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EMBO WORKSHOP REPORT

Calcium signals in the cell nucleus Strasbourg, France, August 20–23, 1998

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Introduction

Just as the great explorations revealed a world far more diverse and rich than had been imagined before, a mass of fast accumulating data is disclosing a much more complex and sophisticated picture of the nucleus than hitherto assumed. The nucleus has long been thought of as some sort of enclosure circumscribed by a double membrane and containing the genetic material of the cell. The inner and outer nuclear membranes join periodically at the nuclear pores, forming complexes supposed to create large channels for the free diffusion of ions and small macromolecules. In this view, the flow of calcium between nucleus and cytoplasm would appear unrestricted, with nuclear Ca^{2+} signals originating from cytosolic Ca^{2+} waves merely by passive transmission through the nuclear pore complex (NPC). However, recent evidence has changed the *mappa mundi* of the cell. It has been shown that nuclear Ca^{2+} can be regulated independently of cytosolic Ca^{2+} changes. And, just as the *Terra Incognita* discovered beyond the Ocean Sea proved a fabulous New World, the nuclear envelope (NE) between the cytoplasm and nucleoplasm appears to play an essential role in the regulation of Ca^{2+} signals inside the nucleus. It contains proteins that regulate and respond to changes in nucleosolic Ca^{2+} concentration ($[Ca^{2+}]_{nucleosol}$). Furthermore, several specific Ca^{2+}-dependent nuclear functions have been described and the list is growing. The regulation of nuclear Ca^{2+} signals, and the role of nuclear Ca^{2+} as a specific regulator of nuclear events through Ca^{2+}-binding proteins, were the main themes of the recent EMBO workshop entitled 'Calcium signals in the cell nucleus', organized by A.N.Malviya and coworkers in Strasbourg, France.

'That that is, is'

The existence of Ca^{2+} signals at the level of the nucleoplasm has never been a real issue, as the proverbial hermit cited in Shakespeare's *Twelfth Night* would no doubt have assented. Typical resting $[Ca^{2+}]_{nucleosol}$ is in the 100–300 nM range whereas, upon stimulation, values of 350–1200 nM can be reached depending on experimental conditions. What has long been contentious, however, is the source of these signals. Where does the Ca^{2+} liberated inside the nucleus come from? A purely cytosolic origin was the accepted tenet until the early 1990s, but this model came to be questioned by reports documenting rises in nuclear Ca^{2+} levels independently of changes in cytosolic Ca^{2+} despite the presence of NPCs. Such gradients were initially observed at rest, but progress in technology allowed measurements to be performed in stimulated cells upon the passage of global Ca^{2+} waves. Yet these studies have also yielded many negative results. The indicators used, fluorescent radiometric dyes or constructs based on the photoprotein aequorin, tend to produce artefacts and have been blamed for the discrepancies (Malviya and Rogue, 1998). These limitations prompted investigators to develop more reliable methods, and the recent development of Ca^{2+}-sensitive green fluorescent protein (GFP) derivatives fused with calmodulin (cameleons) has raised great expectations. At the workshop, A.Miyawaki (Japan) presented recent data on Ca^{2+} signals visualized by two-photon excitation microscopy using improved cameleons (Miyawaki *et al.*, 1999). He expressed optimism that the teething problems (sensitivity to pH and calmodulin concentrations) outlined during the discussion of his presentation would soon be resolved.

Notwithstanding, results showing that cytosolic and nucleoplasmic Ca^{2+} signals do not equilibrate rapidly cannot be ignored. B.Himpens (Belgium) presented data demonstrating that the amplitude of these nucleocytoplasmic Ca^{2+} gradients depends critically upon experimental conditions. Other arguments were also discussed, which confirm that the diffusion of Ca^{2+} to the nucleus is restricted under some circumstances. For instance, patch–clamping of the NE reveals ionic conductances that close during the translocation of macromolecules through the NPC. The existence of intracellular Ca^{2+} microdomains, as well as localized and transient rises in Ca^{2+} concentrations, also suggests that nucleocytoplasmic Ca^{2+} gradients may be physiological. The idea that under certain conditions the NE could oppose a barrier to the free diffusion of Ca^{2+} was taken to suggest that nuclear Ca^{2+} signals might also originate from sources within the nucleus. Different results have since corroborated this concept. On the whole, there appears to be a variety of situations depending on the cell type, its state of differentiation or proliferation and agonist concentration. Thus, the nuclear Ca^{2+} signal can be derived from the cytosolic Ca^{2+} wave by passive diffusion through the NPC. It can also originate *in nucleo*, and in this case two models have been proposed: either the nuclear Ca^{2+} is derived from the Ca^{2+} released in the immediate perinuclear vicinity of the NE, or it is released from the lumen of the NE considered as the nuclear Ca^{2+} pool (Figure 1).

The sources of nuclear Ca^{2+} signals: cytosolic, perinuclear and nuclear

The study of elementary Ca^{2+} release events in the cytoplasm such as 'Ca^{2+} puffs' [Ca^{2+} microsignals

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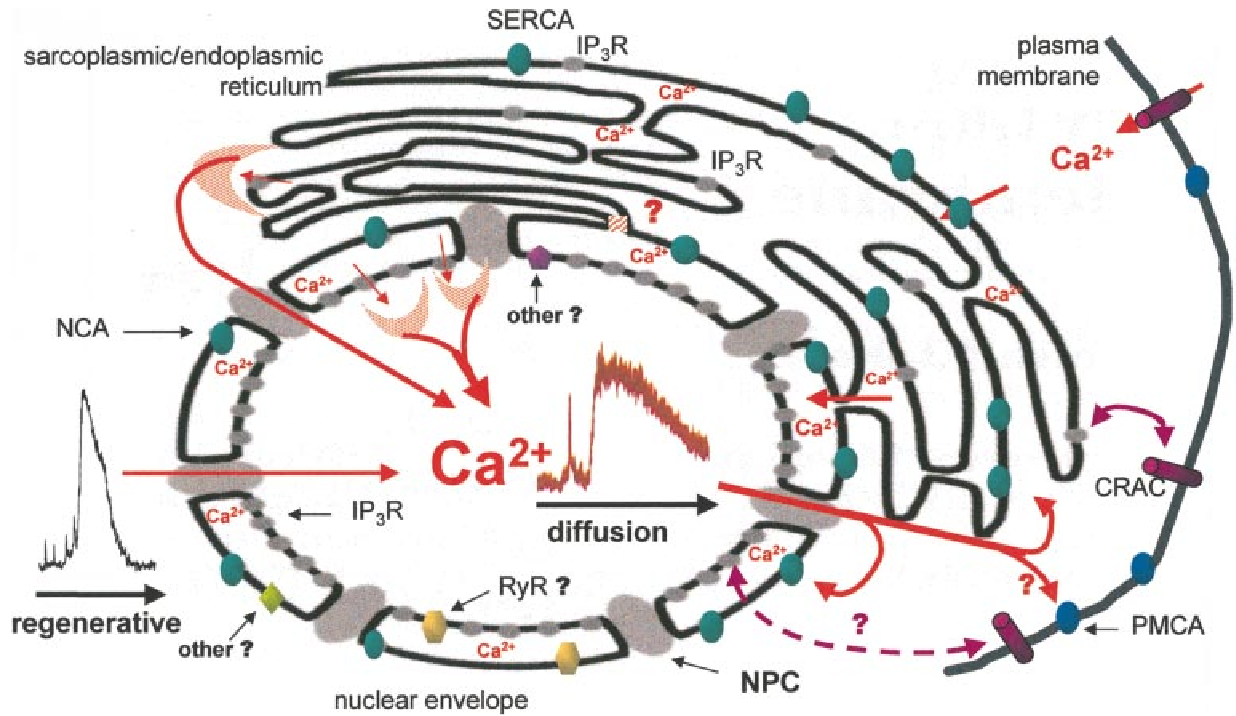

Fig. 1. Regulation of nuclear Ca^{2+} dynamics. The nuclear Ca^{2+} signal can result from the cytosolic Ca^{2+} wave by passive diffusion through the NPC. It can also originate *in nucleo*, and in this case two models have been proposed: either the nuclear Ca^{2+} derives from the Ca^{2+} liberated in the immediate perinuclear vicinity of the NE, or it is liberated from the nuclear reservoir (via IP_3R on the inner membrane, eventually via ryanodine receptors, RyR) located in the lumen of the NE. Whatever its origin, the nuclear Ca^{2+} signal propagates across the nucleoplasm by simple diffusion. It is dissipated by egression through the NPCs followed by sequestration into the ER or the nuclear Ca^{2+} pool. This pool is filled either by continuity with the lumen of the ER or through NCA (nuclear Ca^{2+}-ATPase) IP_4 receptor which is located on the outer membrane of the NE. Nuclear IP_3R receptors could be involved in the capacitative influx of Ca^{2+}. CRAC, Ca^{2+}-release-activated channel; NPC, nuclear pore complex.

produced by Ca^{2+} release from the endoplasmic reticulum (ER) through inositol 1,4,5-trisphosphate receptors (IP_3Rs)], has allowed the identification of a novel mechanism for the production of nuclear Ca^{2+} signals. Indeed, this approach reveals that these elementary cytosolic Ca^{2+} transients can only be transmitted to the nucleoplasm provided they are produced in close enough proximity to the NE. Thus, M.Bootman (UK) showed that 'Ca^{2+} puffs' generated within 6 µm of the nucleus are transmitted almost instantly to the nucleoplasm, whereas those puffs produced further away are not (Lipp *et al.*, 1997). The diffusion through the NPCs of elementary 'Ca^{2+} puffs' generated in the perinuclear vicinity is a mechanism distinct from the transmission of a global cytosolic Ca^{2+} wave to the nucleoplasm. Once inside the nucleus, the evolution of these elementary Ca^{2+} signals depends on the frequency of stimulation. At low frequency, the $[Ca^{2+}]_{nucleosol}$ returns to baseline between each stimulus, but at high frequency, $[Ca^{2+}]_{nucleosol}$ oscillations of increasing amplitude are observed resulting from the progressive accumulation of the Ca^{2+} transmitted by the perinuclear 'puffs'. Above a certain threshold, these perinuclear 'Ca^{2+} puffs' will produce a global nuclear Ca^{2+} wave.

Nuclear Ca^{2+} signals can also have a nuclear origin, by release of Ca^{2+} from the pool in the NE. Thus, an early rise of the $[Ca^{2+}]_{nucleosol}$ on the nuclear rim can be documented under certain circumstances. The mathematical modelling of nuclear Ca^{2+} dynamics by M.Britch (Belarus) similarly suggests that, under certain experimental conditions, exchanges between the NE and the nucleoplasm account for most of the variation in $[Ca^{2+}]_{nucleosol}$, but it is the characterization of specific Ca^{2+} transporters on the NE that provides the major argument in favour of a nuclear origin for nuclear Ca^{2+} signals.

The characteristics of this nuclear Ca^{2+} pool located in the lumen of the NE clearly need to be defined. Permeant fluorescent indicators (e.g. fluo-3-pentaacetoxy methyl ester) confirm that $[Ca^{2+}]_{lumen\ NE}$ (i.e. the Ca^{2+} concentration in the lumen of the NE) can reach the resting values reported for the lumen of the ER, in the range of several hundred micromolar. This is to be expected as the ER is the main intracellular Ca^{2+} reservoir and its lumen is in continuity with that of the NE. It was emphasized during discussion that the use of intact cells to measure $[Ca^{2+}]_{lumen\ NE}$ is preferable to that of isolated nuclei, so as to avoid artefactual leaks. O.Petersen (UK) suggested that filling of the nuclear Ca^{2+} pool may be controlled through negative feedback by the $[Ca^{2+}]_{lumen\ NE}$, just as that of the ER is regulated by the $[Ca^{2+}]_{lumen\ ER}$ (Mogami *et al.*, 1998). However, the relationships between the ER and NE appear complex. For instance, the use of selectively targeted aequorin constructs has revealed that under certain circumstances the lumen of the ER can become compartmentalized, with domains of high $[Ca^{2+}]_{lumen\ ER}$ coexisting with regions where $[Ca^{2+}]_{lumen\ ER}$ is low (Montero *et al.*, 1997). This implies that transient $[Ca^{2+}]$ gradients between the lumen of the NE and that of the ER may also exist. However, such gradients have yet to be identified.

The question of the propagation of nuclear Ca^{2+} signals was also addressed during the workshop. In the cytosol,

global Ca^{2+} signals result from spatially and temporally coordinated recruitment of highly localized release events such as 'Ca^{2+} puffs'. These elementary events dissipate rapidly owing to diffusion in the cytoplasm and sequestration into intracellular stores, and M.Berridge (UK) underscored that it is their recruitment through Ca^{2+}-induced Ca^{2+} release (CICR) that produces the global regenerative Ca^{2+} waves (Berridge, 1993, 1997). However, the interior of the nucleus is devoid of organelles that might serve to store and release Ca^{2+}, and thus a regenerative mechanism such as CICR cannot be supported. M.Nathanson (USA) discussed data obtained by high-speed confocal line-scanning microscopy, demonstrating that nuclear Ca^{2+} waves emanate at the nucleus–cytosol border and then cross the nucleus simply by passive diffusion. A different environment in comparison with the cytosol probably explains why the nucleosol is able to support the propagation of Ca^{2+} waves over significant distances without regenerative Ca^{2+} release. Nuclear Ca^{2+} signals are finally dissipated by egression out of the nucleus through the NPC on the opposite side, followed by subsequent sequestration into the ER (no Ca^{2+} pumps have been identified on the inner leaflet of the nuclear membrane).

Regulation of the nuclear calcium pool

Specific Ca^{2+} transporters have been identified on the NE, which either release Ca^{2+} from the nuclear Ca^{2+} pool or replenish it (Santella and Carafoli, 1997; Malviya and Rogue, 1998). The most intensively studied Ca^{2+}-transporter protein involved in refilling the nuclear Ca^{2+} store is the nuclear Ca^{2+}-ATPase (NCA), a SERCA2b-like Ca^{2+} pump situated on the outer nuclear membrane. A.N.Malviya (France) has shown that NCA is phosphorylated by cAMP-dependent protein kinase (PKA), enhancing its Ca^{2+}-pumping activity (Rogue *et al.*, 1998). A.N.Malviya also demonstrated that the effect of PKA is associated with an accelerated nuclear localization sequence (NLS)-independent transport of intermediate size macromolecules (~10 kDa dextran) into isolated nuclei, probably as a consequence of increased filling of the nuclear Ca^{2+} pool. The principles governing the nuclear localization of PKA, summarized by S.Taylor (USA), are better understood. The two classes of physiological inhibitors of PKA, the regulatory subunits R and PKI (small heat-stable protein kinase inhibitors), seem to be principally responsible. R contains an N-terminus dimerization domain that mediates PKA interaction with AKAPs, anchor proteins involved in the targeting of PKA to specific subcellular sites such as the nucleus, whereas PKI contains a C-terminal nuclear export signal that induces the transport of the catalytic subunit out of the nucleus. Taken together, these results suggest that cross-talk between cAMP- and Ca^{2+}-dependent signalling pathways occurs at the level of the nucleus, and that this cross-talk may be involved in the regulation of nucleo-cytoplasmic transport of macromolecules.

IP_3R, which appears to be located exclusively on the inner nuclear membrane, seems to be the principal release channel for the nuclear Ca^{2+} reservoir. Different nuclear IP_3R isoforms have been identified, depending on cell type. M.Nathanson has identified the presence of type III IP_3Rs in the nucleus of pancreatic acinar cells. The properties of type III receptors, which act as positive-feedback Ca^{2+} channels producing all or none cytosolic Ca^{2+} transients that almost completely empty ER Ca^{2+} stores (Hagar *et al.*, 1998), seem most appropriate with regard to the generation of nuclear Ca^{2+} signals. S.Delisle (USA) presented striking confocal data using type I and III IP_3R–GFP constructs. When these constructs are merged to a myristylation sequence that normally targets proteins to the plasma membrane, surprisingly both IP_3R-1 and IP_3R-3 become distributed away from the plasma membrane and towards the NE. The significance of this observation is unclear but both myristylated IP_3R–GFP constructs increase Ca^{2+} influx into transfected cells to a much greater extent than their non-myristylated counterparts, suggesting a role for nuclear IP_3Rs in capacitative Ca^{2+} entry. Indeed, deletion experiments show that full-length IP_3Rs are required, indicating that they might bridge the space between the nucleus and Ca^{2+} signalling complexes at the level of the plasma membrane. S.Muallem (USA) presented data supporting direct coupling between plasma membrane signalling complexes comprising CRAC-type capacitative Ca^{2+} influx channels and IP_3Rs in the ER (Kiselyov *et al.*, 1998).

The importance of IP_3 in nuclear Ca^{2+} movements raises the question of the origin of this second messenger in the nucleus. The NPCs are permeable to IP_3 so that, a priori, a synthesis *in nucleo* does not appear necessary. Nonetheless, the existence of a nuclear phosphoinositide cycle capable of generating IP_3 has long been recognized. Indeed, phosphatidylinositol (PI), phosphatidylinositol-4,5-biphosphate (PIP_2), 1,2-diacylglycerol (DAG), phospholipase isoforms, DAG kinase isoforms (such as DAGKz, which can be phosphorylated by PKC, leading to its exclusion from the nucleus), PI(4)P kinase, PI(5)P kinase and a PI phosphatase are all present in the nucleus. However, a stimulus-induced production of IP_3 in the nucleus has not yet been observed directly. N.Divecha (The Netherlands) presented evidence for the existence of two distinct nuclear pools of PIP_2 (NE, nucleoplasm). Furthermore, nuclear DAG can derive either from phosphatidylcholine (this DAG pool may play a role in the regulation of the G_1/S transition) or from PIP_2 (this DAG, which is channelled directly into the production of phosphatidic acid catalysed by a DAGK, seems to be involved in the G_2/M transition). Phosphatidylinositol-3,4,5-biphosphate can also be detected in the nucleus, as well as PI3 kinase (Lu *et al.*, 1998). These results suggest that a number of distinct lipid signalling processes are regulated *in nucleo* to provide a variety of second messengers presumably involved in the coordinated regulation of nuclear function. Other second messenger-regulated nuclear Ca^{2+} release channels (such as ryanodine receptors) were discussed, but their role is less well established than that of nuclear IP_3Rs.

How are nuclear Ca^{2+} changes detected?

The nuclear Ca^{2+} signal, be it produced *in nucleo*, in the perinuclear vicinity or through transmission of a global wave from the cytosol, regulates a number of Ca^{2+}-dependent nuclear functions. Ca^{2+} exerts its effects via the different types of calcium-binding proteins present in the nucleus, including several 'EF hand' proteins such as

S100 or calreticulin, but the main target of nuclear Ca^{2+} is calmodulin, which is present in large amounts in the nucleus of most cell types. As underscored by O.Bachs (Spain), nuclear calmodulin has today become an important topic in nuclear signalling, whereas only a few years ago its mere presence there was hotly debated. Some of these Ca^{2+}-binding proteins regulate nuclear functions directly. Thus, J.Naranjo (Spain) described DREAM (Ca^{2+}-dependent nuclear repressor of dynorphin-dependent gene expression), a new transcriptional repressor containing four EF hands (Carrion *et al.*, 1998). Nuclear PKC, which could function as a molecular switch decoding Ca^{2+} and DAG signals (Oancea and Meyer, 1998), is another example. Calpains, Ca^{2+}-dependent proteases, can also be found in the nucleus. L.Santella (Italy) discussed their role in the regulation of the cell cycle. Calpain accumulates in the nucleus during mitosis and meiosis (prior to NE breakdown) and, conversely, calpastatin (calpain inhibitor) arrests or delays the hormone-induced reinitiation of meiosis when injected into the oocyte nuclei.

Ca^{2+}-binding proteins such as calmodulin also modulate nuclear functions by interacting with different binding partners. Several 'calmodulin binding proteins' are members of the growing family of nuclear serine/threonine kinases, such as Ca^{2+}/calmodulin-dependent protein kinase (CaM kinase). Similarly, B.Hemmings (Switzerland) discussed Ndr, an NLS-containing nuclear serine/threonine kinase that interacts with S100A1 or S100B (Millward *et al.*, 1998). Ndr, which is also negatively regulated by protein phosphatase 2A, seems to be involved in cell cycle control and morphogenesis. The list of nuclear functions regulated by these nuclear Ca^{2+}-binding proteins includes not only cell cycle progression (Pesty *et al.*, 1998) but also apoptosis, nucleocytoplasmic transport and gene expression.

Ca^{2+} and the nuclear transport process

Nucleocytoplasmic transport of proteins is another Ca^{2+}-dependent nuclear function. Thus, essentially all nucleocytoplasmic exchange of macromolecules occurs through NPCs, and a wealth of data presented at the workshop demonstrates that NPC permeability is Ca^{2+}-dependent, providing a structural explanation for the Ca^{2+}-dependence of the transport process. For instance, U.Aebi (Switzerland) discussed recent results of unfixed NPCs in a physiological buffer imaged by atomic force microscopy (AFM) showing reversible Ca^{2+}-mediated opening and closing of the NPC baskets (Stoffler *et al.*, 1999). Similar data were obtained by H.Oberleithner (Germany) using isolated NEs from *Xenopus laevis* oocytes, indicating that NPC shape changes are strictly ATP- and Ca^{2+}-dependent and may be attributed to contractile elements (actin–myosin) in the NPC. The high free Ca^{2+} concentrations (>1 μM) required in the medium for this effect could be generated physiologically by the nuclear IP_3Rs located in clusters on the inner membrane in close vicinity to the NPCs. Moreover, a direct relationship between NPC gating and $[Ca^{2+}]_{lumen\ NE}$ was documented by J.Bustamante (Brazil), whose results are in agreement with those presented by A.N.Malviya. It has been suggested that gp210, an NE protein with several intraluminal EF hand motifs and located at the level of the NPC, might mediate the effects of $[Ca^{2+}]_{lumen\ NE}$ on NPC permeability. Thus, an emerging theme in nuclear Ca^{2+} research is that NPCs are components of intracellular signalling routes to the nucleus. The control of NPC permeability and macromolecule transport by signal-transduction (Ca^{2+}) pathways provides another mechanism allowing the cell to synchronize gene expression with IP_3 generation, Ca^{2+} waves and other cytoplasmic signals.

Ca^{2+} can also modulate the nucleocytoplasmic transport process directly. Different reports indicate that NLS-independent exchanges are, at least partially, Ca^{2+}- sensitive. On the other hand, NLS-dependent import relies on GTP and the Ran-regulated transport machinery, and classically this process is Ca^{2+}-independent. However, recent results discussed at the Workshop are challenging this view. Thus, U.Greber (Switzerland), using fluorescently labelled adenovirus visualized with time-lapse fluorescence microscopy in living epitheloid cells, showed that depletion of the nuclear Ca^{2+} pool blocks transport across the NE, both NLS-mediated and by passive diffusion (Suomalainen *et al.*, 1999). J.Hanover (USA) has characterized an NLS-mediated, GTP-independent and Ca^{2+}-/calmodulin-stimulated nuclear protein import pathway. He has proposed a model to account for these outstanding findings, in which the stimulated release of intracellular Ca^{2+} from the ER/NE stores blocks the GTP-dependent NLS-protein import; the cell then switches to the GTP-independent Ca^{2+}- and calmodulin-dependent pathway. O.Petersen presented data indicating that the availabilty of calmodulin is also a limiting factor (up to 50% of the available calmodulin can translocate to the nucleoplasm upon prolonged stimulation). Taken as a whole, these findings, though they require further confirmation, show that Ca^{2+} is a key regulator of NLS-dependent protein import into the nucleus, with a GTP-dependent pathway that is sensitive to $[Ca^{2+}]_{lumen\ NE}$ and inhibited at high $[Ca^{2+}]_{cytosol}$, whereas the GTP-independent and calmodulin-dependent pathway is stimulated by increasing $[Ca^{2+}]_{cytosol}$. The requirements for nuclear protein export, which seem to be unaffected by either depletion of lumenal Ca^{2+} stores or inhibition of calmodulin, appear distinct from those for nuclear import.

Nuclear Ca^{2+} in gene transcription

Although numerous aspects of gene transcription are Ca^{2+}-dependent, it is the CREB (cAMP response element binding protein)-dependent transcription that has been the most studied. There are multiple CREB-kinase candidates, including CaM kinases. Specific isoforms of CaM kinase have been located in the nucleus, such as type II (phosphorylates both activating CREB-Ser^{133} and inhibitory CREB-Ser^{142}) and type IV (only phosphorylates CREB-Ser^{133}). CaM kinase II is activated by autophosphorylation on T^{286}, whereas CaM kinase IV is activated by phosphorylation on T^{196} by an upstream Ca^{2+}- and calmodulin-dependent kinase, CaM kinase kinase β, which is also present in the nucleus. It has been suggested that nuclear CaM kinases could act as frequency decoders with respect to $[Ca^{2+}]_{nucleosol}$ oscillations; indeed, the activity of CaM kinase II has been shown to be sensitive to the frequency of $[Ca^{2+}]$ oscillations (De Konick and Schulman, 1998). S.Finkbeiner (USA) presented data

from hippocampal neurons showing that CREB-Ser^{133} phosphorylation is an early event (within 1 min) following Ca^{2+} entry. In addition, Ca^{2+} entry via L-type Ca^{2+} channels is more effective in activating CRE-mediated transcription of c-*fos* than Ca^{2+} entry through *N*-methyl-D-aspartate (NMDA) receptors, whereas Ca^{2+} influx via either route is sufficient to trigger serum response element (SRE)-mediated c-*fos* expression. Therefore, Ca^{2+} entry through different routes generates different transcriptional responses. H.Bading (UK) discussed transcriptional regulation by spatially distinct Ca^{2+} signals. Nuclear Ca^{2+} stimulates CRE-dependent gene expression, whereas increases in cytosolic Ca^{2+} activate transcription mediated by the SRE. H.Bading has also shown that recruitment of the coactivator CBP by phosphoCREB-Ser^{133} is followed by a second regulatory step involving stimulation of CBP activity by nuclear Ca^{2+} through CaMK IV (Chawla *et al.*, 1998). Thus, a single second messenger (Ca^{2+}) can generate diverse transcriptional responses depending on its route of entry and localization (nucleosolic versus cytosolic).

NF-AT and NF-κB are also Ca^{2+}-dependent transcription factors. Phosphorylation of NF-AT on the regulatory domain by NF-AT kinases results in cytoplasmic localization, whereas calcineurin activation in response to Ca^{2+} transients leads to nuclear translocation through dephosphorylation and unmasking of NLS. In the case of NFκB, one of the IκB inhibitor isoforms is phosphorylated by a CaM kinase leading to its ubiquitination and degradation. These results raise the question of the specificity of the Ca^{2+} signal: how can a Ca^{2+} signal, which activates several transcription factors, produce a specific Ca^{2+}-dependent transcriptional response? Data from the group of R.Lewis (USA) presented by R.Dolmetsch (USA) indicate that $[Ca^{2+}]$ oscillations play a key role, increasing the information content of Ca^{2+} signals as well as their efficiency (Dolmetsch *et al.*, 1998). Indeed, oscillating Ca^{2+} signals represent codes (amplitude or frequency coding) that can be decoded by the transcriptional system. Thus, rapid $[Ca^{2+}]$ oscillations stimulate both NF-AT and NFκB, whereas infrequent oscillations activate only NFκB; and a sustained $[Ca^{2+}]$ elevation activates NF-AT, whereas a transient rise stimulates NFκB. E.Carafoli (Switzerland) discussed the regulation of a target gene, *PMCA*, by calcium.

Nuclear Ca^{2+} and programmed cell death

Nuclear changes are prominent features of apoptosis and the role of Ca^{2+} in triggering the 'death' programme is well established (Yano *et al.*, 1998). Early nuclear events include DNA cleavage into large fragments (Mg^{2+}-dependent) followed by further degradation of these fragments resulting in the classical DNA ladder (Ca^{2+}/Mg^{2+}-dependent). K.Cain (UK) demonstrated that the caspase inhibitor z-VADfmk blocks all the features of apoptosis including the ordered DNA cleavage. Mitochondria are thought to play a key role in apoptosis. Thus, J.-C.Martinou (Switzerland) showed that Bax induces both the release of cytochrome *c* and a loss of membrane potential in isolated mitochondria. However, P.J.Rogue (France) pleaded for caution with respect to the exclusive emphasis currently placed on mitochondria. Recent reports are forcing a re-evaluation of this model, suggesting an alternative view involving multiple apoptotic pathways. The role of nuclear Ca^{2+} in cell death clearly needs to be explored further.

A continent to be explored

The workshop, unanimously hailed as a great success by its participants, confirmed the dynamism of the field of nuclear calcium research. In his closing remarks, E.Carafoli, while stressing the quality and intensity of the different presentations, also emphasized the work that lay ahead. Indeed, it is a whole new continent that has to be explored. The field seems to be at a point akin to that of bioenergetics when the chemiosmotic coupling hypothesis was proposed. In his opening lecture, 1997 Nobel laureate J.Walker (UK) highlighted how the understanding of a complex molecular structure (F_0F_1-ATPase) greatly advanced the understanding of a cellular process (bioenergetics). No doubt that progress in research on nuclear Ca^{2+} dynamics, the regulation of the system and the structure of its components, will greatly accelerate the understanding of nuclear calcium function.

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