Cytosolic Calcium Oscillations in Signal Transduction Pathways

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Abstract: The oscillatory nature of the intracellular calcium signal has been recognized as soon as the methodological developments allowed us to record calcium fluctuations at the single cell level. While the principal mechanisms responsible for the generation of these oscillations have been partially resolved, more attention has been recently focused on signal decoding and more particularly on the role of cell structure organization in transducing this signal to the molecular targets of the calcium messenger.

INTRODUCTION

To the young investigator who nowadays almost routinely determines intracellular calcium in a research laboratory, it may sound astonishing to hear that, what appears now as trivial and common knowledge was a puzzling mystery in cell biology less than twenty years ago. The cell membrane constituted a practically insurmountable barrier preventing access to and measurement of calcium inside an intact cell , and the role of calcium as a widely utilized intracellular messenger was merely suspected.

In the late seventies, aequorin, a photoprotein isolated from a luminescent jellyfish, was the only tool that could be utilized to record intracellular calcium concentration with sufficient accuracy [1,2]: indeed, aequorin's bioluminescence depends upon Ca^{2+} concentration from 0.1 to > 100 µM. However, because of the size of the protein (22'000 kDa), introducing it into cells required harsh and cumbersome procedures, such as permeabilization or osmotic shock, or poorly efficient methods such as micro-injection [2].

The design by Tsien *et al*., in 1982 [3], of the first cell-permeant, highly selective organic fluorescent indicator for calcium, quin2, can be considered as one of the major breakthroughs in cell biology at the end of the twentieth century. For the first time, it became possible to follow dynamic changes in cytosolic calcium concentration in essentially intact living cells, for example in adrenal glomerulosa cells, and to correlate these variations with cellular functional responses [4]. The ingenious and simple principle that allowed quin2 to cross the cell membrane and remain trapped within the cell was then applied to construct more potent fluorescent probes, such as fura-2, the archetypal calcium indicator. Because of its higher quantum yield, this new generation of indicators made it possible to record intracellular calcium changes at the single cell level.

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Surprisingly, though, the first demonstration of an oscillatory pattern in the cytosolic calcium response to an agonist was obtained with aequorin in single hepatocytes [5]. The cells responded to an alpha-adrenergic agonist and to vasopressin with astonishingly repetitive and regular calcium transients, whose frequency depended upon agonist concentration while amplitude remained constant. This initial work was then followed by a wealth of reports describing the observation, mainly obtained with fura-2, of oscillatory calcium responses in practically every cell type, from pituitary cells [6,7] to fibroblasts [8], from oocytes [9] to insulin-secreting cells [10] and adrenal glomerulosa cells [11], to cite only a few examples.

A further impressive progress was next achieved with the availability of very sensitive video cameras that made it eventually possible for researchers to actually "see", sometimes even in three dimensions, calcium changes occurring within single cells [12]. With this enhanced power of resolution, it then became obvious that, in addition to fluctuating, the cytosolic calcium concentration was not homogeneously distributed throughout the cell and this was confirmed again in numerous cell types as different as neuronal cells [13], adrenal chromaffin cells [14], erythroblasts [15], pituitary lactotrophs [16], Xenopus laevis oocytes [17], acinar cells [18], endothelial cells [19] or vascular smooth muscle cells [20], for example. To describe the often spectacular images obtained with these powerful tools, scientists have resorted to illustrative terms ("blips" and "puffs" [21]), sometimes even borrowed from other disciplines such as oceanography ("waves" [22] and "tides" [23]) or astronomy ("sparks" and "quarks" [24]).

SIGNAL MODULATION AND MOLECULAR MECHANISMS

Since the discovery of the oscillatory nature of the calcium signal two fundamental questions have been raised: *How does it work ?* and *What is it for ?* Although no definitive and complete answers have been provided yet, we will describe some of the progress made in our knowledge of the molecular mechanism(s) involved in the generation of calcium oscillations and calcium waves, analyze the role of

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intracellular organelles in the confinement or propagation of this signal, and finally discuss the advantages of a rapidly changing signal for independently modulating several cellular functions, sometimes occurring simultaneously in a same cell, and as diverse as gene expression, differentiation, cell division and proliferation, hormone and neurotransmitter secretion, muscle contraction, or even control of programmed cell death.

Early analysis of the diversity of calcium oscillatory patterns in various cell types led to the suggestion that there probably exists different mechanisms responsible for these periodic fluctuations of calcium: In fact, oscillations in cytosolic calcium can result schematically from fluctuations either in calcium entry through the plasma membrane or in the release from intracellular stores [25]. However, while periodical changes in plasma membrane calcium channel activity principally occur in excitable cells, as, for example, during neuronal firing or heart beating, fluctuations primarily due to calcium release observed in non-excitable cells are also accompanied by secondary calcium influx in order to compensate for calcium extrusion out of the cell and to maintain stores ready for a next burst of calcium. This influx can be mediated either by a capacitative mechanism [26] or through distinct pathways [27]. The requirement for Ca^{2+} store repletion from the extracellular medium is illustrated by the behavior of single bovine glomerulosa cell challenged with a submaximal concentration (0.1 nM) of AngII (Fig. (**1**)). After a delay of several minutes, cells responded to the agonist either with large and regular Ca^{2+} transients (A) or smaller and more stochastic fluctuations (B). Blocking voltage-operated Ca^{2+} channels with nicardipine (Nic) did not affect the amplitude of the signal but reduced the frequency of the spikes, from 0.44 to 0.28 min^{-1} in this particular example, while completely removing extracellular Ca^{2+} with an excess of EGTA immediately stopped the oscillations and even slightly reduced the basal levels. Inhibition of intracellular Ca^{2+} pumps with thapsigargin during a burst of activity (B) prolonged the duration of the next transient and Ca^{2+} was then maintained at higher levels, the larger fluctuations having ceased. Together, these observations suggest that upon glomerulosa cell stimulation with low concentrations of the agonist, Ca^{2+} enters the cell and is pumped from the cytosol into the endoplasmic reticulum to be subsequently explosively released. While, in this case, calcium influx is probably essentially mediated by a capacitative mechanism, Ca^{2+} flow through additional channels (both T-type or L-type channels are sensitive to 2 µM nicardipine) apparently participates to store repletion, and modulates the frequency of transients.

Even when the oscillations are generated through intracellular calcium release, variations in the shape of the calcium transients suggest that different mechanisms occur from one cell type to the other. Before describing the various mechanisms involved in different cells, we have to briefly review the various proteins that may modify cytosolic calcium concentration (Table 1). Together, these proteins are part of the "*calcium signaling toolkit*" recently described in detail by Berridge *et al*. [21]. These effectors are classically divided in two groups: 1) *calcium channels*, intracellularly located or embedded in the plasma membrane, which carry calcium very efficiently down its electrochemical gradient and are activated by several mechanisms such as membrane depolarization, calcium store depletion, or binding of specific ligands; and 2) *calcium pumps and exchangers*, also

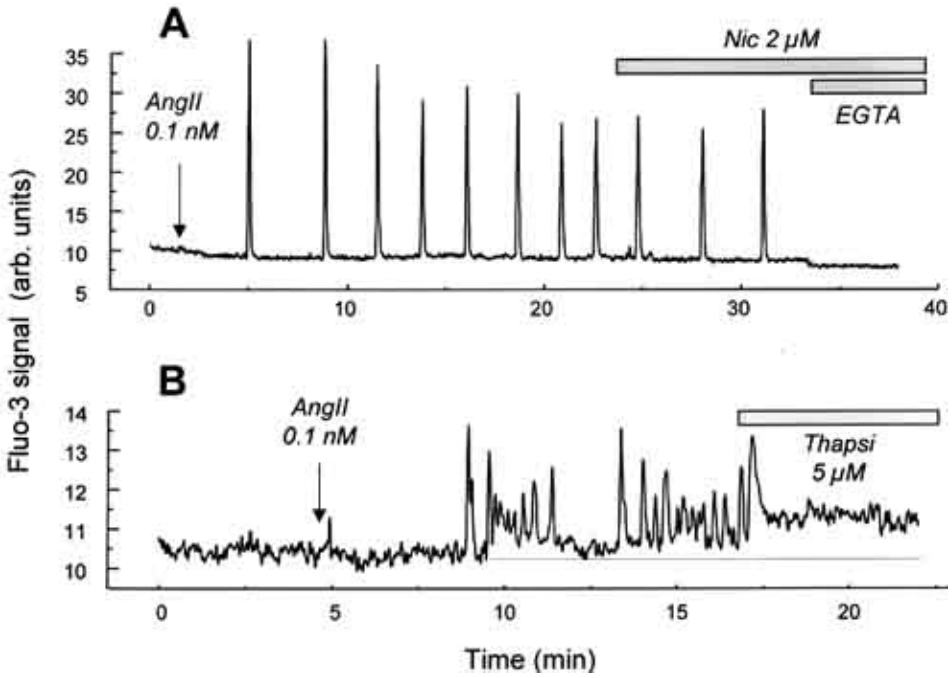

Fig. (1) *Angiotensin II-induced calcium oscillations in single bovine adrenal glomerulosa cells.* Cytosolic calcium fluctuations in the presence of 0.1 nM AngII were monitored with the fluorescent probe fluo-3 on a Zeiss Axiovert 10 inverted microscope equipped with a Bio-Rad CRS-400 microfluorimeter. The fluorescent signal was sampled at 2 Hz and recorded using the CRS-400 software. Nic: nicardipine, 2 µM; EGTA, 2 mM; Thapsi: thapsigargin, 5 µM.

Table 1. Main Cellular Effectors Responsible for Shaping the Calcium Signal

tools	molecular structure	isoforms	activation (modulation)	driving force	subcellular localization
Voltage-operated channels *(VOC)*	multi- or monomeric (α_1, β, γ, $\alpha_2\delta$)	10 α_1 : A-I, S	cell depolarization (G prot, kinases)	electrochemical gradient	p.m.
Store-operated channels *(SOC)*	homo- or heterotetrameric	>20 : TRPC1-7 TRPV1-6 TRPM1-8	store depletion (PKC, CaM/Ca^{2+}, TK)	electrochemical gradient	p.m.
Receptor-operated channels *(ROC)*	heteromeric	> 9	spec. ligand binding (kinases)	electrochemical gradient	p.m.
Inositol trisphosphate receptors *(IP_3R)*	homo- or heterotetrameric	3 : IP_3R1-3	$InsP_3$ (Ca^{2+}, ATP, cAMP, FKBP12)	chemical gradient	e.r.
Ryanodine receptors *(RyR)*	homotetrameric	3 : RyR1-3	cADP-Ribose (Ca^{2+}, ATP, FKBP12)	chemical gradient	e.r., s.r.
Mitochondrial calcium uniporter	not cloned	?	Ca^{2+} (Mg^{2+})	electrical gradient	mitoch.
Sarco-endoplasmic reticulum pumps *(SERCA)*	multimeric	3 : SERCA1-3	Ca^{2+} (PKA, phospholamban)	ATP hydrolysis	e.r., s.r.
Plasma membrane pumps *(PMCA)*	monomeric	4 : PMCA1-4	Ca2+	ATP hydrolysis	p.m.
Na/Ca exchangers (NCX)	monomeric	3 : NCX1-3	Ca^{2+} (ATP)	Na^+ electrochem. gradient	p.m., mitoch.

present on both intracellular and plasma membranes, which require energy to accumulate calcium against its gradient and are directly activated by a rise of calcium concentration in the cytosol. Each of these effectors is subject to complex modulation, either by calcium-dependent mechanisms (feedback control) or by independent intracellular messengers (crosstalk between signaling pathways). For example, the activity of the inositol 1,4,5-trisphosphate receptor (IP_3R) is modulated by ATP [28], the production of which is increased upon Ca^{2+} release and subsequent entry of the cation into mitochondria, or Ca^{2+} pumping by SERCA is regulated by cAMP-dependent phosphorylation of the associated protein phospholamban [29]. Regulation can also occur upstream of the effector, affecting the generation of activators like $InsP_3$ or cyclic ADP-ribose. Moreover, the complexity and diversity of calcium signaling also results from the multiplicity of effector isoforms (and splice variants), each with specific properties. Various isoforms are often expressed together in a single cell. For example, RyR1 is mostly expressed in skeletal muscle and RyR2 in cardiomyocytes, but many cell types simultaneously express the three types of IP_3R's.

In view of the diversity of the intervening players, it is not surprising that different cell types, each expressing its specific set of tools, display Ca^{2+} signals with different characteristics. Even within a cell line such as the insulinoma HIT cells, individual cells have been shown to respond to successive applications of a muscarinic agonist with reproducible patterns in term of amplitude, frequency and shape of Ca^{2+} transients, but these patterns markedly vary from one cell to the other, suggesting that each cell displays its own "Ca^{2+} fingerprint" [10]. Another example of different behaviors in response to the same stimulus has already been mentioned in Fig. (**1**).

Increasing the concentration of the agonist that elicits Ca^{2+} oscillations can result either in a higher frequency or in a higher amplitude of the transients. Indeed, while changing vasopressin or phenylephrine concentration profoundly affected the frequency, but not the amplitude, of the calcium oscillations in hepatocytes, as described by Woods *et al*. in their seminal paper [30], in other systems, such as lacrimal acinar cells stimulated with a muscarinic agonist [31], activation results in Ca^{2+} oscillations of constant frequency but of variable amplitude. As we will see later, modulating the signal in amplitude or in frequency can have dramatically different consequences on gene expression, and probably also on many other cellular functions.

With this in mind, we can now start to discuss more in detail the molecular mechanisms responsible for generating store-dependent periodical transients of calcium in the cytosol. The fact that Ca^{2+} oscillations are generally observed in response to a relatively narrow window of agonist concentrations is very informative. Lower levels of activation probably do not reach the threshold required for triggering massive Ca^{2+} release, and only minor, highly local and barely detectable events such as *puffs* and *sparks* [21] can occur under these conditions.

At higher levels, real bursts of calcium appear, leading to waves of calcium throughout the cell and probably reflecting some chain reaction to insure propagation of the signal. Finally, when agonist concentration becomes too high, or upon Ca^{2+} overload in the cell, distortion of the signal

occurs because the re-uptake phase is absent and high concentrations of cytosolic calcium are maintained. Under these conditions, calcium influx is probably no more buffered by the organelles. Although the permissive window is probably different from one cell to the next, when they are connected through gap junctions, Ca^{2+} waves can travel across and generate oscillations in neighboring cells. One can therefore expect that under these conditions the most sensitive cell will serve as a sort of pace maker for controlling the activity of surrounding cells. This could partly explain why *in vitro*, secretion is generally triggered at lower concentrations of the agonist when cells are cultured at higher density, a condition more favorable to the formation of gap junctions [32].

Four mechanistic models have emerged in the literature to explain the generation of Ca^{2+} oscillations in non excitable cells and have been analyzed in details by Harootunian *et al*. [33]. These models can be classified depending on whether Ca^{2+} exerts a positive or a negative feedback on its own release from internal stores and whether $InsP_3$ also undergoes oscillations linked to the Ca^{2+} spikes (Fig. (**2**)).

In the first model (①), it is assumed that Ca^{2+} released by $InsP_3$ exerts a negative feed back regulation on the $InsP_3$ receptor (IP_3R), on the cytosolic side, and that simultaneously, the decreasing Ca^{2+} concentration in the lumen of the e.r. is responsible for a reduction of the affinity of this receptor for its ligand. Thus, after Ca^{2+} release, the IP_3R remains refractory until cytosolic calcium is low again and the stores have been refilled with sufficient Ca^{2+}. This mechanism is really attractive, particularly in cells devoid of ryanodine-sensitive pools and displaying a quantal release of Ca^{2+} by $InsP_3$ [34]. Moreover, cytosolic calcium is expected to oscillate only in a window of agonist concentration and to be modulated in frequency by extracellular Ca^{2+}. Because receptor activation and $InsP_3$ production remain constant and only the sensitivity of the stores to $InsP_3$ fluctuate, this model can be tested with caged $InsP_3$ that can be released (upon strong illumination) at different phases of the oscillations. This compound is expected to affect the calcium signal only during the rising (responsive) phase, but not between peaks, when the IP_3R is refractory. This model fits best with the type of Ca^{2+} transients shown in Fig. (**1**), as observed upon stimulation of bovine glomerulosa cells with AngII [35].

The two next models involve a *positive* modulation by cytosolic Ca^{2+} on either a ryanodine receptor (RyR) (②), or on the $InsP_3$-producing enzyme, PLC (③). Although a positive action of Ca^{2+} helps to understand how Ca^{2+} waves can spread from place to place throughout the cell, it cannot satisfactorily explain the transient nature of the signal. Indeed, while the decreasing phase of the transients can be simply due to pool exhaustion, the latter must remain refractory to accumulate enough Ca^{2+} until the next burst of release and refilling of the stores is expected to be slower

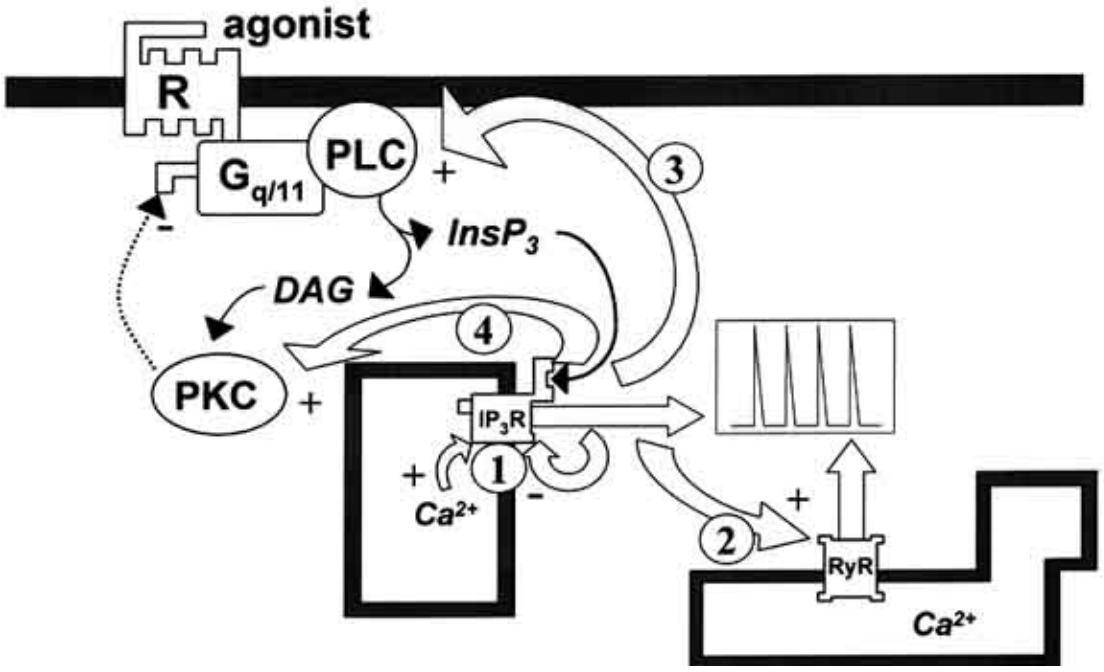

Fig. (2). Mechanisms of the generation of calcium oscillations.

The main mechanisms (1 to 4) proposed in the literature to underline the generation of cytosolic calcium oscillations are indicated (see text for more detailed information). Abbreviations used are: *R*: receptor; *G* : G protein of the q/11 family; *PLC*: phosphoinositide-specific phospholipase C; *$InsP_3$*: inositol 1,4,5-trisphosphate; *DAG*: diacylglycerol; *PKC*: protein kinase C; *IP_3R*: $InsP_3$ receptor; *RyR*: ryanodine receptor.

than extrusion out of the cytosol. In fact, both models 2 and 3 could coexist with model 1 in the same cell. Each can be specifically tested, either by overloading cells with Ca^{2+}, which should induce Ca^{2+} oscillations even in the absence of agonist if mechanism 2 is involved, or with GTPγS, which sensitizes PLC to Ca^{2+} and should therefore cause oscillations at lower [Ca^{2+}] in case of model 3. The latter model predicts that $InsP_3$ levels should fluctuate in parallel with cytosolic calcium and this has been shown in fibroblasts upon stimulation with vasopressin [8].

Finally, the last model (④) involves a transient uncoupling of the receptor from PLC through a Ca^{2+}-dependent activation of PKC. In this case, PKC-dependent receptor phosphorylation could be responsible for a transient decrease in $InsP_3$ levels, and therefore for a reduction in Ca^{2+} release. Under these conditions, Ca^{2+} is removed from the cytosol and PKC is slowly inactivated, the duration of the lag time to the next Ca^{2+} transient being also a function of the availability of phosphatases responsible for receptor dephosphorylation. This model can also be tested quite easily by altering PKC activity, for example through activation or down regulation of the enzyme with phorbol esters. The existence of such a mechanism has been particularly well illustrated in a study analyzing the ability of metabotropic glutamate receptor (mGluR) isoforms to elicit calcium oscillations when expressed in HEK293 or NIH3T3 cells [36]. While stimulation of the type 5 mGluR leads to regular Ca^{2+} oscillations, the response of the type 1 mGluR is a sustained elevation of Ca^{2+}. Construction of chimeras allowed to delimit a small region of the receptor controlling the pattern of the Ca^{2+} response that is located immediately after the 7th transmembrane domain and that interferes with a Gq protein. The single diverging amino acid in this region (a Thr in type 5 is replaced by an Asp in type 1) is subject to phosphorylation by PKC. Moreover, targeted mutation of this amino acid aimed at preventing PKC phosphorylation suppressed the oscillatory response of the type 5 receptor.

To summarize, it appears that the mechanisms involved in the generation of the calcium signal in a given cell and consequently the pattern of this signal tightly depend upon the various effectors present in this cell, as well as upon the right proportion of modulators and other calcium binding proteins. One important actor is however still missing in this succinct description of cellular Ca^{2+} handling, and its essential role in calcium signal confinement, buffering and signal decoding will be specifically discussed in the next sections; this new intervening actor is the *mitochondrion*.

ROLE OF MITOCHONDRIA IN CALCIUM SIGNALING

Because of their ability to rapidly sequester high amounts of calcium during the rising phase in the cytosol and to release it after some delay in a sodium-dependent manner, mitochondria play an important role in shaping both the amplitude and the spatio-temporal pattern of calcium signals [37,38,39].

Calcium uptake into mitochondria directly depends on the organelle's internal negative potential, which is generated through H^+ extrusion by the respiratory chain and can reach values as negative as -180 mV. Inhibitors of mitochondrial respiration or H^+ ionophores have been shown to markedly increase the amplitude of the cytosolic Ca^{2+} signal, clearly demonstrating the Ca^{2+} buffering function of these organelles in various cell types.

Other mechanisms, in addition to the sequestration of Ca^{2+}, are probably also involved in the modulation of the cytosolic signal by mitochondria. Indeed the slow release of Ca^{2+} into the cytosol by these organelles could be responsible for maintaining neighboring IP_3R in a refractory state for a longer period of time after the initial burst; alternatively, by producing ATP in response to a rise of Ca^{2+} in the matrix, mitochondria should be able to regulate the activity of SERCA pumps that are located in close proximity. In some cells, mitochondria have been shown to prevent diffusion of calcium throughout the cytosol, confining the signal in a restricted area, as for example in acinar pancreatic cells [40]. Finally, because ATP positively modulates IP_3R activity [28], Ca^{2+} release sites in the vicinity of the mitochondria could be under local control by these organelles, which would result in a true crosstalk between the endoplasmic reticulum and the mitochondria.

With the availability of recombinant aequorin and fluorescent probes that can both be targeted to the mitochondrion [41,42], it has become clear that this organelle is able to sense cytosolic calcium oscillations and to relay and even amplify them within the mitochondrial matrix, for example in mammalian CHO.T cells or hepatocytes [42,43].

In fact, the efficiency of mitochondrial Ca^{2+} uptake is probably due in major part to their close apposition to some regions of the endoplasmic reticulum, as noticed in many cells [44], an observation suggesting that mitochondria are located in microdomains of high calcium, next to Ca^{2+} release sites [45,46,47], or even that Ca^{2+} can be delivered through "quasi synaptic" connections [48].

Indeed, coupling between the sarco/endoplasmic reticulum and the mitochondria can be so tight in some cells that oscillations generated upon repeated Ca^{2+} release are detected only in mitochondria, and not in the cytosol. In C2C12 myotubes, for example, the oscillatory Ca^{2+} signal induced by cell depolarization with potassium is exclusively detected in the mitochondria [49]. The sarcoplasmic reticulum origin of the oscillatory mitochondrial signal was confirmed in this study by demonstrating that caffeine is able to induce similar oscillations in mitochondria, while cyclopiazonic acid, a SERCA inhibitor, prevented KCl-induced mitochondrial oscillations, probably by perturbing Ca^{2+} loading of the stores. In these cells, the "pace making" activity is thus apparently due to fluctuations of the ryanodine receptor activity. Interestingly, cell depolarization with a nicotinic agent, or $InsP_3$ production mediated by activation of the purinergic P receptor resulted in different mitochondrial Ca^{2+} patterns, suggesting that not only the proximity of the RyR and of the mitochondrial uniporter is relevant in the generation of the matrix signal, but also the

spatial relationship of the organelles to the plasma membrane receptors.

In other cell types [48,50], clamping the bulk cytosolic Ca^{2+} at resting levels with EGTA did not affect transduction of the IP_3R- or RyR-mediated Ca^{2+} signal into the mitochondria, once again supporting the idea of a localization of mitochondria in high calcium microdomains, in vicinity of Ca^{2+} release sites. The machinery of local signaling between the sarco/endoplasmic reticulum and mitochondria, as well as the morphological organization of these structures has been recently reviewed by Hajnóczky *et al*. [44].

This cellular organization strongly suggests that the role of the mitochondria is probably not limited to that of a barrier confining Ca^{2+} signal in a specific place of the cytosol [40], but that these organelles act as Ca^{2+} sensors to translate the cytosolic signal into specific responses like steroid synthesis or ATP production (see below).

DECODING THE MESSAGE CONVEYED BY CALCIUM OSCILLATIONS

As mentioned earlier, the initial discovery of oscillatory intracellular calcium patterns in hepatocytes [5] started a period of abundant production of works reporting calcium oscillations in practically every cell type examined and aimed at understanding the mechanisms underlying these patterns [25,51]. The next task obviously was then to analyze how cells translate spatially and temporally dynamic signals into a functional response.

Depending on the cell type and specific function, the response to a calcium-mobilizing agonist will predominantly depend upon intracellular calcium changes being sensed and decoded by distinct organelles such as mitochondria for metabolic responses, the nucleus for genomic effects or secretory granules for exocytosis.

Many processes linked to cellular oxidative metabolism occur within mitochondria and it has been known for quite some time that three dehydrogenases located within the mitochondrial matrix are sensitive to calcium changes [52,53]. In fact, single intramitochondrial calcium spikes have been shown to modulate simultaneously the redox state of flavoproteins and pyridine nucleotides, a parameter of mitochondrial oxidative metabolism. The frequency of these oscillating calcium signals can thus be decoded by mitochondria to control mitochondrial dehydrogenase activities [42] and therefore to match locally and temporally ATP production to physiological needs, as for example in contracting muscle cells.

Another cell type in which mitochondria play a crucial role in differentiated function is the steroid producing cell from various steroidogenic tissues such as the testis, the ovary or the adrenal gland. Indeed, the rate limiting step of steroidogenesis and most of the enzymes metabolizing cholesterol, the precursor of all steroid hormones, into the final products are located within the mitochondria. For example, the zona glomerulosa of the adrenal cortex makes the mineralocorticoid, aldosterone, in response to two physiological stimulators, the octapeptide hormone, AngII, and extracellular potassium, which both lead to profound changes in intracellular calcium homeostasis [4]. Again, in zona glomerulosa cells, changes in cytosolic calcium are amplified within the mitochondrial matrix [54] and it was possible to show, by blocking calcium influx into mitochondria with ruthenium red, that AngII-mediated activation of aldosterone biosynthesis was prevented, due to blockade of calcium-sensitive cholesterol import into mitochondria [55,56].

The expression of numerous nuclear genes is known to be controlled by calcium [57]. Recently, it has been demonstrated that the expression of certain genes can be modulated by the frequency of cytosolic calcium oscillations [58]. Indeed, in T lymphocytes, transcription driven by three proinflammatory factors (NF-AT, Oct/OAP and NF-κB) was activated as a function of oscillation frequency: when rapid oscillations were elicited, all three transcription factors were activated, while, at lower frequencies, only NF-κB was responsive. Similarly, using a caged derivative of InsP to produce intracellular calcium spikes at various frequencies, Li *et al*. [59] observed that, in activated T cells, gene activation through the same transcription factors, as measured with a reporter gene, could be optimized by varying spike frequency. While amplitude modulation may also play a role, these reports provide the first evidence for selectivity of the response to calcium based on frequency of calcium oscillations.

Finally, secretory granules may also represent targets for calcium signaling. For example, spontaneous oscillations of intracellular calcium have been linked with growth hormone secretion in normal rat pituitary cells [60]. Similarly, in rat gonadotropes, gonadotropin-releasing hormone (GnRH)-induced calcium oscillations lead to rythmic exocytosis, as measured by membrane capacitance changes, each oscillation being accompanied by a burst in the rate of exocytosis [61].

Although the detailed mechanisms are far from being understood, the few above examples provide striking demonstrations of the way cells decode oscillatory calcium signals for a selective and specific activation of the cell's functional responses.

CONCLUSION

Our knowledge of the molecular mechanisms and of the physiological significance of intracellular Ca^{2+} oscillations has considerably evolved since the first observation of fluctuating Ca^{2+} in single hepatocytes [30]. This has led us to a new vision of cell signaling. Indeed, while massive stimulation has been generally obtained, *in vitro*, by suddenly exposing naive cells to rather elevated concentrations of agonists in order to investigate Ca^{2+} signaling, *in vivo*, modifications of circulating Ca^{2+}-mobilizing hormones and factors are much more subtle and continuous. Under such conditions, the sensitivity resulting from fine tuning of the signal through either frequency or amplitude modulation becomes highly relevant in an

organism undergoing slow and limited changes in homeostasis.

ABBREVIATIONS

AngII = Angiotensin II

Nic = Nicardipine

IP_3R = $InsP_3$ receptor

SERCA = Sarco-endoplasmic reticulum calcium ATPase

$InsP_3$ = Inositol 1,4,5-trisphosphate

RyR = Ryanodine receptor

e.r. = Endoplasmic reticulum

PLC = Phosphoinositide-selective phospholipase C

PKC = Protein kinase C

mGluR = Metabotropic glutamate receptor

GnRH = Gonadotropin-releasing hormone

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