invertebrate and mammalian cells, NAADP is active in the nanomolar range (Lee & Aarhus, 1995; Albrieux et al., 1998; Bak et al., 1999; Cancela et al., 1999).

A series of analogues of NAADP have been synthesized, providing information on their structure-function relationships. The importance of the 2′-phosphate group was shown by attaching a caging group to it, which eliminates its Ca^{2+} releasing activity (Lee et al., 1997*a*). Exposure of caged NAADP to ultraviolet light photolyzes the caging group and regenerates the biological activity. Nicotinic acid adenine dinucleotide, a common metabolite of NAD which is structurally identical to NAADP except without the 2′-phosphate, is likewise inactive biologically (Lee & Aarhus, 1995). The exact position of the phosphate is less critical. Derivatives with

either 3′-phosphate or with a phosphate group cyclically attached to both the 2′- and the 3′-position retain substantial Ca^{2+} releasing activity (Lee & Aarhus, 1997).

The amino group at the 6-position of the adenine of NAADP is also crucial since its conversion to a hydroxyl group increases the half-maximal concentration of NAADP from about 20 nM to higher than 10 μM (Lee & Aarhus, 1997). Retaining the 6-nitrogen of the amino group but cyclically attaching an etheno group linking N6 and N1 of the adenine ring produces a more active agonist, 1,N^6-etheno-NAADP, which is also fluorescent (Lee & Aarhus, 1998). A similarly active derivative, 1,N^6-etheno-2-aza-NAADP, has excitation and emission maxima, respectively at 360 nm and 470 nm (Lee & Aarhus, 1998), making it potentially suitable for visualizing the NAADP-sensitive Ca^{2+} stores in cells. These structure-function studies indicate that the Ca^{2+} releasing activity of NAADP has exquisitely specific structural requirements.

Enzymatic Synthesis of NAADP

NAADP can be chemically synthesized from NADP by an alkaline treatment at elevated temperature (Clapper et al., 1987; Lee & Aarhus, 1995). Commercial preparations of NADP are commonly contaminated with sufficient amounts of NAADP to account for all its apparent Ca^{2+} release activity (Clapper et al., 1987; Lee & Aarhus, 1995). Enzymatically, it is synthesized by ADP-ribosyl cyclase, the same enzyme that catalyzes the cyclization of NAD to produce cADPR (Aarhus et al., 1995). As shown in Fig. 1, NAADP is a long linear molecule bearing little resemblance with the cyclic structure of cADPR (Lee et al., 1989, 1994*b*). It is thus rather remarkable that a single enzyme can use two different substrates, NAD and NADP, to synthesize two different products. More so is the fact that both products possess novel Ca^{2+} signaling functions. In addition to cyclizing NADP (or NAD), the cyclase can also catalyze the exchange of the nicotinamide group of the pyridine nucleotide with nicotinic acid to form NAADP (Aarhus et al., 1995). This base-exchange reaction is dominant over the cyclization reaction at acidic pH (Aarhus et al., 1995).

The cyclase was first described in sea urchin egg homogenates and has since been shown to be ubiquitous (Clapper et al., 1987; Rusinko & Lee, 1989; Lee & Aarhus, 1991; Lee & Aarhus, 1993). The cyclase from *Aplysia* is a soluble protein of 30 kDa (Hellmich & Strumwasser, 1991; Lee & Aarhus, 1991). X-ray crystallography shows that it is a dimer in the form of a donut with a central cavity (Prasad et al., 1996). Each monomer is a bean-shaped molecule composed of two domains separated by a central cleft. The two monomers associate in a head-to-head fashion such that the two central clefts envelop and form the central cavity. Dynamic laser light scattering (Munshi et al., 1998) and chemical cross-linking (Bruzzone et al., 1998; Munshi et al., 1998) show that the cyclase is also a dimer in solution. All the β-sheets are concentrated in the C-domain of the monomer while the N-domain is consisted of mainly α-helixes (Prasad et al., 1996). The cyclase has ten cysteine residues and all are paired in disulfide linkages, three in the N-domain and two in the C-domain.

The cyclase has been co-crystallized with nicotinamide, a substrate for the base-exchange reaction (Aarhus et al., 1995; Munshi et al., 1999). X-ray crystallography shows that nicotinamide is bound in a pocket near the central cleft of each monomer. The Glu179 in the binding pocket is found by site-directed mutagenesis studies to be most critical for the enzymatic activities. Changing it to Gly leads to loss of both the cyclase and the base-exchange activity, but the mutant enzyme retains the ability to bind NAD as shown by photoaffinity labeling using ^{32}P-8-azido-NAD (Munshi et al., 1999). Similar loss of activity is observed with more conservative substitutions, such as changing Glu179 to Gln, Asn, Asp, or Leu. These results indicate Glu179 is likely to be the catalytic residue.

The cyclase is homologous to CD38, a lymphocyte antigen, sharing about 30% amino acid identity and a perfect alignment of the cysteine residues (States et al., 1992). The similarity extends to function and CD38 was also found to be a multifunctional enzyme catalyzing both the cyclization of NAD and the synthesis of NAADP via the base-exchange reaction (Howard et al., 1993; Aarhus et al., 1995; Lee et al., 1993; Takasawa et al., 1993*b*). Additionally, CD38 also catalyzes the effective hydrolysis of cADPR, which is completely resistant to other hydrolytic enzymes such as NADase, phosphatases or phosphodiesterases (Takahashi et al., 1995; Graeff et al., 1997). Thus, CD38 can serve as a degradation pathway for breaking down cADPR to ADP-ribose and terminating its Ca^{2+} signaling function. Although CD38 was first described as a surface antigen on lymphocytes, it has since been found to be ubiquitously expressed in many tissues, on cell surfaces as well as associated intracellular organelles (Koguma et al., 1994; Yamada et al., 1997). Indeed, the NAADP synthesizing activity widely observed among mammalian tissues may well be due to CD38 (Chini et al., 1995; Chini & Dousa, 1995). The enzymatic properties and the widespread biological functions of CD38 has been reviewed recently (Lee et al., 1997*b*, 1999).

Although the cyclase was first discovered in sea urchin eggs, the egg cyclase has not yet been purified. Nonetheless, important regulation mechanisms of the sea urchin egg cyclase have been described. The enzyme is activated by a cGMP-dependent process most likely via a protein kinase (Galione et al., 1993; Graeff et al.,

1998). Treatment of the eggs with nitric oxide can elevate the cellular cGMP levels and activate the cyclase, resulting in mobilization of the cADPR-sensitive Ca^{2+} stores (Clementi et al., 1996; Willmott et al., 1996). The cyclase activity of the eggs can also be activated by cAMP, but the end result is different. Under this condition, NAADP, instead of cADPR, synthesis is stimulated (Wilson & Galione, 1998). Two types of cyclase coexist in the eggs, a soluble and a membrane-bound form. The soluble form is cGMP-sensitive, while the membranous form is responsible for synthesizing NAADP (Graeff et al., 1998; Wilson & Galione, 1998). It thus appears that the synthesis of the two Ca^{2+} messengers in the eggs can be differentially regulated by different cyclic nucleotides.

Calcium Signaling

The Ca^{2+} release activity of NAADP was first described in sea urchin egg homogenates (Clapper et al., 1987; Lee & Aarhus, 1995). Addition of NADP to the homogenates elicited a rapid Ca^{2+} release, which was found to be due to the contaminating NAADP. That the NAADP mechanism is functional in live cells was directly demonstrated by microinjection of NAADP into intact eggs (Lee & Aarhus, 1995; Perez-Terzic et al., 1995). Rapid release of NAADP in the entire egg by whole-cell illumination to photolyze caged NAADP preloaded into the cell can activate a prolonged Ca^{2+} oscillation (Aarhus et al., 1996). Localized release of NAADP, on the other hand, can initiate a propagative Ca^{2+} wave as shown in Fig. 2. The output of a nitrogen laser was focused to a spot about 5–10 μm in diameter at the edge of an egg preloaded with caged NAADP and fluo 3. A single pulse of laser light initiated a localized Ca^{2+} increase, which then propagated across the entire egg in about 20 sec (Fig. 2). The speed of the Ca^{2+} wave is very similar to that observed at fertilization (Eisen et al., 1984). The NAADP-mechanism is thus highly versatile and is capable of producing either propagative Ca^{2+} waves or Ca^{2+} oscillations, depending on the mode of excitation.

The NAADP-mechanism can be distinguished pharmacologically from other Ca^{2+}-signaling mechanisms. It is insensitive to antagonists of the cADPR and the IP_3-receptors, 8-amino-cADPR (Walseth & Lee, 1993) and heparin, respectively (Lee & Aarhus, 1995). The NAADP-sensitive Ca^{2+} release can be blocked by L-type Ca^{2+} channel blockers, suggesting similarity (Genazzani et al., 1996, 1997). Fractionation studies indicate the NAADP-sensitive Ca^{2+} stores in sea urchin eggs are physically separate from those sensitive to cADPR or IP_3 (Lee & Aarhus, 1995) and they appear to possess a thapsigargin-insensitive Ca^{2+}-ATPase (Genazzani & Galione, 1996). In yeast, a similar Ca^{2+}-ATPase (PMR1) has been shown to be associated with the Golgi (Sorin et al., 1997). A homologue of PMR1 has also been cloned from rat (Gunteski-Hamblin et al., 1992). In PC12 cells, thapsigargin-insensitive Ca^{2+} stores have been described (Fasolato et al., 1991), suggesting this type of Ca^{2+} store may be widespread.

Indeed, systems responsive to NAADP include invertebrate, sea urchin eggs (Clapper et al., 1987; Lee & Aarhus, 1995) and *Ascidian* oocytes (Albrieux et al., 1998), as well as mammalian, mouse pancreatic acinar cells (Cancela et al., 1999) and rat brain microsomes (Bak et al., 1999). In all cases, the effective NAADP concentration is in the range of 10–50 nM except for the brain microsomes, which is higher at about 1 μM. The pharmacology is also similar, the effects of NAADP being sensitive to L-type channel blockers but insensitive to 8-amino-cADPR and heparin. In the case of the pancreatic acinar cells, it is proposed that NAADP-induced Ca^{2+} release is highly localized, but the resulting Ca^{2+} signal is then amplified by the Ca^{2+}-induced Ca^{2+} release mechanism mediated by both the cADPR- and the IP_3-mechanisms (Cancela et al., 1999). Thus inhibiting the amplification mechanisms by 8-amino-cADPR (Walseth & Lee, 1993) or heparin can lead to apparent inhibition of the NAADP-sensitive Ca^{2+} release. That NAADP can induce highly localized Ca^{2+} release is also seen in *Ascidian* oocytes. Diffusion into the oocytes of as low as 10 nM NAADP through a patch pipette can induce a rapid and Ca^{2+}-dependent decrease in ion current at the oocyte membrane. The NAADP-induced Ca^{2+} release is apparently restricted only to the cortical region of the oocytes and does not extend into the bulk of the cytoplasm (Albrieux et al., 1998). Clearly, cells possess multiple Ca^{2+} stores and multiple signaling molecules to mobilize them. Through restricted distribution and selective interactions among them, Ca^{2+} signaling can be localized or amplified into global changes. This kind of versatility may well be one of the main reasons why cells need more than one Ca^{2+} messenger. The implications of the apparent proliferation of Ca^{2+} messengers will be explored further in a later section.

Receptor Desensitization

There is ample evidence that the NAADP-sensitive Ca^{2+} release is mediated by a specific receptor. The structure-function studies described above show the exquisitely specific structural requirements of NAADP itself (Lee & Aarhus, 1997), consistent with recognition by a receptor. Direct demonstration comes from ^{32}P-NAADP binding in sea urchin egg microsomes, which is specifically competed by NAADP with a half-mximal concentration of about 10 nM, similar to the half-maximal effective concentration of the Ca^{2+} release (Aarhus et al., 1996). The L-type channel blockers, although effective in inhibiting the NAADP-sensitive Ca^{2+} release, do not compete for

^{32}P-NAADP binding (Genazzani et al., 1997), suggesting their site of action is the Ca^{2+} release channel and not the ligand activation site.

An interesting property of the NAADP-mechanism that needs to be discussed in relation to its *in vivo* function is self-inactivation or desensitization (Aarhus et al., 1996; Genazzani et al., 1996). In egg homogenates, the mechanism can be inactivated by prior exposure to NAADP and will not respond to subsequent challenge with NAADP. The desensitization occurs at the receptor level since it can be demonstrated directly using specific NAADP-binding to microsomes (Aarhus et al., 1996). If this self-inactivation also operates *in vivo*, it would suggest that the mechanism can only work once. This does not appear to be the case since self-inactivation is fully reversible after NAADP removal. In egg homogenates, removal of NAADP can be done by simple dilution (*unpublished data*). Removal can also be done effectively using common hydrolytic enzymes such as alkaline phosphatase or nucleotide pyrophosphatase (Lee et al., 1997*a*). Endogenous NAADP hydrolysis activity has also been detected in sea urchin egg and a variety of mammalian tissue extracts (Chini & Dousa, 1995; Wilson & Galione, 1998). The general presence of these enzymes in cells ensures that NAADP is degraded after its signaling function is complete and the mechanism can thus respond again to NAADP. Indeed, activation of the degradation pathway can potentially be an integral part of the NAADP signaling mechanism and regulate how long the NAADP-mechanism remains refractory after initial activation.

Self-desensitization is, in fact, not unique to the NAADP-mechanism but is also widely seen in the IP_3- and cADPR-mechanisms (Clapper et al., 1987; Dargie et al., 1990; Pietri et al., 1990; Hajnoczky and Thomas, 1994; Genazzani et al., 1996; Oancea and Meyer, 1996; Dufour et al.,). The NAADP-mechanism, however, does appear to be more susceptible to such self-modulation (Aarhus et al., 1996; Genazzani et al., 1996).

A Proliferating Family of Calcium Messengers

It is generally accepted that IP_3 is a ubiquitous Ca^{2+} messenger for mobilizing the Ca^{2+} stores in the endoplasmic reticulum (reviewed in Berridge (1993)). The fact that cells do possess other Ca^{2+} stores releasable by other Ca^{2+} messengers makes it of interest to consider possible reasons that this may confer significant advantages to the cells.

Mediating Spatially Localized Ca^{2+} Changes

Localization of a particular type of Ca^{2+} store in a selective region of the cell, in principle, can allow Ca^{2+} changes to occur only in that region. The *Ascidian* oocyte is such a case. The cADPR- and NAADP-sensitive Ca^{2+} stores appear to be restricted only to the cortex of the oocyte. Ca^{2+} release from these stores effectively activates membrane events such as cortical exocytosis and modulation of the membrane ion current, but does not affect the cytoplasmic Ca^{2+} concentration (Albrieux et al., 1998). The IP_3-sensitive Ca^{2+} stores, on the other hand, are widely distributed in the cytoplasm, allowing global activation of the oocyte.

A similar situation is seen in mouse oocytes, which were previously thought to be responsive only to IP_3 since microinjection of micromolar concentrations of cADPR failed to elevate cytoplasmic Ca^{2+} (Kline & Kline, 1994). However, when the Ca^{2+}-dependent cortical exocytosis is specifically measured, cADPR is shown to be highly effective with a half-maximal concentration of 5 nM (Ayabe et al., 1995). Thus, mobilization of Ca^{2+} stores can result in highly localized Ca^{2+} changes and cytoplasmic Ca^{2+} elevation is not necessarily an obligatory response.

Segregation of Ca^{2+} stores is also seen in parotid acinar cells. Using the fluorescent probe, BODIPY-ryanodine, the ryanodine receptor containing Ca^{2+} stores are localized to the basal pole of the cells. Both cADPR and ryanodine compete for the fluorescent labeling indicating the stores are sensitive to cADPR (Zhang et al., 1999). The IP_3-receptor, on the other hand, is segregated to the apical pole. Since the diffusion of Ca^{2+} in cells is very limited (Allbritton et al., 1992), localization of Ca^{2+} stores should result in localized Ca^{2+} changes. Selective regions of the cell can thus be activated without affecting other parts.

Subserving Distinct Cellular Functions

Although cells possess multiple Ca^{2+} stores, a stimulus, by coupling to a specific messenger, can selectively mobilize only one specific type. This is the case when plant cells are activated by the plant hormone, abscisic acid (ABA). ABA regulates the expression of a complex series of genes, which enables plants to respond to adverse environmental cues. Microinjection of cADPR into plant cells activates the similar gene expression as ABA. Conversely, 8-amino-cADPR, an antagonist of cADPR (Walseth & Lee, 1993), completely eliminates the effects of ABA on gene expression. No such inhibition is seen with heparin, an inhibitor of the IP_3-receptor (Wu et al., 1997). The ABA action is thus specifically linked to the cADPR-sensitive stores. Similar selective activation of Ca^{2+} stores has also been reported in lacrimal acinar cells, where α-adrenergic stimulation mobilizes the cADPR-sensitive stores, while acetylcholine activates the IP_3-pathway (Gromada et al., 1995*a*,*b*).

In *Ascidian* oocytes, fertilization triggers a rapid

turning off of the membrane Ca^{2+} current, initiates the cortical exocytosis and a long term cytoplasmic Ca^{2+} oscillation (Albrieux et al., 1997, 1998). Both NAADP and cADPR are highly effective in modulating the membrane current, with a half-maximal concentration of about 1 nM. Additionally, cADPR can trigger the cortical exocytosis, which neither IP_3 nor NAADP are capable of mediating (Albrieux et al., 1998). On the other hand, only IP_3, but not cADPR or NAADP, is able to initiate the cytoplasmic Ca^{2+} oscillation. It thus appears that the three Ca^{2+} signaling molecules are each involved in mediating distinct functions in the oocytes.

Not all cells possess multiple types of Ca^{2+} stores. The pancreatic β-cell appears to have only the cADPR-sensitive stores (Takasawa et al., 1993*a*). Normal β-cells respond to glucose challenge with elevation of endogenous cADPR and increased insulin secretion. In diabetic β-cells, such as from *ob/ob* mouse islets or from the RINm5F cell line, the cADPR signaling system is replaced by the IP_3-mechanism, and these aberrant cells also lose the ability to respond to glucose (Takasawa et al., 1998). Similar exclusivity in Ca^{2+} stores is seen in intestinal muscles. The longitudinal muscle possesses only the cADPR-sensitive Ca^{2+} stores while the IP_3-mechanism is in the circular muscle (Kuemmerle & Makhlouf, 1995).

Generating Complex Ca^{2+} Signaling Patterns Through Interaction

The mechanism of Ca^{2+} release activated by the three Ca^{2+} agonists are different. The action of cADPR, in conjunction with calmodulin, is to greatly increase the Ca^{2+} sensitivity of the Ca^{2+}-induced Ca^{2+} release mechanism such that it can be activated by Ca^{2+} even when it is in nanomolar range (Lee, 1993; Lee et al., 1994*a*, 1995). IP_3-induced Ca^{2+} release also shows dramatic dependence on Ca^{2+} (Bezprozvanny et al., 1991; Finch et al., 1991; Dufour et al., 1997) and, in *Xenopus* oocytes, is required for the generation and propagation of Ca^{2+} waves (DeLisle & Welsh, 1992). The NAADP-mechanism, however, shows no dependence on Ca^{2+} (Chini & Dousa, 1996; Genazzani et al., 1996, Bak et al., 1999). This and the self-inactivation property make NAADP ideally suited for functioning as a Ca^{2+} trigger. Indeed, in pancreatic acinar cells, NAADP has been proposed as the Ca^{2+} trigger responsible for initiating the Ca^{2+} spiking observed following treatment with physiological concentrations of cholecystokinin (Cancela et al., 1999). The localized Ca^{2+} released by NAADP, through interaction with the Ca^{2+}-induced Ca^{2+} release mechanisms mediated by cADPR and IP_3, is then amplified into Ca^{2+} spiking. Thus, inactivation of the NAADP-receptor completely blocks cholecystokinin from initiating the spiking (Cancela et al., 1999).

The generation of a Ca^{2+} wave is another Ca^{2+} signaling pattern commonly seen in cells. That a localized increase of NAADP, and the subsequent interaction with other Ca^{2+} release mechanisms, can generate a propagating wave is shown in Fig. 2.

The *Ascidian* oocyte is another example of a cell in which various types of Ca^{2+} stores functionally interact. Thus, both the IP_3-induced and the fertilization-induced cytoplasmic Ca^{2+} oscillations can be greatly dampened by prior activation of the cortical NAADP-stores (Albrieux et al., 1998). Similarly, in the case of sea urchin eggs, the long-lasting Ca^{2+} oscillation activated by photolyzing caged NAADP has been proposed to be the consequence of the interaction between the NAADP- and the cADPR-sensitive stores (Aarhus et al., 1996; Lee et al., 1997*a*).

Differentiating Diverse Ca^{2+} Stimuli

Activation of surface receptors by agonists generally leads to IP_3 production (Berridge, 1993). Receptor activation can also result in production of cADPR as is shown in lymphocytes following stimulation of the T-cell receptor by antibody (Guse et al., 1999). However, Ca^{2+} mobilization is not exclusively linked to surface receptor activation. Nitric oxide, a permeant messenger, whose action bypasses the surface receptor, can likewise signal through Ca^{2+} mobilization via the cADPR-pathway (Clementi et al., 1996; Willmott et al., 1996). In fact, Ca^{2+} mobilization is involved in processes totally intrinsic to cells, such as circadian rhythm and cell division (Twigg et al., 1988; Ding et al., 1998). It is thus intriguing to think that cells may possess a battery of Ca^{2+} messengers, each linked to a specific class of stimuli. In this manner, diverse signals can be differentiated.

Concluding Remarks

The picture emerging is that various types of Ca^{2+} stores in cells are not necessarily distributed uniformly. The segregation of Ca^{2+} stores allows highly localized Ca^{2+} signaling in selected regions without having to affect the cell globally. Thus, elevation of cytoplasmic Ca^{2+} is not necessarily an obligatory consequence of store-mobilization. These local Ca^{2+} changes, nevertheless, can be specifically linked to particular physiological responses. Through interactions with other Ca^{2+} signaling mechanisms, these localized Ca^{2+} changes can be, in some cases, amplified into global changes or propagated as Ca^{2+} waves. Therefore, depending on which type of Ca^{2+} stores are mobilized the biological consequences can be very different. Ca^{2+} mobilization as a signaling mechanism is thus versatile and highly specific. The ex-

istence of a multitude of Ca^{2+} messenger systems and Ca^{2+} stores can, therefore, provide the cells with the means to respond to and differentiate between diverse stimuli.

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