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REVIEW: MOLECULAR CELL BIOLOGY

DECODING CALCIUM SIGNALS IN LIVING CELLS

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ABSTRACT

The regulation of calcium ion concentration outside and inside the cell, together with the movement of calcium ions from one compartment to another, are key factors that determine the fate of a cell: growth, stimulation and death. Calcium is the most ubiquitous second messenger encountered in a plethora of physiological functions from hormonal release and muscular contraction to gene expression. The level of increase in calcium concentration within a cell is finely modulated in terms of the quantity of ions, time and space, thus constituting a complex language that can only be interpreted by specific proteins.

Keywords: ***Signalling; Excitation-contraction coupling; Exocytosis; Microdomains; Ion channels***

[I] PHYSIOLOGICAL FUNCTIONS

The homeostasis of calcium ion concentration within a cell is a fine-tuned regulated parameter which presides over many pivotal physiological functions. For example, in the heart, the contraction of cardiac muscle cells is triggered by depolarizing events that induce a small influx of calcium ions, which (through L-type calcium channels or LCCs) in turn leads to the massive release of calcium from intracellular pools (sarcoplasmic reticulum or SR) via calcium release channels known as ryanodine receptors (RyRs). This released calcium binds to the contractile apparatus, which mediates a conformational change and the movement of the myofilaments and therefore a shortening of the cardiomyocyte. This process is referred to as excitation-contraction coupling [1]. In contrast, skeletal muscle cells exhibit a direct mechanical coupling between LCCs and RyRs [2]. In neurones, the cellular localization of calcium channels determines their particular function. At presynaptic sites, calcium channels regulate the release of neurotransmitters by exocytosis. In dendritic spines (that contain the post-synaptic densities where the neurotransmitter receptor complexes are present), calcium signals are responsible for the modulation of learning and memory processes [3]. In particular intracellular inositol-trisphosphate receptors (or $InsP_3Rs$, calcium release channels that are located on the membrane of the endoplasmic reticulum or ER) play a major role as signal detectors and integration centres [4]. In non-excitable cells, $InsP_3Rs$ are the central components of the calcium-induced calcium release (CICR)

phenomenon, but not the only components, since RyRs can also be present and functional such as in the pancreas where RyRs mediate calcium release [5].

Calcium signals can be found in various compartments and organelles of the cell where they display distinct functions. Mitochondria, for instance, serve as calcium buffering systems, and alteration of this pathway leads to pathophysiological states in many varieties of tissues such as in the heart, brain, pancreas and kidney [6]. Moreover, lysosomes also represent calcium stores, and the calcium release from acidic organelles is mediated by nicotinic acid adenine dinucleotide phosphate (NAADP) receptors identified as two-pore channels [7]. It is now accepted that the nucleus itself is involved in the occurrence of calcium signals [8, 9]. These nucleoplasmic calcium signals activate distinct pathways that control gene expression very specifically and independently of cytosolic calcium [10].

Hence, calcium controls many aspects of life at the cellular but also at the organism level, from fertilization of mammalian, sea urchin, fish and frog eggs through development, differentiation and proliferation to the activation of transcription factors and apoptosis [11].

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[II] PATHWAYS INVOLVED IN THE INCREASE OF THE INTRACELLULAR CALCIUM CONCENTRATION

There are 3 main pathways by which a rise in intracellular calcium can occur. They are: i) calcium entry through voltage-gated calcium channels, ii) calcium entry through ligand-gated channels, and iii) calcium release from internal stores.

While the voltage-gated, ligand-gated and store depletion pathways have been studied intensively, the involvement of polymodal (responsive to temperature, light, voltage, ligand, pH and mechanical stimuli) transient receptor potential (or TRP) channels has been the subject of recent investigations [12-14]. These channels are thought to be involved in many physiological functions such as blood pressure, the regulation of mineral absorption/reabsorption, gut motility and airway responsiveness, pain and taste transductions, thermo- and mechano-sensations and cell proliferation/death [15].

In many neuronal and non-neuronal cell types, various molecules (such as peptides, hormones and transmitters) have been shown to specifically bind to their receptors and upon stimulation trigger an influx of calcium *via* activation of phospholipase C (PLC) and adenylate cyclase (AC) pathways, thus exerting cellular functions [16]. Calcium release from stores in mammalian cell types primarily occurs as a result of the activation of metabotropic ligand-binding receptors, which use second messenger signalling cascades to indirectly activate intracellular ion channels.

[III] DIFFERENT TYPES OF CALCIUM SIGNALS

3.1. Calcium sparklets

Optical recordings of localized calcium influx events *via* LCC present in the plasma membrane are called “calcium sparklets”. When a single LCC opens, this leads to calcium influx, resulting in an increase in the total amount of calcium in the cleft between the LCC in the plasma membrane and the opposing cluster of RyRs in the SR membrane. These increases can be recorded as calcium signals named calcium sparklets [17]. These sparklets reveal an unexpected feature of these channels, depending on their activity. In cerebral arterial myocytes, they are of two types, i.e. exhibiting low or high persistent activity, and both are activated by PKC/PKA [18]. Highly elevated PKA-dependent calcium sparklets are observed in cerebral arterial smooth muscle during acute hyperglycemia, vascular dysfunction and diabetes.

3.2. Calcium sparks

The cluster of RyRs that faces the sparklet senses the calcium ions, leading to the synchronous opening of the entire cluster of RyRs and to a highly localised release of calcium out of the SR into the membrane cleft [19]. These elementary signals are thought to sum up to underlie global calcium transients to initiate contraction in the heart. Calcium sparks are usually characterised by the amplitude, the duration, the rise time, the spatial spread of the Ca^{2+} signal, and by the frequency (of occurrence of events) of individual spark sites or the frequency of all the sparks within one cell [20]. In smooth muscles, sparks can occur spontaneously or after activation by many factors such as caffeine, calcium entry via L-type calcium channels, increase in SR calcium content and stretch (see for details: [21]). It should be noted that calcium sparks are the only small physiologically relevant elementary calcium release events in cellular calcium signalling and that the corresponding homogeneous calcium release remains unexplained. However, calcium sparks have been shown to be a major signalling pathway for excitation-contraction coupling.

3.3. Calcium quarks

There is yet another phenomenon in which highly localized SR calcium release in both skeletal and cardiac muscles may be triggered under certain conditions, i.e. calcium quarks [22]. By using two-photon photolytic activation of localized calcium release in cardiac myocytes, it has been shown that a single calcium spark actually consists of the summation of unitary calcium events characterised by tiny amplitudes, a very short lifetime and tight spatial confinement. Hence, each individual RyR could give rise to calcium releases in the dyadic cleft called calcium quarks [22]. However, this phenomenon remains unclear (see also: [23]).

3.4. Calcium puffs

Local intracellular calcium signals caused by the opening of clustered InsP3 receptors in the ER or SR membrane are known as calcium puffs [8, 24]. Calcium puffs display a longer lifetime in comparison to calcium sparks and a wider spread. Each calcium puff would be composed of elementary events named calcium blips [25]. Calcium puff events have been characterised in neurones [26] and in smooth muscle cells [27].

3.5. Calcium marks, scraps and blinks

Microdomain miniature calcium transients in single mitochondria are termed "marks" and SR luminal calcium depletion transients are termed "scraps".

In the cardiac cell line H9C2, it has been shown that subsequent to calcium spark events, rapid increases in calcium in the neighbouring mitochondria can occur [28]. It was proposed that calcium marks are triggered by RyRs through a process involving the travel of calcium from spark sites into the mitochondria.

Attempts were made to monitor calcium movements within the SR lumen, which led to the observation of local depletions of the intracellular calcium concentration (“scraps”), mirrors of the calcium transients recorded in the cytosol [29]. Likewise, rapid and substantial depletions of calcium from the nanometer-sized luminal stores in heart cells have been shown and called "calcium blinks", blinks meaning the confined SR depletions from single calcium sparks [30, 31]. Therefore, the visualization of these local store calcium signals can be an important key to understand cardiac physiopathology.

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3.6. Calcium waves

Calcium waves are global increases of the intracellular calcium concentration from the SR or ER that propagate throughout the cell, sustained by the phenomenon of CICR. These events have a longer lifetime and a larger amplitude than the elementary events previously described and can appear in several forms (spirals, U- and V-shapes and circular waves). The analysis of their kinetics gives indications about the activity of proteins involved in buffering systems such as SR/ER calcium ATPase (SERCA) pumps [32].

All these different types of calcium signals modulate various physiological functions specific to particular cell types.

[IV] CALCIUM HANDLING MECHANISMS

The common method to detect changes in the intracellular calcium concentration is to use fluorescent calcium probes (Fura-2, Fluo-3, Fluo-4, Indo-1, Calcium Green, Rhod-2, or Oregon Green 488 BAPTA-1, this list being obviously not exhaustive) and to monitor the corresponding signals over time. However, one has to bear in mind that such techniques only enable the investigator to detect free calcium ions, i.e. calcium which is not bound to proteins, whereas most of the calcium ions in the cell are bound. The role of such calcium-binding proteins is not only important for creating a buffering system, but also for interpreting calcium signals and triggering molecular responses accordingly. Indeed, it seems difficult to relate the individual, short and sometimes highly localized increases of calcium concentration to other signalling processes such as phosphorylation events, which one assumes would be more global and of longer onset/duration.

Recently, a nice study has provided the beginning of an answer to that question. The authors expressed the calcium-dependent protein kinase C (PKC) isoform alpha fused to various fluorescent proteins in HEK293 and COS1 cells. By confocal imaging, they could monitor the PKC translocation events from the cytosol to the plasma membrane. They successfully demonstrated for the first time the relationship between global and local calcium signals on the one hand, and global and local PKC translocation events on the other hand. It should be noted that they evidenced that calcium signals and PKC translocations were concomitant [33].

Therefore, PKCalpha is a cellular "calcium sensor" following the spatio-temporal characteristics of each calcium signal. Whether these PKC translocation events are associated with phosphorylation events is extremely likely but still needs to be investigated.

Calcium fluctuations are sensed by a wide array of "calcium sensors" which have one common feature: they are all endowed with calcium-binding sites, such as in calcium-sensitive enzymes, with different affinities for calcium, and the binding of the latter modifies their activity by changing their conformation, which therefore triggers or discontinues diverse biochemical processes, which in turn control various cellular reactions [34]. The duration and kinetics of intracellular calcium fluctuations control the timing of calcium-binding/unbinding to the "calcium sensors", thus forming the basis for the temporal coding of calcium signalling events [35]. There are a few main intracellular compartments intimately involved in creating, encoding and decoding calcium signals, which are the cytoplasm, the Golgi complex, lysosomes, secretory granules, the nucleus, the endoplasmic reticulum and the mitochondria [36]. Furthermore, the formation of intracellular free calcium fluctuations is controlled by calcium channels and transporters, which, being activated by physiological stimulations, precisely regulate the calcium ion concentration [35].

[V] CALCIUM CLEARANCE MECHANISMS

Calcium clearance mechanisms are important to maintain calcium homeostasis within a range of concentrations that allow biochemical reactions to take place but are not toxic to cellular functions [37]. Indeed calcium removal (or calcium clearance) is even decisive for the fate of the cell, which includes the "cell death" process when the cell has lost the capacity to extrude or reuptake the extra amount of calcium [38, 39]. The mechanisms involved in intracellular calcium buffering and restoration to basal levels include calcium pumps in both intracellular compartments and the plasma membrane, mitochondrial calcium uptake, plasma membrane sodium/calcium exchange and calcium-binding proteins in the cytosol **[Figure-1]**. Depending on the cell type, the mechanisms of calcium homeostasis and the implications of each of the clearance systems mentioned above will be different.

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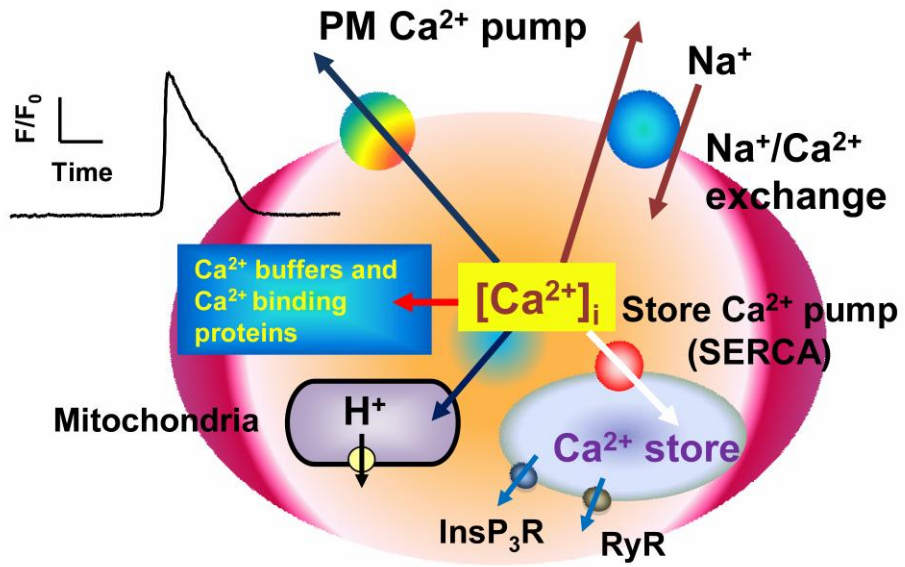

Fig: 1. Schematic representation of possible calcium clearance mechanisms in neuronal, muscle and non-excitable cells: Arrows suggest that increased cytosolic calcium can be extruded *via* plasma membrane (PM) Ca^{2+} pump, Na^{+}/ Ca^{2+} exchange, stored by mitochondria, intracellular compartments (*via* sarco/endoplasmic reticulum Ca^{2+} pump, SERCA), Ca^{2+} buffers and Ca^{2+} binding proteins. Abbreviations: ryanodine receptor (RyR), inositol-trisphosphate receptor ($InsP_3R$), intracellular calcium concentration ($[Ca^{2+}]_i$) and normalized change of the calcium-dependent fluorescence (F/F_0). A representative $[Ca^{2+}]_i$ transient (recorded with a fluorescent calcium indicator) is depicted in the top left corner of the cartoon.

[VI] CONCLUSION

Calcium is the key to a wide variety of cellular processes. Monitoring and deciphering calcium signals can be a useful tool to predict how cells are likely to behave. Besides the understanding of essential physiological phenomena, the exploration of the readout of calcium events by calcium detectors (fluorescent or endogenous calcium-binding proteins) could have applications in detecting pathophysiological remodelling, arrhythmic cardiac patterns and neurological disorders such as Alzheimer's disease [40]. Once typical calcium signal patterns can be identified and defined, this will be useful in developing drug screening strategies and drug therapies.

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