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The Silence of the Cell

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A Brief History of Cell Calcium

Some of the most abundant metals in Earth´s crust as iron, magnesium and calcium have significant roles in the regulation of cellular processes [1,2]. Biochemically, these metals are electrophiles capable of modifying the electron ion flow in biomolecules, by binding and orienting them [3]. One of the most versatile metals in cell physiology is the calcium ion (Ca^{2+}). Nowadays, many fundamental functions of the cell are Ca^{2+} dependent. Paradoxically, early life emerged in a scant free Ca^{2+} environment (~100 nM) because the cation was maintained as insoluble salt by the high seawater pH. In these conditions, photosynthesis was developed by autotrophs releasing molecular oxygen (O_2) from splitting water. Because O_2 easily permeate through cell lipid bilayers generating many toxic effects, the incorporation of this molecule in cell metabolism through cellular respiration reduced its harmful effects, but carbon dioxide (CO_2) was produced as a waste product. The rich CO_2 environment generated by cellular respiration induced a slight acidification of seawater that progressively releases Ca^{2+}. In contrast to O_2, the fatty inner core of the lipid bilayers prevented large Ca^{2+} influxes, but some Ca^{2+} permeation occurred through imperfect junctions between phospholipid domains and ionic channels [4,5]. In early life, Mg^{2+} controlled the cellular physiology, but when Ca^{2+} arise in the environment, many Mg^{2+} interacting proteins begin to be Ca^{2+} interacting proteins, even when cytosolic Mg^{2+} concentration is various orders of magnitude higher than Ca^{2+}. These ions have similar biochemical properties, but Ca^{2+} can coordinate many bonds than Mg^{2+}, and the variable hydration of Ca^{2+} allows to the ion to react faster than Mg^{2+} [2]. Then, the triggering action of Ca^{2+} in cell signaling is particularly valuable. Nevertheless, Ca^{2+} overload is potentially harmful to the cell since destabilize cytoskeleton, activate hydrolytic enzymes and cause damage in membrane lipid bilayers [2,6-8]. The key for cell survival in a Ca^{2+} rich environment was to establish a well-organized regulation of Ca^{2+} cytosolic concentrations ($[Ca^{2+}]c$). In this concern, cells have developed a noteworthy complex machinery of Ca^{2+} binding proteins, transporters that control the membrane Ca^{2+} flow, and a highly specialized Ca^{2+} compartmentalization system. In addition, the development of a cellular code for Ca^{2+} signaling composed by Ca^{2+} transient events that take place in microdomains or globally was crucial to prevent the cell Ca^{2+} overload.

Ca^{2+} binding proteins

Key molecules in the regulation of cellular Ca^{2+} are the Ca^{2+} binding proteins. The interaction of Ca^{2+} with specific binding site in proteins can cause coordinate bonds with a great variability in number, angle and distances, and Ca^{2+} rapidly exchange its hydration degree allowing deeper protein sites binding [2,9]. Although Ca^{2+}-binding proteins have a great structural diversity, it is commonly observed in these proteins a helix-loop-helix structural domain named EF-hand. The EF-hand displays many conformational states that operate as a multifunctional domain with different sensitivity to Ca^{2+} [10,11].

Other Ca^{2+}-binding domain in proteins is C_2, a concave hole in which multiple Ca^{2+} ions binds occur. The C_2 domain proteins are involved in the binding of membrane phospholipids in a Ca^{2+}-dependent manner and thereby act as cellular Ca^{2+} effectors [12]. It is common to find the EF-hand and C_2 domains in the same protein [1].

The extracellular and cytosolic Ca^{2+} environments

The large electrochemical gradient of Ca^{2+} across the plasma membrane generates two different environments inside and outside the cell. The extracellular Ca^{2+} concentration in marine animals, as the squid, is around 10 mM, while in mammals cells are maintained in the range of 2.5 to 5 mM. Noteworthy, free basal $[Ca^{2+}]c$ in all cells is maintained in nanomolar range [1]. Since bacteria to vertebrates, resting $[Ca^{2+}]c$ are around 100 to 300 nM [1,4,13]. This resting $[Ca^{2+}]c$ reduces the possibility of multiple Ca^{2+} binds in a protein. In this sense, it is known that the bind of a single Ca^{2+} ion causes little or no change in the protein conformation, but multiple Ca^{2+} binding is essential to induce protein structural changes [1,14].

Extracellular Ca^{2+} levels are almost stable in comparison with cytosolic. $[Ca^{2+}]c$ increment takes place under specific signaling and arises from two sources, the extracellular environment and the intracellular Ca^{2+} stores. The extracellular environment is an infinite source for Ca^{2+} that is accessible through various selective and non selective plasma membrane Ca^{2+} channels. The intracellular Ca^{2+} stores represent a finite source of Ca^{2+}. The most important Ca^{2+} store is the endoplasmic reticulum (or sarcoplasmic reticulum in muscles), although almost all cell organelles can store Ca^{2+} [15]. Ca^{2+}-release from sarco-endoplasmic reticulum occurs via activation of inositol 1,4,5-trisphosphate (IP_3) or by Ca^{2+}-induced-Ca^{2+}-release (CICR) receptor-channels. IP_3 is a second messenger produced in response of plasma membrane receptor activation. Ca^{2+} release from sarco-endoplasmic reticulum can be induced by cytosolic Ca^{2+} increments or cyclic ADP ribose through CICR sensitive channels [16].

Two systems remove Ca^{2+} from cytoplasm, the Ca^{2+} pumps that have high affinity, but low capacity, and the and the Na^+/Ca^{2+} exchanger that has lower affinity but much larger capacity [17]. Ca^{2+} pumps are located in both plasma membranes (PMCA) and sarco-endoplasmic reticulum (SERCA) and are known as the "fine-tuner" of cytosolic Ca^{2+} concentration [17,18]. It has proposed that SERCA pumps are involved in signal transduction while PMCA pumps are crucial in cell survival [19].

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Cell Ca^{2+} regulation

It has been proposed that the sarcoplasmic reticulum is a superficial buffer barrier that regulates Ca^{2+} spread between myoplasm and the extracellular environment [20]. In airways smooth muscle we observed that the buffer properties of sarcoplasmic reticulum act also in an inverse way, that is, sarcoplasmic reticulum can buffer the Ca^{2+} exit from the cell [21].

Cytosolic Ca^{2+} regulation is fundamental for cell signaling. This regulation is achieved by several mechanisms, including Ca^{2+} channels distribution in the plasma membrane, sarco-endoplasmic reticulum and other Ca^{2+} stores as mitochondria or Golgi [15]. In general, when a channel is open, a concentrated plume of Ca^{2+} is formed around the channel mouth and dissipates rapidly by diffusion [22]. If ryanodine Ca^{2+} channels are sequentially reached by the concentrated Ca^{2+} plume, this initially local transient can be propagated as a wave until global cellular signal occurs. These waves adopt different shapes according to their emission frequency, wavelength and velocity, and they can appear as solitary, target or spiral waves [23].

Ca^{2+} signaling can be regulated also by the periodicity of Ca^{2+} transients. Some cellular functions only needs a single or few transient Ca^{2+} signals, as exocytosis and contraction, but others, as cellular proliferation, requires prolonged Ca^{2+} signals generated by repetitive Ca^{2+} transients. Then, Ca^{2+} transients are essentially the cellular universal code of Ca^{2+} signalization. Ca^{2+} dispersion restriction can create temporal and spatial limited domains and functionally, these domains have a vital role avoiding unspecific or global Ca^{2+}-induce signals [24]. In this context, an additional message could be added to Ca^{2+} code, the $[Ca^{2+}]c$ undershoot.

$[Ca^{2+}]c$ undershoot

Many excitable and non excitable cells show a fast$[Ca^{2+}]c$ drop below basal $[Ca^{2+}]c$ after the withdrawal of agonists that induced the mobilization of Ca^{2+} from sarco-endoplasmic reticulum [12,25-30] electrical stimulation [31,32], spontaneous Ca^{2+} transients [33] or during Ca^{2+} oscillations [34] (Figure 1). This phenomenon known as $[Ca^{2+}]c$ undershoot has been observed in muscles [31-37], neurons [26,27,38-40], microglia [41,42], oligodendrocytes [29], spermatozoa [28,43] and chromaffin [44] and prostate stromal [45] cells. It has been widely demonstrated that the $[Ca^{2+}]c$ drop during the undershoot is induced by the refilling of Ca^{2+} in sarco-endoplasmic reticulum by SERCA [25-27]; while the undershoot recovery is produced by extracellular Ca^{2+} entry through voltage Ca^{2+} channels [30] or capacitative Ca^{2+} entry [36]. Recently, it has been observed that $[Ca^{2+}]c$ undershoot alterations induced by pharmacological treatments are associated to changes in sarcoplasmic reticulum Ca^{2+} loading mechanisms [35,36].

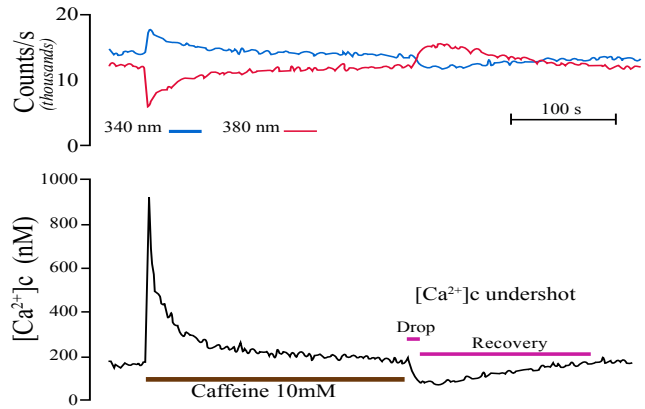

Figure 1: Typical recording of a $[Ca^{2+}]c$ undershoot induce by caffeine removal in an isolated smooth muscle cell from bovine trachea. Upper traces display fluorescence recordings at 340 and 380 nm and lower trace shows $[Ca^{2+}]c$.

Role of $[Ca^{2+}]c$ undershoot in cell physiology: The silence of the cell

Sarco-endoplasmic reticulum is a key controller of $[Ca^{2+}]c$ that can potentiate or attenuate cellular responses [21,27]. Ca^{2+} uptake by sarco-endoplasmic reticulum during undershoot has a powerful effect by reducing abruptly the $[Ca^{2+}]c$. The transient reduction of $[Ca^{2+}]c$ after Ca^{2+} stimulation diminished the possibility of activation of Ca^{2+}-binding proteins, process that probably silence some signaling cascades and avoid an overload of Ca^{2+}.

Other consequence of Ca^{2+} undershoot is the modification of the spatiotemporal distribution of Ca^{2+}. For example, the silences between phasic $[Ca^{2+}]c$ rises during caffeine-induced oscillations in neurons are attributed to Ca^{2+} undershoot [40]. In addition, in seizure-like events in hippocampal slice cultures, it has been proposed that the $[Ca^{2+}]c$ undershoot could be involved postictal depression period [39]. Then, it is possible that undershoot of $[Ca^{2+}]c$ is involved in the synchronization and rhythmic cell activity.

In conclusion, the deficit of intracellular Ca^{2+} produced by the refilling of sarco-endoplasmic reticulum during undershoot could be crucial to the triggering role of Ca^{2+} in cellular activity. This, Ca^{2+} "silences" could be a mechanism that regulates the cell excitability restricting transiently Ca^{2+} signaling.

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