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Minireview

Ca^{2+}-storage organelles

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Intracellular Ca^{2+}-storage organelles are found in virtually all eukaryotic cells. They play an important role in the regulation of the cytosolic free Ca^{2+} concentration and, thereby, in the regulation of cellular activity. Ca^{2+}-storage organelles consist, in the simplest model of a Ca^{2+} pump, of a Ca^{2+}-storage protein and a Ca^{2+}-release channel. The primary structure of these functionally important proteins of Ca^{2+}-storage organelles is similar in different cell types and conserved through evolution. In contrast, their spatial arrangement and, thus, the architecture of Ca^{2+}-storage organelles may vary dramatically from one cell type to another.

Ca^{2+} pump; Ca^{2+} release channel; Ca^{2+} storage protein; Sarcoplasmic reticulum; Calsiosome; Endoplasmic reticulum

1. INTRODUCTION

Virtually all eukaryotic cells possess intracellular Ca^{2+} stores. Together with the plasma membrane, these Ca^{2+} stores play a crucial role in the regulation of the cytosolic free Ca^{2+} concentration, $[Ca^{2+}]_i$. In the resting cell, they lower $[Ca^{2+}]_i$ to values of around 100 nM; in the activated cell they rapidly release Ca^{2+} and raise $[Ca^{2+}]_i$ to micromolar levels. These changes of $[Ca^{2+}]_i$, albeit small compared to the millimolar extracellular Ca^{2+} concentration, are sufficient to activate or inactivate a variety of intracellular processes and to influence thereby virtually every aspect of the physiology of a cell (for recent reviews see [1,2]).

Given this central role of Ca^{2+} in cell regulation, it is not astonishing that the primary structure of functionally important proteins of Ca^{2+} stores is conserved through evolution. For example, the Ca^{2+} storage protein calreticulin, as cloned from rat skeletal muscle, shares a high degree of homology with a Ca^{2+}-binding protein from human lymphocytes, but also with proteins from the insect *Drosophila* and the parasite *Onchocerca volvulus* [3]. Similarly, the intracellular Ca^{2+}-pump protein of yeast shows significant similarities with the primary structure of its mammalian counterpart [4].

However, given the extreme variety of cell types whose activity is regulated by Ca^{2+}-storage organelles (from neutrophils to muscle fibers) and the variety of cellular functions that are regulated by Ca^{2+} (from phago-lysosome fusion to fiber contraction), it is also not astonishing that the functional properties and the spatial organization of Ca^{2+}-storage organelles seem to vary dramatically between different cell types.

This review will therefore consist of two parts. I will first summarize our present knowledge on functionally important proteins of Ca^{2+} stores and will then discuss the putative heterogeneity of Ca^{2+}-storage organelles. In this short review, many important aspects had to be omitted and the interested reader should also consult other recent reviews on this subject [5–7].

2. FUNCTIONALLY IMPORTANT PROTEINS OF Ca^{2+}-STORAGE ORGANELLES

A Ca^{2+}-storage organelle consists, in the simplest model, of a Ca^{2+} pump, a Ca^{2+}-storage protein and a Ca^{2+}-release channel.

2.1. *Ca^{2+} pumps*

Tissue-specific expression of three intracellular Ca^{2+}-pump proteins has been demonstrated: the type I or fast-type Ca^{2+}-ATPase is found in fast-twitch skeletal muscle; the type II or slow-type Ca^{2+}-ATPase is found in slow-twitch skeletal and cardiac muscle; and a non-muscle form of the slow-type Ca^{2+}-ATPase is found in non-muscle cells [8,9].

All three proteins are approximately 100 kDa Ca^{2+}-ATPases that can form a phosphorylated intermediate in a Ca^{2+}-dependent manner (e.g. [10,11]). They are structurally distinct from the plasma membrane Ca^{2+}-ATPase [12]. While a separate gene codes

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for the fast-type Ca^{2+}-ATPase, the slow-type and the non-muscle slow-type Ca^{2+}-ATPases are generated by alternative splicing of the same gene [8,9]. So far, expression of only one type of Ca^{2+}-ATPase per cell type could be found. However, functional studies in non-muscle cells suggest that certain cell types might have at least 2 types of Ca^{2+}-pump mechanisms (e.g. [13]). Therefore, other types of intracellular Ca^{2+} pumps have been proposed. A 140 kDa protein that crossreacts with monoclonal antibodies raised against fast-type Ca^{2+}-ATPase has been suggested to be the Ca^{2+}-pump of the Ins(1,4,5)P_3-sensitive Ca^{2+} pool in adrenal chromaffin cells [14]. In pancreatic acinar cells, Ca^{2+} uptake into the InsP_3-sensitive Ca^{2+} pool has been proposed to occur through a mechanism that is insensitive to vanadate, but sensitive to the proton ionophore nigericin [13]. However, no data on the structure of these putative Ca^{2+} pump proteins is available.

2.2. *Ca^{2+}-storage proteins*

In order to function as intraluminal Ca^{2+} buffer, Ca^{2+}-storage proteins of intracellular Ca^{2+} stores should bind Ca^{2+} with a high capacity, but low affinity. In mammalian tissues, at least four intracellular proteins with these properties have been described: skeletal muscle calsequestrin [15], cardiac calsequestrin [16], calreticulin [17,18] and endoplasmin/grp94/ERp99 (presumably endoplasmin, grp 94, and ERp99 are identical proteins, however, no unifying terminology exists; see [19]). Each of these putative Ca^{2+}-storage proteins shows a distinct tissue distribution. Cardiac and skeletal muscle calsequestrins are found mainly in cardiac and skeletal muscle sarcoplasmic reticulum. In addition, proteins closely related to calsequestrins seem also to be expressed in smooth muscle and chicken cerebellum [20,21]. Calreticulin is found in virtually all cell types [17]. In subcellular fractionation studies in some non-muscle cells, it co-purifies with Ins(1,4,5)P_3-sensitive Ca^{2+} stores [22]. Endoplasmin/grp94/ERp99 is found in a variety of non-muscle cells [19]. Although a role in Ca^{2+} storage by intracellular organelles has been proposed for all four proteins, such a role has never been experimentally proven.

All four putative Ca^{2+}-storage proteins have been cloned. Skeletal muscle and cardiac calsequestrins, although products of different genes, show 65% sequence similarity [16]. Skeletal muscle calsequestrin and calreticulin contain two short stretches with similar sequence [18]. No sequence similarity of endoplasmin/grp94/erp99 with calsequestrins has been described. Calreticulin and endoplasmin/grp94/erp99 share the C-terminal Lys-Asp-Glu-Leu sequence, usually referred to in the one letter code as KDEL sequence. However, this sequence is found in many non-secreted proteins [23] and cannot be considered as an indication of sequence homology.

The most obvious role of Ca^{2+}-storage proteins is to act as Ca^{2+} buffer in the lumen of Ca^{2+}-storage organelles. They thereby reduce the lumen/cytosol Ca^{2+} gradient and diminish the risk of Ca^{2+} precipitations due to extensively high intraluminal Ca^{2+} concentrations. However, Ca^{2+}-storage proteins may not only be passive Ca^{2+} buffers but also be actively involved in the regulation of the Ca^{2+} release. Indeed, cardiac calsequestrin interacts Ca^{2+}-dependently with sarcoplasmic reticulum membrane proteins [24]. Interestingly, Ca^{2+} binding to calsequestrin is driven exclusively by entropy gain, a feature typically observed with proteins that act as Ca^{2+} vectors in signal transduction rather than as simple Ca^{2+} buffers [25].

No data in favour of an active role of non-muscle Ca^{2+}-storage proteins in signal transduction are available. However, it has been postulated that in non-muscle cells the filling state of the intracellular Ca^{2+} store might (i) affect its Ca^{2+}-release properties [26] and (ii) regulate Ca^{2+} influx across the plasma membrane [27]. Such a role of the filling state of the intracellular Ca^{2+} pool is only conceivable if there is an intraluminal Ca^{2+} sensor. Both calreticulin and endoplasmin/grp94/ER99 would be ideal candidates for such a function.

2.3. *Ca^{2+}-release channels*

Ca^{2+}-release channels must be able to release Ca^{2+} during cellular activation. Thus, in addition to a Ca^{2+} channel portion, they need a sensor that recognizes a cellular activation signal. In the case of many non-muscle cells, this activation signal is inositol(1,4,5) trisphosphate, Ins(1,4,5)P_3, which is generated in response to cell-surface activation by a variety of agonists [28]. Indeed, an Ins(1,4,5)P_3-sensitive Ca^{2+}-release channel, usually referred to as the Ins(1,4,5)P_3 receptor, is widely distributed among non-muscle cells [29].

In muscle cells, another Ca^{2+}-release channel is found, the ryanodine receptor [30]. This channel becomes Ca^{2+}-conductive in response to ryanodine, caffeine and Ca^{2+}, however, the physiological activation signal for this channel is still a matter of debate and might depend on the cell type. Recent studies show that a ryanodine receptor is also found in certain non-muscle cells [31].

Molecular cloning of the Ins(1,4,5)P_3 receptor showed a neuronal and a non-neuronal form of the protein, derived by alternative splicing [32]. The comparison of the primary structure of the ryanodine receptor and Ins(1,4,5)P_3 receptor revealed some areas of high homology between the two proteins. Thus, there seems to be a genetically related family of intracellular Ca^{2+} release channels.

3. HETEROGENEITY OF Ca^{2+} STORES

The above described proteins, Ca^{2+} pumps,

Ca^{2+}-storage proteins and Ca^{2+}-release channels, are the basic construction elements of a cell for the assembly of Ca^{2+}-storage organelles. However, does the relative simplicity of their construction elements lead to a uniform structure of Ca^{2+} stores? Indeed, until some years ago, the well-known role of the sarcoplasmic reticulum as a Ca^{2+} store of muscle cells was thought to be uniformly assumed by its presumed counterpart, the endoplasmic reticulum (ER), in non-muscle cells. Subcellular fractionation, as well as morphological studies in some cellular systems, were in favour of this hypothesis (for review see [33]). However, some old and many new results suggest that this hypothesis is too simplistic.

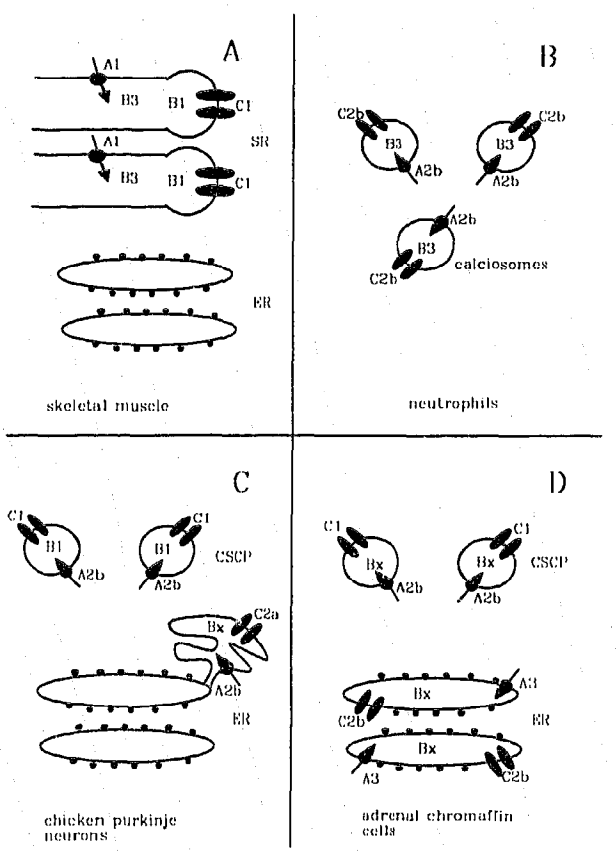

Fig. 1. Models of Ca^{2+}-storage organelles and their relationship with the endoplasmic reticulum in various cellular systems. Proteins of Ca^{2+}-storage organelles are coded as follows (see also text): fast-type Ca^{2+}-ATPase = A1; non-muscle slow-type Ca^{2+}-ATPase = A2b; putative 140 kDa Ca^{2+} pump of adrenal chromaffine cells = A3; skeletal muscle calsequestrin = B1; calreticulin = B3; unknown = Bx; Ryanodine receptor = C1; $Ins(1,4,5)P_3$ receptor, neuronal form = C2a; $Ins(1,4,5)P_3$ receptor, non-neuronal form = C2b. The model depicted in panel A shows the generally accepted model of the skeletal muscle sarcoplasmic reticulum. The other panels are partially speculative adaptations of experimental data in non-muscle cells. Neutrophils (panel B, [36]) are devoid of endoplasmic reticulum and contain only one type of $Ins(1,4,5)P_3$-sensitive Ca^{2+} store (= calciosomes). In chicken Purkinje neurons (panel C, [42]) smooth-surfaced cisternae of the endoplasmic reticulum might serve as $Ins(1,4,5)P_3$-sensitive Ca^{2+} stores and a calsequestrin containing compartment as Ca^{2+}/caffein-sensitive Ca^{2+} pool (CSCP). In adrenal chromaffin cells (panel D, [14]), the entire endoplasmic reticulum, endowed with a putative 140 kDa Ca^{2+} pump might serve as $Ins(1,4,5)P_3$-sensitive Ca^{2+} store, while a separate organelle, endowed with the non-muscle slow-type Ca^{2+}-ATPase might serve as Ca^{2+}/caffein-sensitive Ca^{2+} pool (CSCP).

(i) The sarcoplasmic reticulum is not a muscle analogue of the ER, but rather a specialized intracellular organelle containing a highly specific subset of proteins. The sarcoplasmic reticulum is probably formed by 'budding off' from the ER [34].

(ii) Some non-muscle cells with entirely normal intracellular Ca^{2+} stores, e.g. neutrophils and platelets, synthesize only a few proteins and accordingly contain very little ER. In particular, mature neutrophils are virtually devoid of ER [35,36]. Thus, even if their Ca^{2+} stores stem from the ER, they must somehow be able to lose selectively the protein synthesizing part of the ER without losing the Ca^{2+}-storing part. Not surprisingly, studies in these ER-poor cell types have always identified structures other than the ER as Ca^{2+}-storage sites (dense tubular system in platelets and calciosomes in neutrophils) [36–38].

(iii) Many recent subcellular fractionation studies in various cell types found no correlation between the distribution of ER markers and $Ins(1,4,5)P_3$-induced Ca^{2+} release or $Ins(1,4,5)P_3$ binding (for review see [5,6]). Again, this does not exclude a role of the ER in Ca^{2+} storage, as the ER breaks during homogenization and a subfraction of the ER, involved in Ca^{2+} homeostasis, might have been purified. However, the latter results are not compatible with the whole ER being the Ca^{2+} store in these cell types.

(iv) Functional studies in various cell types suggest the existence of various intracellular Ca^{2+} stores: (a) $Ins(1,4,5)P_3$-sensitive Ca^{2+} stores, i.e. Ca^{2+} stores that release Ca^{2+} in response to $Ins(1,4,5)P_3$, presumably via the $Ins(1,4,5)P_3$ receptor; (b) Ca^{2+}/caffeine-sensitive Ca^{2+} stores, i.e. Ca^{2+} stores that release Ca^{2+} in response to $[Ca^{2+}]_i$ elevations and to caffeine, presumably via the ryanodine receptor; (c) $Ins(1,4,5)P_3$-insensitive and Ca^{2+}-insensitive Ca^{2+} stores. No physiological release mechanism for this type of Ca^{2+} store is known. In many cases these stores seem to be physically separated, as $Ins(1,4,5)P_3$-induced Ca^{2+} release does not affect the Ca^{2+} content of the Ca^{2+}-sensitive Ca^{2+} store and vice versa. The idea that all of these stores are contained within the ER is difficult to reconcile with the concept that the ER is a continuous endomembrane system.

(v) Morphological studies on the subcellular distribution of the non-muscle 100 kDa Ca^{2+}-ATPase in various cellular systems show a pattern clearly distinct from the ER [14,39].

(vi) Morphological studies on the subcellular distribution of calreticulin in various cellular systems show an ER pattern in some cell types [7,20] and a pattern different from the ER in other cell types [40].

(vii) Morphological studies on the subcellular distribution of the Ins(1,4,5)P_3 receptor in Purkinje neurons show this protein highly concentrated in smooth-surfaced cisternae of the ER, but not in the rough ER [41].

It is impossible at this point to propose one unifying hypothesis that explains the contradictory observations in different cell types. However, one might consider that the seemingly conflicting results are due to a 'customized' assembly of the basic construction elements of Ca^{2+}-storage organelles in different cell types. Although there is no proof for such an extreme individualism of cells with respect to Ca^{2+}-storage organelles, this possibility deserves attention. In order to illustrate this putative heterogeneity, I will assign codes to the basic construction elements of Ca^{2+}-storage organelles:

(A) Ca^{2+} pumps: fast-type Ca^{2+}-ATPase = A1; slow-type Ca^{2+}-ATPase = A2a; non-muscle slow-type Ca^{2+}-ATPase = A2b; putative 140 kDa Ca^{2+} pump of adrenal chromaffine cells = A3; unknown = Ax

(B) Ca^{2+}-storage proteins: skeletal muscle calsequestrin = B1; cardiac calsequestrin = B2; calreticulin = B3; endoplasmin/grp94/ERp 99 = B4; unknown = Bx

(C) Ca^{2+}-release channels: Ryanodine receptor = C1 ; Ins(1,4,5)P_3 receptor, neuronal form = C2a; Ins(1,4,5)P_3 receptor, non-neuronal form = C2b; unknown = Cx

In addition, I will assign a code for the relationship of the Ca^{2+}-storage organelle with the ER.

(E) Relationship ER/Ca^{2+} store: identical with the rough ER = E1; specialized portion of the smooth ER = E2; not part of the ER = E3; relationship with the ER not known = Ex

On the basis of this code, the fast-twitch skeletal muscle sarcoplasmic reticulum would be an A1,B1,B3,C1,E3 Ca^{2+} store. Calciosomes as described in neutrophils and related myeloid cells [36] would be A2b,B3,C2b,E3 Ca^{2+} stores. The two Ca^{2+} stores proposed in adrenal chromaffin cells [14] would be A3,Bx,C2b,E1 (Ins(1,4,5)P_3-sensitive Ca^{2+} store) and A2b,Bx,C1,E3 (Ca^{2+}/caffein-sensitive Ca^{2+} store). The potential spatial organization of some of these Ca^{2+} stores is schematically represented in Fig. 1. (The possibility of a differential spatial organization of Ca^{2+}-uptake sites and Ca^{2+}-release sites, as for example seen in sarcoplasmic reticulum, is not taken into account (see [6]). In summary, despite many new insights into the molecular mechanisms of Ca^{2+} homeostasis, our knowledge of the structure of Ca^{2+}-storage organelles remains limited. The apparently conflicting findings obtained in various cellular systems might indicate tissue-dependent heterogeneity. Future biochemical and morphological studies, using specific antibodies against functionally important proteins of Ca^{2+}-storage organelles, will be necessary for the better understanding of the structure of Ca^{2+}-storage organelles.

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