J. Membrane Biol. 153, 83–92 (1996)

The Journal of
Membrane
Biology

Topical Review

The Cell Nucleus: An Eldorado to Future Calcium Research?

L. Santella
Laboratory of Cell Biology, Stazione Zoologica ''A. Dohrn,'' Villa Comunale, 80121, Napoli, Italy

Received: 26 February 1996/Revised: 15 May 1996

Introduction

Calcium signaling is one of the most intensively investigated areas of cell biology: topics like membrane transport (pumps, channels, receptors, exchangers), the decoding of the calcium message by proteins like calmodulin, and the diverse categories of calcium-modulated enzymes, have become popular objects of study. All this, however, has so far largely concerned the cytosolic compartment of the cell: surprisingly, the nucleus has essentially remained a black box to calcium research. The literature conveys an impression of reluctance in accepting the nucleus and its specific functions as *bona fide* prime targets of calcium signaling: especially controversial is the area of nuclear/cytoplasmic calcium fluxes and their control. Important new information has appeared during the last few years, but the nucleus has not yet become a favored object of calcium research. One purpose of this topical review is to convey these exciting new developments to help convince calcium signaling researchers that the nucleus could become an eldorado for future advancement.

The Traffic of Calcium across the Nuclear Envelope: Unrestricted Diffusion *vs.* Controlled Permeability

By general consensus the nuclear pores only restrict the permeability of molecules larger than about 20 kDa, and therefore simple ions like calcium should in principle diffuse freely in and out of the nucleus. Unless mechanisms exist to somehow control the traffic of calcium through the pores, the nuclear interior would thus not be shielded from the changes in the cytoplasmic concentration of calcium required to modulate specific cytoplasmic functions. Recent work has documented the existence in the nucleus of numerous calcium-modulated functions that are specific to it, i.e., not common to the cytoplasm: intuitively, one would thus like to think that their modulation, in an environment as special as that within the nucleus, would demand a calcium homeostasis separated from that of the cytoplasm.

The literature on the control of the concentration of calcium in the nucleus under different conditions has become abundant and is to a large extent conflicting. Depending on the cell system studied, on the method used, and on the physiological condition considered, the nuclear concentration of calcium has been found to be lower, higher or equal to that of the cytoplasm (Williams, Becker & Fay, 1985; Neylon et al., 1990; Bachs, Agell & Carafoli, 1992, 1994; Himpens, De Smedt & Casteels, 1994; Lin, Hajnóczky & Thomas, 1994). More recent work has emphasized the likelihood of errors in some of the earlier measurements (Al-Mohanna, Caddy & Bolsover, 1994), and, most importantly, has shifted the emphasis from the long-term difference in concentration to the possibility and the magnitude of time delays in the transmission of the cytoplasmic calcium waves to the nucleus: this has now become an important topic, and will thus be discussed in some detail. One preliminary caveat, however, is in order: measurements (e.g., using fluorescent calcium indicators), in cell systems in which the cytoplasmic compartment is spatially minor with respect to the nuclear one, have yielded substantially different results with respect to those on cells in which large

Key words: Nuclear calcium—Calcium signaling—Calmodulin
Correspondence to: L. Santella

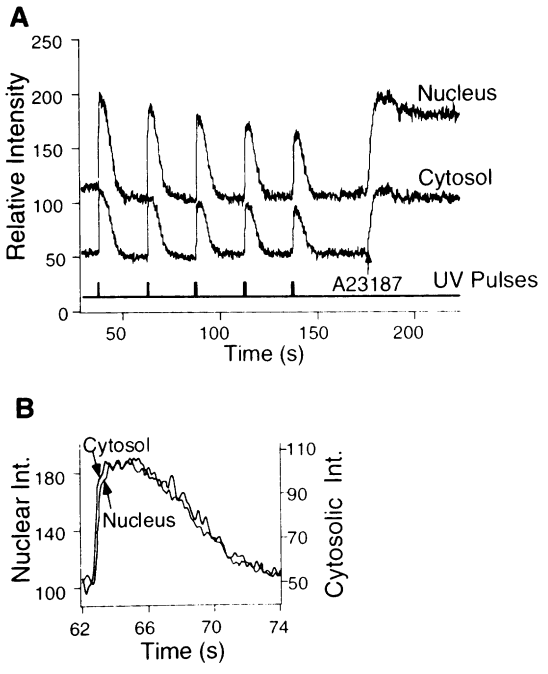

Fig. 1. Nuclear and cytosolic calcium responses to pulses of caged $InsP_3$ into the cytosol. The figure shows: (*A*) fluorescence intensity traces from nuclear and cytosolic locations during multiple UV laser pulses to release $InsP_3$ from the caged state. The cells were microinjected with a mixture of 70 kDa calcium green dextran, 70 kDa calcium green dextran targeted to the nucleus with a nuclear localization sequence, and caged $InsP_3$. (*B*) Expansion of the time scale of the second calcium spike of *A.* The fluorescence intensities of the nuclear and cytosolic traces were normalized so that maximal and minimal intensities are coincident. (*Modified from* Allbritton et al., 1994).

cytoplasmic and nuclear compartments coexist (e.g., oocytes). Although it would have been advisable to compare cells that have similar (spatial) characteristics, this has not been generally done: as a result, the situation is presently highly controversial. The possibility that the nuclear-cytoplasmic calcium traffic would occur with properties that are inherently different in different cell systems must be given serious consideration.

With this general caveat in the background, the most significant recent contributions to the problem of the nuclear-cytoplasmic calcium traffic can be summarized as follows: targeting of the calcium indicators aequorin (Brini et al., 1993) and calcium green dextran (Allbritton et al., 1994) to the nucleus of basophilic leukemia and Hela cells by coupling them to nuclear localization sequences has shown that calcium waves generated in the cytosol by the emptying of stores were transmitted to the nucleoplasm with insignificant time delays. Figure 1 shows an experiment (Allbritton et al., 1994) with nuclear-targeted 70 kDa calcium green dextran in rat basophilic leukemia cells: the cytosolic calcium transients induced by inositol 1,4,5-trisphosphate ($InsP_3$)[1] were transmitted nearly immediately to the nucleoplasm, with a delay of the order of 200 msec. The conclusion of these studies with nuclear-targeted indicators was therefore that the nucleoplasm was not insulated from cytosolic calcium: i.e., the nuclear pores were freely and essentially continuously permeable to it. At variance with these results, a recent study using confocal microscopy of cultured neuronal cells treated with the fluorescent indicator fluo-3 has shown that small changes of cytosolic calcium were indeed rapidly transmitted to the nucleus, (Al-Mohanna et al., 1994) whereas larger (>300 nM) cytosolic calcium increases were clearly attenuated in the latter. Two possible explanations were offered for the attenuation phenomenon: either the buffering of nucleoplasmic calcium by a high capacity ligand, or the closing of the nuclear pores at cytosolic calcium concentrations in excess of 300 nM.

The existence of ionic concentration gradients across the nuclear envelope (NE)[1] had been originally indicated by earlier work by Loewenstein and Kanno (1963), Ito and Loewenstein (1965), Paine, Moore and Horowitz (1975), and Feldherr, Kallenbach and Schultz (1984). The transit of the macromolecules destined to penetrate into the nucleus, or to emerge from it has been shown to occur through the central channel of the nuclear pore complexes (NPCs)[1] (*see* Hinshaw, Carracher & Milligan, 1992 for a 3-dimensional model of these structures) which span the inner and outer nuclear membranes (*reviewed in* Wiese & Wilson, 1993; Gerace & Foisner, 1994; Davis, 1995; Goldberg & Allen, 1995). The permeability of the NPCs to small ionic species like calcium has never been specifically considered, but the experiments of Al-Mohanna et al. (1994) on the fluxes of calcium between the cytosol and the nucleus of neuroblastoma cells have led the authors to conclude that the entire area of the nuclear pores, i.e., the central hole and the openings between the spokes, was available for calcium diffusion. The NPCs could however exist in both open and closed states, as suggested, and experimentally supported, by electrophysiological work by various authors (Mazzanti et al., 1990; Mazzanti, DeFelice & Smith, 1991; Bustamante, 1992; Bustamante, 1994; Dale et al., 1994; Bustamante et al., 1995): Patch-clamping of isolated nuclei has indeed revealed ionic channels (conduc-

[1] The abbreviations used are: NE, nuclear envelope; NPCs, nuclear pore complexes; GVBD, germinal vesicle breakdown; 1-MA, 1-methyladenine; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′,-tetraacetic acid; $InsP_3$, inositol 1,4,5-trisphosphate; $InsP_4$, inositol 1,3,4,5-tetrakisphosphate; cADPr, cyclic ADP-ribose; MLCK, myosin light chain kinase, CFS, cytostatic factor; CaMK II, calmodulin-dependent kinase II; hnRNPs, heterogeneous ribonucleoprotein particles.

tance of 200 pS) in the nuclear envelope: a study by Tabares et al. (1991) has shown that the species transported through the channels was chloride. Since the patch area examined always contained several pores, the necessary conclusion of these studies (Mazzanti, Innocenti & Rigatelli, 1994) was that the pores, which were proposed to correspond to the ionic channels, were not freely permeable to small ionic species. Importantly, these ionic channels, were also detected when the nuclear envelope was patch clamped within the cell (Mazzanti et al., 1994), ruling out the objection (Bustamante, 1994) that the channels were artifactual and due to the absence of cytosolic components in the essay medium.

Some of these studies (DeFelice & Mazzanti, 1993; Dale et al., 1994) were performed on the isolated nuclei of starfish oocytes, using patches that contained more than 100 pores. The germinal vesicle (nucleus) of these oocytes has a diameter of about 60 μm and is located at the animal pole, away from the endoplasmic reticulum structures that envelope the nucleus of most of the cell types used in calcium-imaging experiments. When isolated from the ovary, these oocytes are arrested at the late prophase of the first meiotic division. The resumption of meiosis, including the breakdown of the large germinal vesicle (GVBD)[1] and the formation of the two polar bodies, is triggered by the interaction of the hormone 1-methyladenine (1-MA)[1], secreted from the follicle cells that surround the oocytes (Kanatani & Shirai, 1967; Kanatani et al., 1969; Shilling et al., 1989; Chiba et al., 1993) with the plasma membrane of the latter. Numerous studies have suggested a role for intracellular calcium in meiosis reinitiation in the oocytes of invertebrates (Moreau et al., 1978; Deguchi & Osanai, 1994; Santella & Kyozuka, 1994), amphibians (Masui et al., 1977; Moreau, Guerrier & Dorée, 1980) and mammals (De Felici, Dolci & Siracusa, 1991; Kaufman & Homa, 1993; Lefèvre, Pesty & Testart, 1995). Calcium transients have been associated with the breakdown of the nuclear envelope before mitosis in sea urchin embryos (Poenie et al., 1985; Steinhardt & Alderton, 1988), in mouse eggs following parthenogenetic activation (Tombes et al., 1992) and during the first cell cycle of sea urchins (Steinhardt, 1990; Ciapa et al., 1994; Harris, 1994). Calcium has been proposed to participate also in the regulation of other aspects of the cell division cycle (*reviewed in* Whitaker & Patel, 1990; Hepler, 1994; Means, 1994).

The very large size of the starfish oocytes nuclei permits their direct impalement *in vivo*, allowing the selective introduction into them of calcium-indicators or chelators, of calcium itself, and of various modulators of calcium fluxes and functions. Surprisingly, microinjection experiments (Santella & Kyozuka, 1994) have shown that fura-2 injected into the nucleus remained there for at least 60 min (Fig. 2*A*): i.e., it apparently failed to diffuse freely across the nuclear envelope.When fura-2 (or calcium green conjugated to a 10 kDa dextran) was instead injected into the cytoplasm, it apparently penetrated into the nucleus, since the latter stood out as very brilliant against the background of the cytosol (Fig. 2*B*). However, the manual extraction of the nucleus from the fluorescent cytoplasm (Fig. 2*C*) revealed that the dye had in fact failed to enter the nucleus, whose intense fluorescence was evidently artifactual, and due to the layers of cytoplasm surrounding it. The possibility that dye had become lost from the nucleus during the procedure of its extraction from the cell was ruled out by experiments in which the extraction was performed after injecting fura-2 into the nucleus; the nucleus maintained its intense fluorescence throughout the entire procedure

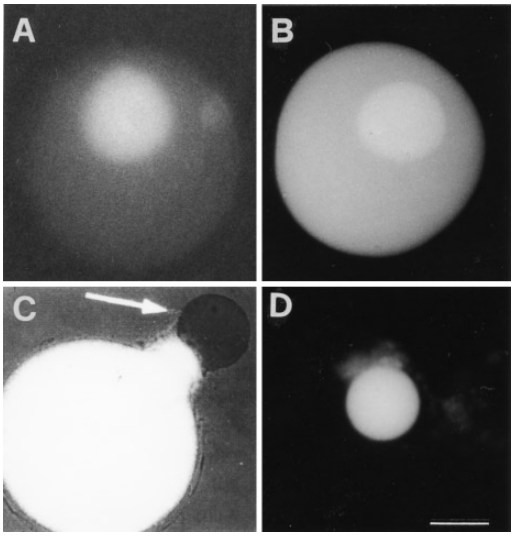

Fig. 2. Microinjection of fura-2 into starfish oocytes. (*A*) The indicator was injected directly into the nucleus of *Asterina pectinifera* oocytes and remained there, i.e., it did not diffuse to the cytoplasm, for at least 1 hr. (*B*) The oocytes were injected with fura-2 into the cytoplasm. After the injection the nucleus became even more brilliant than the cytoplasm, apparently indicating that the dye had diffused into it from the cytoplasm. However, panel *C* shows that this had not been the case: 1 hr after the injection of the indicator into the cytoplasm, manual extrusion of the nucleus (arrow) revealed that it was not fluorescent, i.e., fura-2 had not penetrated into it. It may be pertinent to mention at this point that in the previously quoted electrophysiological work on starfish oocytes by Dale et al. (1994) the dye lucifer yellow did not diffuse out of the nucleus when injected into it, whereas, as the authors of the study put it, it ''apparently'' diffused into it when injected into the cytoplasm. Possibly, also in that study the diffusion of the dye from the cytoplasm to the nucleus had been only apparent. To optimally visualize the dark nucleus in panel (*C*) phase-contrast microscopy was used. (*D*) Isolation of the nucleus previously injected with the indicator from an oocyte. The nucleus retained its intense fluorescence throughout the entire procedure of its extraction. Thus, the lack of fluorescence in the nucleus in panel *C* was not due to the loss of the indicator (and of calcium) during the procedure of manual extraction of the nucleus. Bar = 50 μm. (*Modified from* Santella & Kyozuka, 1994).

(Fig. 2*D*). Apparently, then, under the ''natural'' in vivo conditions the nuclear envelope of the starfish oocytes was not freely permeable to small molecules like fura-2 (700 Da) and calcium. The starfish oocytes work has also shown that 1-MA triggered three calcium transients, two in the cytoplasm and one in the nucleus: the latter was temporally sandwiched between the two cytosolic transients, following the first by about 20 sec (Fig. 3*A*). Elimination of the cytoplasmic calcium transients by injecting calcium chelators into the cytosol suppressed the nuclear transient: nuclear calcium, however, still rose gradually and GVBD was delayed (Fig. 3*B*). By contrast, the injection of the chelators into the nucleus left the first cytosolic calcium transient unchanged but suppressed the second and blocked GVBD (Fig. 3*C*). In these oocytes, the cytosolic and nuclear calcium pools were evidently separate, and their cross-talk was bidirectional: i.e., the cytoplasmic calcium transients (the first cytosolic peak after 1-MA treatment) were transmitted to the nucleoplasm, and the nuclear transient was in turn transmitted to the cytosolic compartment, inducing the second cytosolic peak which was essential for re-entry into meiosis.

Two last important findings, which open interesting perspectives on the possible mechanisms for the regulation of the permeability of the pores, must be mentioned to complete the discussion of the permeability of the envelope to small molecules, including calcium. A recent study, (Greber & Gerace, 1995) has used a combination of ionophores and the inhibitor of the endoplasmic reticulum calcium pump, thapsigargin, to deplete calcium from the lumen of the endoplasmic reticulum (and from the nuclear envelope, which is its extension). Under these conditions, the signal-mediated transport of proteins, but also the ''passive'' diffusion of small molecules from the cytoplasm to the nucleus became potently (and reversibly) inhibited: Stehno-Bittel et al. (1995) have instead depleted the calcium store of isolated nuclei, or of isolated nuclear membrane ghosts by $InsP_3$, or calcium chelators and have observed that the diffusion of intermediate-size molecules into the nucleus (but not, apparently, that of species smaller than 500 kDa) was inhibited. These striking results show that the permeability of the nuclear pores is indeed modulated, and thus offer a convenient way out of the dilemma of whether the nuclear pores are permanently open or, somehow, sealed: apparently, they can exist in either state depending on conditions.

The Traffic of Calcium across the Nuclear Envelope: How Many Transport Systems?

Most studies on the exchange of calcium between the cytosol and the nucleoplasm have so far concentrated on the nuclear pores. One of them (Al-Mohanna et al., 1994) has even elaborated a mathematical diffusion model in which calcium was predicted to pass through the entire area of the pore complex, and not just through its central hole which is generally assumed to allow the transit of macromolecules. The problem with the pores, however, is that the nuclear envelope also contains specific calcium-transporting systems: a calcium pump (Nicotera et al., 1989; Lanini, Bachs & Carafoli, 1992), an $InsP_3$-sensitive calcium channel (Nicotera et al., 1990; Malviya, 1994; Miyazaki, 1995; Gerasimenko et al., 1995; Stehno-Bittel et al., 1995) a receptor for inositol 1,3,4,5-tetrakisphosphate ($InsP_4$)[1] (Köppler et al., 1993; Malviya, 1994), and a cyclic ADP-ribose (cADPr)[1] (ryanodine) modulated channel (Gerasimenko et al., 1995). It has also been shown that the nucleus contains the enzymatic pathway for the production of $InsP_3$ (Divecha et al., 1993). The presence of these systems in the envelope is hardly surprising, considering that the latter is an extension of the endoplasmic reticulum, which contains these systems: Indeed, recent work has established that the calcium pump of the envelope is identical to that of endoplasmic reticulum (Lanini et al. 1992). These transporting systems must evidently be integrated with the pores in the overall process of the regulation of nuclear calcium homeostasis. A reasonable proposal would be that the pump, which is likely to be located on both membranes of the envelope, would accumulate calcium into the lumen of the latter. From there, calcium would be discharged to both the cytosol and the nucleoplasm (Bachs et al., 1990; Gerasimenko et al. 1995; Malviya, 1994) through one of the two channels mentioned above: which one of the two predominates would probably depend on the cell type. The recent direct demonstration of the presence of the $InsP_3$ channels in the inner membrane of the envelope (Humbert et al., 1996) nicely rationalizes the finding that the addition of $InsP_3$ (and cADPr) to isolated liver nuclei indeed induced a transient intranuclear calcium elevation (Gerasimenko et al., 1995) evidently, in that experiment $InsP_3$ (and cADPr) had penetrated into the nucleus to activate these channels. Similarly, the injection of $InsP_3$ into the nucleus of *Xenopus laevis* oocytes, under conditions in which the $InsP_3$-sensitive channels into the cytoplasmic membranes had been blocked by the injection of heparin, induced elevation of nuclear calcium (Hennager et al., 1995). Finally, the injection of caged $InsP_3$ into the nucleus of starfish oocytes, and calcium green dextran 10kDa induced an immediate elevation of nuclear calcium upon illumination (Santella, 1996), and the injection of ruthenium red, the blocker of the (ryanodine) cADPr-sensitive channel (Santella & Kyozuka, *unpublished results*) into the nucleus of these oocytes prevented the reinitiation of meiosis. It may be relevant to mention at this point that the fusion of nuclear vesicles derived from the disassembled nuclear envelope in

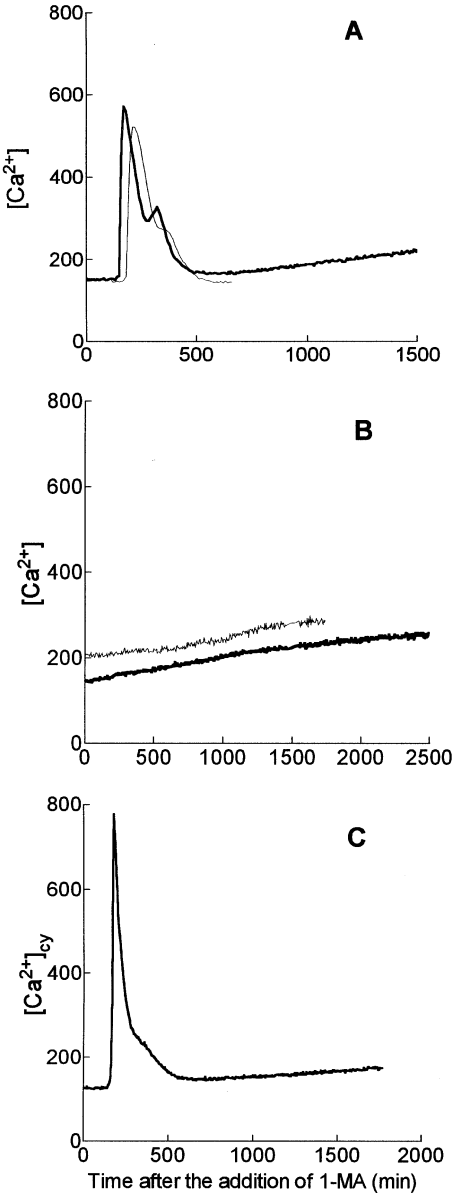

Fig. 3. Calcium transients in the cytoplasm and in the nucleus of microinjected starfish oocytes. (*A*) Response of a starfish oocyte to 1-MA following injection of fura-2 into the cytoplasm and into the nucleus. Thick line, cytosolic calcium; thin line, nuclear calcium. The quasi-simultaneous measurements of calcium in the two compartments (the 50 μm measuring ring was placed over a zone of the cytoplasm, over the nucleus, or alternatively, every 5 sec, over both) showed that 2 min after the addition of the hormone a cytosolic calcium transient was produced which was followed about 20 sec later by a nuclear transient and then by a second cytosolic transient. (*B*) Injection of BAPTA (1 mM) into the cytoplasm of another starfish oocyte abolished both the cytosolic and the nuclear transients. However, a gradual increase in baseline calcium occurred in both cell compartments; under these conditions GVBD occurred with a delay of about 30 min. (*C*) Measurement of cytosolic calcium following injection of BAPTA into the nucleus of a third oocyte. Both the nuclear and the second cytosolic calcium transients were abolished, blocking the reinitiation of meiosis. (*Modified from* Santella & Kyozuka, 1994).

Xenopus egg extracts has been shown to depend on the release of calcium through $InsP_3$-modulated channels in the membranes of the vesicles (Sullivan & Wilson, 1994).

In summary, it could be suggested that the calcium pump decreases the concentration of calcium in the nucleoplasm by pumping it into the lumen of the envelope. $InsP_3$ and cADPr will then return part of the calcium of the lumen to the nucleoplasm or to the cytosol, as originally proposed by Bachs, Agell & Carafoli, 1992. The combined operation of these systems could provide for fluctuations, even of large amplitude, in selected nucleoplasmic microdomains: one is reminded here of the situation in sarcomeric muscles, where the massive release of calcium from the terminal cisternal compartment of the sarcoplasmic reticulum through the ryanodine-sensitive channels provides for the very high local calcium concentration necessary to activate the low affinity Ca^{2+}-exporting Na^+/Ca^{2+}-exchanger of the adjacent sarcolemmal membrane. The nuclear pores, which may exist in both calcium-permeable and calcium-sealed states, would be modulated safety devices, which ''buffer'' calcium throughout the entire nucleoplasmic content independent of the pump/channels systems, and more rapidly than the latter could do. Whether the state of depletion of the endoplasmic reticulum cytoplasmic calcium stores is the only means to regulate the opening/closing of the pores (Greber & Gerace, 1995) cannot be decided at the moment. But the flow of calcium through the pores, once they are brought to the calcium-permeable state, could be either into the nucleus, or out of it, depending on the direction of the nuclear-cytoplasmic calcium gradient: the cartoon of Fig. 4 offers a pictorial representation of these concepts.

Targets of Calcium (and Calmodulin) Signaling in the Nucleus

The existence of an independently regulated nuclear calmodulin pool is still a debated issue, partly because the small size of calmodulin would be compatible with its free diffusion across the pore complexes. Calmodulin is present in the nucleus, as shown directly by immunocytochemical work on the nuclei in various cells (Harper et al., 1980; Guerriero, Rowley & Means, 1981; Mata & Fink, 1988), and indirectly by the finding that several enzyme systems which are specific to the nucleus are calmodulin regulated (Bachs et al., 1994). Related to this is the discovery (Srinivasan, Edman & Schulman, 1994) that one isoform of an important calmodulin-modulated enzyme, calmodulin-dependent kinase II (CaMK II)[1], is specifically targeted to the nucleus. In line with the idea that the nuclear calmodulin pool is independently regulated is the recent finding that calmodulin, in spite of its small size (17 kDa) does not

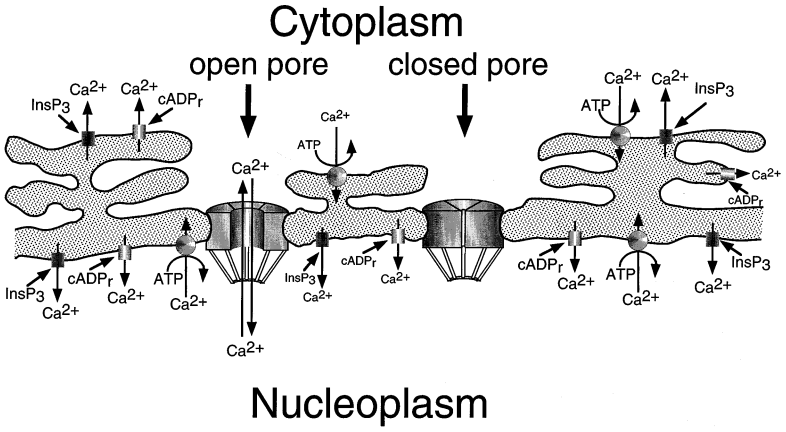

Fig. 4. The nuclear envelope in the regulation of nuclear calcium. Pore complexes are represented in both the calcium permeable and calcium sealed states. The latter state is indicated graphically by the adjoining of the pore subunits. The model shows the calcium transporting systems of the envelope: the Ca^{2+}-ATPase, the $InsP_3$-sensitive calcium release channels, and the cADPr (ryanodine) sensitive channel, which are visualized on both the inner and outer membranes of the envelope. The $InsP_4$-sensitive channel which has been recently shown to be present in the envelope has been omitted, since its function is still obscure.

diffuse freely through the pores, but is admitted into the nucleus via a carrier-mediated process (Pruschy et al., 1994).

Some of the targets of calcium-calmodulin signaling are specific to the nucleus, others are not. The latter include a number of proteins related to the actin motility system, e.g., α-spectrin, myosin light chain kinase (MLCK)[1], and caldesmon (Bachs, Agell & Carafoli, 1994). The role of the motility-related proteins in the nucleus is obscure, but their presence suggests an intranuclear contractile system patterned on that of (some) muscle cell types: this system would be linked to the nuclear envelope since the motility related calmodulin binding proteins are associated with it. Thus, the system could be involved in the regulation of the nuclear-cytoplasmic protein traffic: it has indeed been shown that anti-actin antibodies inhibit the transport of proteins into isolated nuclei (Schindler & Jiang, 1990). Interestingly, actin, MLCK, and calmodulin itself have been shown to be upregulated in the nucleus of proliferating hepatocytes (Bachs et al., 1990). Other calmodulin-modulated proteins found in the nucleus but not specific to it include two enzymes involved in the phosphorylation and dephosphorylation of proteins, i.e., CaMK II (Srinivasan et al., 1994, *see above*), and calcineurin (protein phosphatase 2B) (Bosser et al., 1993), as well as the protease calpain which appears to have a role in the mitotic cycle (Schollmeyer, 1988) and which is transported into the nucleus by an ATP-requiring process. In the nucleus, calpain cleaves a number of high molecular weight proteins (Mellgren, 1991). These are nuclear matrix proteins: interestingly, the m-form of calpain, which requires millimolar calcium for activity, hydrolyzes them at Ca^{2+} concentrations 1000-fold lower when DNA is present (Mellgren et al., 1993). Also of interest is the finding that a new form of calpain, named p-94, has been detected in nuclei of muscle cells in vivo (Sorimachi et al., 1993): mutations in the gene for this form of calpain induce muscular dystrophy (Richard et al., 1995). Protein kinase C, a target of calcium but not of calmodulin, has also been detected in the nucleus (Masmoudi et al., 1989; and *reviewed in* Buckner, 1995; Nishizuka, 1995).

Exciting information has recently become available on the nuclear targets of calmodulin-kinase II and of the calmodulin-dependent phosphatases calcineurin: the former phosphorylates a number of transcription factors and thus promotes gene transcription: the case of the transcription factor CREB, which is permanently bound to the DNA of the promoter of the *c-fos* immediate-early gene, and must thus by definition be phosphorylated by calmodulin kinase II in the nucleus, is particularly striking (Sheng, McFadden & Greenberg, 1990; Roche & Prentki, 1994; Zimprich, Torok & Bolsover, 1995). Calmodulin kinase II and calcineurin apparently also play a role at specific steps of the cell cycle (Means, 1994; Whitaker, 1995) and the kinase is involved in the inactivation of the Cytostatic Factor (CSF)[1] responsible for the block of meiosis II of *Xenopus* oocytes at the metaphase stage. Upon fertilization, CSF and the M phase-promoting factor, which are also required for the progression of meiosis (Lorca et al., 1993), are inactivated by CaMK II. Calmodulin is involved in chromosome segregation of *Saccharomices cerevisiae* and in the polarized growth required for bud formation (Davis, 1992; Sun et al., 1992): very interesting, (Geiser et al., 1991) the interaction of calmodulin with its yeast nucleus target, the mitotic component of the spindle body, has been shown not to require calcium. The conclusion was based on the observation that yeast strains completely devoid of calmodulin were not viable (Davis et al., 1986), whereas strains in which calmodulin had been mutated in a way to eliminate its ability to bind calcium grew well. Although alternative explanations would be possible, the authors considered them as unlikely, and favored the conclusion that in their case calmodulin did not act as a calcium receptor, but performed an as yet unidentified calcium-independent function. As for calcineurin, whose existence in the nuclei of several cell types is now

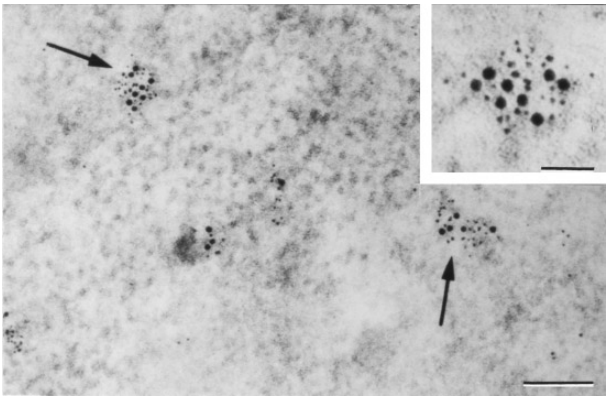

Fig. 5. Immunoelectron microscopy analysis of nuclei within liver hepatocytes. Ultrathin liver sections were incubated with anti-calmodulin and anti-protein p-36 antibodies conjugated to small or large gold particles. The small particles correspond to anti-calmodulin reactivity, the large ones to anti-p36 reactivity. The arrows indicate clusters of calmodulin and p-36 protein. The underlying electron-dense region corresponds to hnRNPs. The scale bar in the large panel corresponds to 0.2 μm, that in the inset to 0.5 μm. (*Adapted from* Bosser et al., 1995).

well documented (Sayoun et al., 1984; Bosser et al., 1993), it has recently been shown to have a function in DNA replication in *Aspergillus nidulans* (Rasmussen et al., 1994). These cell-cycle-related observations can be conveniently linked to previous findings showing increased translocation of calmodulin into the nuclei of cells activated to proliferate: a three-to-fourfold increase in nuclear calmodulin has been reported in liver cells (Serratosa et al., 1988) during the G_1/S transition.

As for the nuclear-specific calmodulin binding proteins, the recent finding that calmodulin inhibits transcription factors of the helix-loop-helix structural group is of great potential interest (Rasmussen et al., 1994; Corneliussen et al., 1994). But the most exciting development in the area is perhaps the discovery that calmodulin inhibits the phosphorylation of a number of nuclear targets of casein kinase II in the liver (Bosser et al., 1993): the inhibition is due to the direct interaction of calmodulin with these targets, thus preventing their phosphorylation by the kinase (Corneliussen et al., 1994; Bosser et al., 1995). What makes these discoveries particularly interesting is the finding that at least two of these targets, the A2 (36 kDa) and C (40 to 42 kDa) proteins, are components of the heterogeneous ribonucleoprotein particles (hnRNPs)[1], (Fig. 5) which contain a number of proteins that complex nascent RNA transcripts of RNA polymerase II: One of their functions would be to alter the structure of hnRNA to facilitate its interaction with factors involved in the processing of pre-mRNA (Bosser et al., 1995; Pinõl-Roma & Dreyfuss, 1991). They could, however, also be involved in the export of mRNA to the cytoplasm (*reviewed in* Dreyfuss et al., 1993) and in the cytosolic metabolism of mRNA. The work on the hnRNPs has now been extended to the nucleus of starfish oocytes, where these particles are much better defined (L. Santella & K. Kyozuka, submitted) and has considerably expanded the information on the interaction of calmodulin with them and, most interestingly, with the nucleolus.

In prophase-arrested nuclei of starfish oocytes, the hnRNPs are visible as electron-dense bodies of about 0.4 μm in diameter (Fig. 6*A*): their identification as hnRNPs is based on immunogold-labeling experiments with antibodies against calmodulin and against the 36 kDa (p-36) hnRNP protein, which shows the two proteins colocalized on the electron-dense bodies (high-magnification inset of Fig. 6*A*). Upon activation of the oocytes with 1-MA, the hnRNPs disappear from the nucleoplasm (Fig. 6*B*) and calmodulin and the p-36 protein translocate to the nucleoplasm and the cytosol: the translocation of the proteins to the latter compartment evidently occur through the nuclear pores, since it precedes the breakdown of the nuclear envelope (Fig. 6*B, inset*). These observations, which are clearly related to the proposed role of the hnRNP proteins in pre-mRNA processing, mRNA export, and cytoplasmic mRNA metabolism, significantly extend the potential area of calmodulin function in the nucleus.

However, the work on the nuclei of starfish oocytes has produced clues on the function of calmodulin in the nucleus also in another direction: at the time when the treatment with 1-MA had caused the disintegration of the hnRNPs and the migration of calmodulin and the p36 protein away from them, the nucleolus, which was only poorly labeled in prophase-arrested cells (Fig. 6*C*), became strongly labeled with the antibodies against the two proteins (Fig. 6*C, inset*). Since the nucleolus is recognized as an important actor in rDNA transcription (*reviewed in* Scheer & Weisenberger, 1994; Mélèse, 1995), a role for calmodulin in the synthesis and processing of pre-ribosomal RNA, and ultimately in the biogenesis of the ribosome, thus appears possible.

Concluding Remarks

The aim of this contribution was to present a succinct way the relatively large body of information now available on the control and function of calcium and calmodulin in the nucleus. Comparatively more attention has been devoted to areas where opinions still differ, e.g., the regulation of calcium fluxes in and out of the nucleus and the permeability of the pores, as well as to areas where new avenues of investigation have begun to appear. Special attention has been devoted to calmodulin, not only because of its role as the principal decoder of the calcium signal in eukaryotic cells, but also because of the recent findings on new, nuclear-specific targets of its modulation. A point that was not considered in detail in the

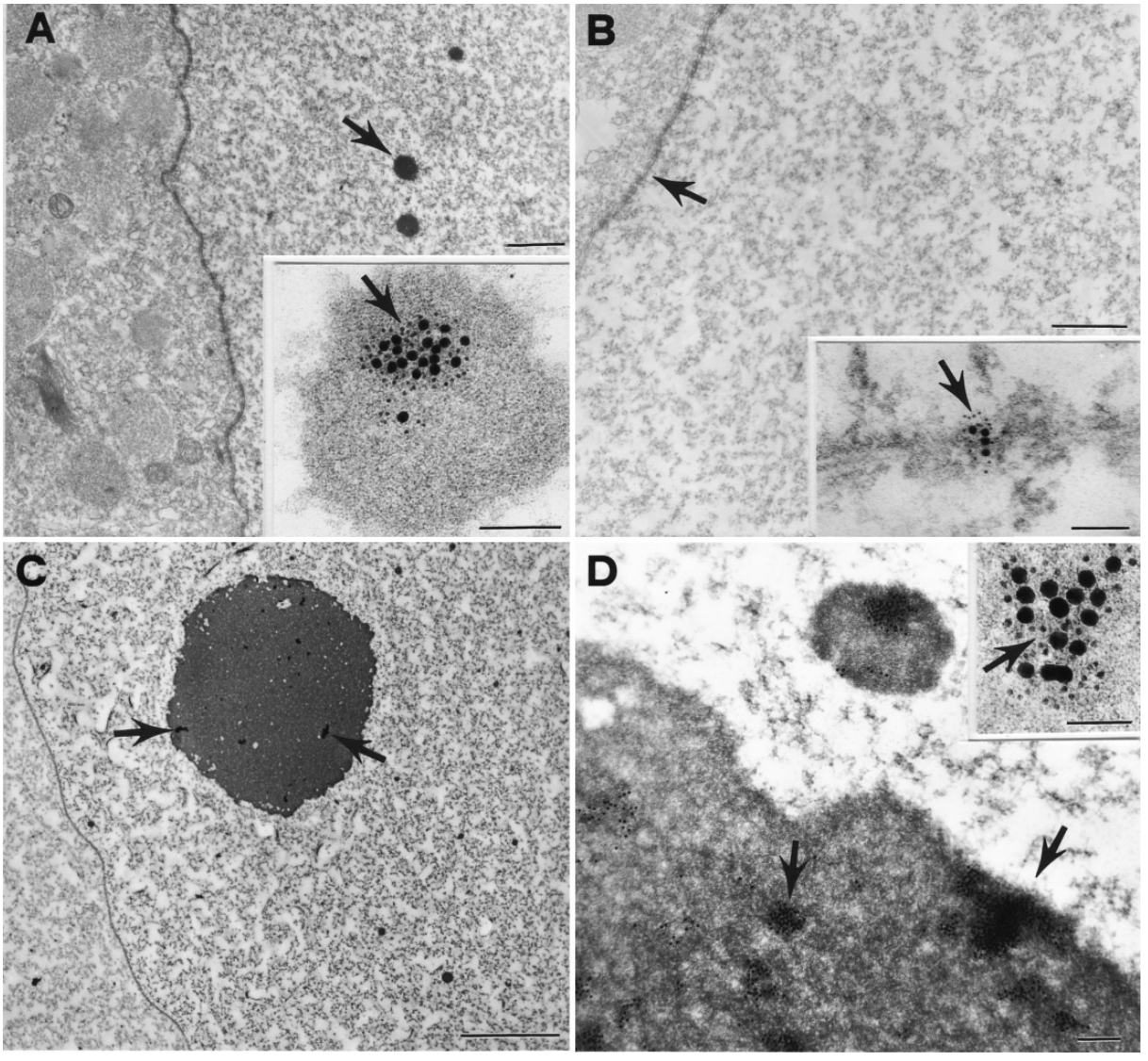

Fig. 6. Immunogold labeling experiments on starfish oocytes using antibodies against calmodulin and the p-36 protein of the hnRNPs. (*A*) Electron micrograph of a prophase arrested oocyte showing electron-dense bodies (about 0.4 μm in diameter) scattered in the nucleoplasm (arrow). Bar = 1 μm. Antibodies against calmodulin (large gold particles) and against the 36 kDa (p-36) hnRNP protein (small gold particles) are clustered on one of the electron-dense particles in the high magnification inset of Fig. 6*A* (arrow). The electron-dense bodies are thus hnRNPs. Bar = 100 nm. (*B*) Addition of 1-MA to the oocytes induces the reinitiation of meiosis and the disappearance of the hnRNPs from the nucleoplasm: 10 min after the treatment with the hormone the hnRNPs are no longer visible in it. Calmodulin and the p-36 protein, which were originally associated with them, are evidently translocated to the cytosol through the nuclear pore complexes which at the time of the observation are still present in the intact envelope (arrow). Bar = 1 μm. The inset shows the two types of gold particles in the process of crossing the envelope into the cytoplasm (arrow). The definition of the details in the inset is lower than in the remainder of the figure because of the type of fixation required by the immunocytochemistry method. Bar = 100 nm. (*C*) A nucleolus in a prophase arrested nucleus showing some labeling with the antibodies against calmodulin and the p-36 protein (arrows). The dark granules indicated by the arrows are aggregates of small and large gold particles insufficiently resolved at this magnification to reveal the individual components. Bar = 5 μm. (*D*) 10 min after treatment with 1-MA the nucleolus became strongly labeled (arrows). Bar = 200 nm. The high magnification inset shows the large and small gold particles (arrows). Bar = 50 nm.

contribution is the role of nuclear calcium in programmed cell death; this is now an intensively studied topic (*reviewed in* Nicotera, Zhivotovsky & Orrenius, 1994), and evidence for the involvement of a calcium-dependent nuclear enzyme, an endonuclease, has appeared (Arends, Morris & Wyllie, 1990). The enzyme has been shown to fragment DNA in thymocytes (Arends et al., 1990) but also in other cell types, e.g., hepatocytes, where its activation is triggered by submicromolar concentration of calcium (Jones et al., 1989). The area of

nuclear calcium in apoptosis, however, is still in its infancy, and it was thus felt premature to discuss it in detail in this topical review.

The title of this review was meant to impress the reader with the great potential of nuclear calcium and calmodulin in future calcium research: processes of far-reaching biological significance such as the processing of mRNA and the biogenesis of the ribosomes may soon become prime actors in the area of calmodulin function. The relative emphasis placed on the nuclei of starfish oocytes may reflect a professional bias, but it is hoped that the information provided will convince the reader that starfish oocytes are indeed a convenient object of study.

The author is indebted to Dr. Neus Agell (Barcelona) for the antibodies against the p-36 protein and to Dr. Ernesto Carafoli (Zürich) for many helpful discussions. She is also indebted to Dr. K. Kyozuka (Asamushi) for his collaboration in the experiments described in Figs. 2 and 3 and to Mr. R. Moser (Zürich) for his help in preparing Fig. 4.

References

Allbritton, N.L., Oancea, E., Kuhn, M.A., Meyer, T. 1994. *Proc. Natl. Acad. Sci. USA* **91:**12458–12462

Al-Mohanna, F.A., Caddy, K.W.T., Bolsover, S.R. 1994. *Nature* **367:**745–750

Arends, M.J., Morris, R.G., Wyllie, A.H. 1990. *Am. J. Pathol.* **136:**593–608

Bachs, O., Agell, N., Carafoli, E. 1992. *Biochim. Biophys. Acta* **1113:**259–270

Bachs, O., Agell, N., Carafoli, E. 1994. *Cell Calcium* **16:**289–296

Bachs, O., Lanini, L., Serratosa, J., Coll, M.J., Bastos, R., Alliguè, R., Rius, E., Carafoli, E. 1990. *J. Biol. Chem.* **265:**18595–18600

Bosser, R., Aliguè, R., Guerini, D., Agell, N., Carafoli, E., Bachs, O. 1993. *J. Biol. Chem.* **268:**15477–15483

Bosser, R., Faura, M., Serratosa, J., Renau-Piqueras, J., Pruschy, M., Bachs, O. 1995. *Mol. Cell. Biol.* **15:**661–670

Brini, M., Murgia, M., Pasti, L., Picard, D., Pozzan, T., Rizzuto, R. 1993. *Embo J.* **12:**4813–4819

Buckner, K. 1995. *Eur. J. Biochem.* **228:**211–221

Bustamante, J.O. 1992. *Pfluegers Arch* **421:**473–485

Bustamante, J.O. 1994. *J. Membrane Biol.* **138:**105–112

Bustamante, J.O., Hanover, J.A., Liepins, A. 1995. *J. Membrane Biol.* **146:**239–241

Chiba, K., Kontani, K., Tadenuma, H., Katada, T., Hoshi, M. 1993. *Mol. Biol. Cell* **4:**1027–1034

Ciapa, B., Pesando, D., Wilding, M., Whitaker, M.J. 1994. *Nature* **368:**875–878

Corneliussen, B., Holm, M., Waltersson, Y., Onions, J., Hallberg, B., Thornell, A., Grundström, T. 1994. *Nature* **368:**760–764

Dale, B., DeFelice, L.J., Kyozuka, K., Santella, L., Tosti, E. 1994. *Proc. R. Soc. Lond. B* **255:**119–124

Davis, L.I. 1995. *Annu. Rev. Biochem.* **64:**865–896

Davis, T.N. 1992. *J. Cell Biol.* **118:**607–617

Davis, T.N., Urdea, M.S., Masiarz, F.R., Thorner, J. 1986. *Cell* **47:**423–431

De Felici, M., Dolci, S., Siracusa, G. 1991. *J. Exp. Zool.* **260:**401–405

DeFelice, L.J., Mazzanti, M. 1993. Biophysics of the nuclear envelope. *In:* Principles of cell physiology and biophysics. N. Sperelakis, editor. pp. 351–366. Acad. Press, New York

Deguchi, R., Osanai, K. 1994. *Dev. Biol.* **163:**162–174

Divecha, N., Banfic, H., Irvine, R.F. 1993. *Cell* **74:**405–407

Dreyfuss, G., Matunis, M.J., Pinõl-Roma, S., Burd, C. 1993. *Annu. Rev. Biochem.* **26:**289–321

Feldherr, C.M., Kallenbach, E., Schultz, N. 1984. *J. Cell Biol.* **99:**2216–2222

Geiser, J.R., van Tuinen, D., Brockerhoff, S.E., Neff, M.M., Davis, T.N. 1991. *Cell* **65:**949–959

Gerace, L., Foisner, R. 1994. *Trends in Cell Biol.* **4:**127–131

Gerasimenko, O.V., Gerasimenko, J.V., Tepikin, A.V., Petersen, O.H. 1995. *Cell* **80:**439–444

Goldberg, M.W., Allen, T.D. 1995. *Curr. Opin. Cell Biol.* **7:**301–309

Greber, U.F., Gerace, L. 1995. *J. Cell Biol.* **128:**5–14

Guerriero, V., Rowley, D.R., Means, A.R. 1981. *Cell* **27:**449–45820

Harper, J.F., Cheung, W.Y., Wallace, R.W., Levine, S.N., Steiner, A.L. 1980. *In:* Calcium and Cell Function. W.Y. Cheung, editor. pp. 273–290. Academic Press, New York

Harris, P.J. 1994. *Dev. Biol.* **161:**370–378

Hennager, D.J., Welsh, M.J., DeLisles, S. 1995. *J. Biol. Chem.* **270:**4959–4962

Hepler, P.K. 1994. *Cell Calcium* **16:**322–330

Himpens, B., De Smedt, H., Casteels, R. 1994. *Cell Calcium* **16:**239–246

Hinshaw, J.E., Carracher, B.O., Milligan, R.A. 1992. *Cell* **69:**1133–1141

Humbert, J.P., Matter, N., Artault, J.C., Köppler, P., Malviya, A.N. 1996. *J. Biol. Chem.* **271:**478–485

Ito, S., Loewenstein, W.R. 1965. *Science* **150:**909–910

Jones, D.P., McConkey, D.J., Nicotera, P., Orrenius, S. 1989. *J. Biol. Chem.* **264:**6398–6403

Kanatani, H., Shirai, H. 1967. *Nature* **216:**284–286

Kanatani, H., Shirai, H., Nakanishi, K., Kurosawa, T. 1969. *Nature* **221:**273–274

Kaufmann, M., Homa, S.T. 1993. *J. Exp. Zool.* **265:**69–76

Köppler, P., Matter, N., Malviya, A.N. 1993. *J. Biol. Chem.* **268:**26248–26252

Lanini, L., Bachs, O., Carafoli, E. 1992. *J. Biol. Chem.* **267:**11548–11552

Lefèvre, B., Pesty, A., Testart, J. 1995. *Exp. Cell Res.* **218:**166–173

Lin, C., Hajnóczky, G., Thomas, A.P. 1994. *Cell Calcium* **16:**247–258

Loewenstein, W.R., Kanno, Y. 1963. *J. Gen. Physiol.* **46:**1123–1140

Lorca, T., Cruzalegui, F.H., Fesquet, D., Cavadore, J.C., Méry, J., Means, A., Dorée, M. 1993. *Nature* **366:**270–273

Malviya, A.N. 1994. *Cell Calcium* **16:**301–313

Masmoudi, A., Labourdette, G., Mersel, M., Malviya, A.N. 1989. *J. Biol. Chem.* **264:**1172–1179

Masui, Y., Meyerhof, P.G., Miller, M.A., Wasserman, W.J. 1977. *Differentiation* **9:**49–57

Mata, M., Fink, D.J. 1988. *Brain Res.* **475:**297–304

Mazzanti, M., DeFelice, L.J., Cohen, J., Malter, H. 1990. *Nature* **343:**764–767

Mazzanti, M., DeFelice, L.J., Smith, E.F. 1991. *J. Membrane Biol.* **121:**189–198

Mazzanti, M., Innocenti, B., Rigatelli, M. 1994. *Faseb J.* **8:**231–236

Means, A.R. 1994. *FEBS Lett.* **347:**1–4

Mélèse, T., Xue, Z. 1995. *Curr. Opin. Cell Biol.* **7:**319–324

Mellgren, R.L. 1991. *J. Biol. Chem.* **266:**13920–13924

Mellgren, R.L., Song, K., Mericle, M.T. 1993. *J. Biol. Chem.* **268:**653–657

Miyazaki, S. 1995. *Curr. Opin. Cell Biol.* **7:**190–196

Moreau, M., Guerrier, P., Dorée, M., Asley, C.C. 1978. *Nature* **272:**251–253

Moreau, M., Vilain, J.P., Guerrier, P. 1980. *Dev. Biol.* **78:**201–214

Neylon, C.B., Hoyland, J., Mason, W.T., Irvine, R.F. 1990. *Am. J. Physiol.* **259:**C675–C686

Nicotera, P., McConkey, D.J., Jones, D.P., Orrenius, S. 1989. *Proc. Natl. Acad. Sci. USA* **86:**453–457

Nicotera, P., Orrenius, S., Nilsson, T., Berggren, P.O. 1990. *Proc. Natl. Acad. Sci. USA* **87:**6858–6862

Nicotera, P., Zhivotovsky, B., Orrenius, S. 1994. *Cell Calcium* **16:**279–288

Nishizuka, Y. 1995. *FASEB J.* **9:**484–496

Paine, P.L., Moore, L.C., Horowitz, S.B. 1975. *Nature* **254:**109–114

Pinõl-Roma, S., Dreyfuss, G. 1991. *Science* **253:**312–314

Poenie, M., Alderton, J., Tsien, R.J., Steinhardt, R.A. 1985. *Nature* **315:**147–149

Pruschy, Y.J., Spitz, L., Carafoli, E., Goldfarb, D.S. 1994. *J. Cell Biol.* **127:**1527–1536

Rasmussen, C., Garen, C., Brining, S., Kincaid, R.L., Means, R.L., Means, A.R. 1994. *EMBO J.* **13:**2545–2552

Richard, I., Broux, O., Allamand, V., Fougerousse, F., Chiannilkuchai, N., Bourg, N., Brenguer, L., Devaud, C., Pasturaud, P., Roudaut, C., Hillair, C., Passos-Bueno, M., Zatz, M., Tischfield, J.A., Fardeau, M., Jackson, C.E., Cohen, D., Beckmann, J.S. 1995. *Cell* **81:**27–40

Roche, E., Prentki, M. 1994. *Cell Calcium* **16:**331–338

Santella, L. 1996. *Invert. Reprod. & Develop.* (*in press*)

Santella, L., Kyozuka, K. 1994. *Biochem. Biophys. Res. Commun.* **203:**74–680

Sayoun, N., Levine, H. III., Bronson, D., Cuatrecas, P. 1984. *J. Biol. Chem.* **259:**9341–9344

Scheer, U., Weisenberger, D. 1994. *Curr. Opin. Cell Biol.* **6:**354–359

Schindler, M., Jiang, L.W. 1990. *In:* Biochemical and Structural Analysis of the Cell Nucleus. E. Wang, J.L. Wang, S. Chien, W.Y. Cheung, C. Wu, editors. pp. 249–263. Academic Press, San Diego

Schollmeyer, J.E. 1988. *Science* **240:**911–913

Serratosa, J., Pujol, M.J., Bachs, O., Carafoli, E. 1988. *Biochem. Biophys. Res. Commun.* **150:**1162–1169

Sheng, M., McFadden, G., Greenberg, M.E. 1990. *Neuron* **4:**571–582

Shilling, G., Chiba, K., Hoshi, M., Koshimoto, T., Jaffe, L.A. 1989. *Dev. Biol.* **133:**605–608

Sorimachi, H., Toyama-Sorimachi, N., Saido, T.C. Kawasaki, H., Sugita, H., Miyasaka, M., Arahata, K., Ishiura, S., Suzuki, K. 1993. *J. Biol. Chem.* **268:**10593–10605

Srinivasan, M., Edman, C.F., Schulman, H. 1994. *J. Cell Biol.* **126:**839–852

Stehno-Bittel, L., Lückhoff, A., Clapham, D.A. 1995. *Neuron* **19:**163–167

Stehno-Bittel, L., Perez-Terzic, C., Clapham, D.E. 1995. *Science* **270:**1835–1838

Steinhardt, R.A. 1990. *J. Reprod. Fert.* **42:**191–197

Steinhardt, R.A., Alderton, J. 1988. *Nature* **332:**364–366

Sullivan, K.M.C., Busa, W.B., Wilson, K.L. 1993. *Cell* **73:**1411–1422

Sun, G.H., Hirata, Y., Ohya, Y., Anraku, Y. 1992. *J. Cell Biol.* **119:**1625–1639

Tabares, L., Mazzanti, M., Clapham, D.E. 1991. *J. Membrane Biol.* **123:**49–54

Tombes, R.M., Simerly, C., Borisy, G.G., Shatten, G. 1992. *J. Cell Biol.* **117:**799–811

Whitaker, M. 1995. *In:* Advances in Second Messenger and Phosphoprotein Research. A. Means, editor. pp. 299–307. Raven Press, New York

Whitaker, M., Patel, R. 1990. *Development* **108:**525–542

Wiese, C., Wilson, K.L. 1993. *Curr. Opin. Cell Biol.* **5:**387–394

Williams, D.A., Becker, P.L., Fay, F.S. 1987. *Science* **235:**1644–1648

Zimprich, F., Torok, K., Bolsover, S.R. 1995. *Cell Calcium* **17:**233–238