

## Minireview

 $\text{Ca}^{2+}$ -storage organelles

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

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

## 1. INTRODUCTION

Virtually all eukaryotic cells possess intracellular  $\text{Ca}^{2+}$  stores. Together with the plasma membrane, these  $\text{Ca}^{2+}$  stores play a crucial role in the regulation of the cytosolic free  $\text{Ca}^{2+}$  concentration,  $[\text{Ca}^{2+}]_i$ . In the resting cell, they lower  $[\text{Ca}^{2+}]_i$  to values of around 100 nM; in the activated cell they rapidly release  $\text{Ca}^{2+}$  and raise  $[\text{Ca}^{2+}]_i$  to micromolar levels. These changes of  $[\text{Ca}^{2+}]_i$ , albeit small compared to the millimolar extracellular  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  in cell regulation, it is not astonishing that the primary structure of functionally important proteins of  $\text{Ca}^{2+}$  stores is conserved through evolution. For example, the  $\text{Ca}^{2+}$  storage protein calreticulin, as cloned from rat skeletal muscle, shares a high degree of homology with a  $\text{Ca}^{2+}$ -binding protein from human lymphocytes, but also with proteins from the insect *Drosophila* and the parasite *Onchocerca volvulus* [3]. Similarly, the intracellular  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$ -storage organelles (from neutrophils to muscle fibers) and the variety of

cellular functions that are regulated by  $\text{Ca}^{2+}$  (from phago-lysosome fusion to fiber contraction), it is also not astonishing that the functional properties and the spatial organization of  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$  stores and will then discuss the putative heterogeneity of  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$ -STORAGE ORGANELLES

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

2.1.  $\text{Ca}^{2+}$  pumps

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

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

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for the fast-type  $\text{Ca}^{2+}$ -ATPase, the slow-type and the non-muscle slow-type  $\text{Ca}^{2+}$ -ATPases are generated by alternative splicing of the same gene [8,9]. So far, expression of only one type of  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$ -pump mechanisms (e.g. [13]). Therefore, other types of intracellular  $\text{Ca}^{2+}$  pumps have been proposed. A 140 kDa protein that crossreacts with monoclonal antibodies raised against fast-type  $\text{Ca}^{2+}$ -ATPase has been suggested to be the  $\text{Ca}^{2+}$ -pump of the  $\text{Ins}(1,4,5)\text{P}_3$ -sensitive  $\text{Ca}^{2+}$  pool in adrenal chromaffin cells [14]. In pancreatic acinar cells,  $\text{Ca}^{2+}$  uptake into the  $\text{InsP}_3$ -sensitive  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  pump proteins is available.

## 2.2. $\text{Ca}^{2+}$ -storage proteins

In order to function as intraluminal  $\text{Ca}^{2+}$  buffer,  $\text{Ca}^{2+}$ -storage proteins of intracellular  $\text{Ca}^{2+}$  stores should bind  $\text{Ca}^{2+}$  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 endoplasmic/grp94/ERp99 (presumably endoplasmic, grp 94, and ERp99 are identical proteins, however, no unifying terminology exists; see [19]). Each of these putative  $\text{Ca}^{2+}$ -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  $\text{Ins}(1,4,5)\text{P}_3$ -sensitive  $\text{Ca}^{2+}$  stores [22]. Endoplasmic/grp94/ERp99 is found in a variety of non-muscle cells [19]. Although a role in  $\text{Ca}^{2+}$  storage by intracellular organelles has been proposed for all four proteins, such a role has never been experimentally proven.

All four putative  $\text{Ca}^{2+}$ -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 endoplasmic/grp94/erp99 with calsequestrins has been described. Calreticulin and endoplasmic/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  $\text{Ca}^{2+}$ -storage proteins is to

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

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

## 2.3. $\text{Ca}^{2+}$ -release channels

$\text{Ca}^{2+}$ -release channels must be able to release  $\text{Ca}^{2+}$  during cellular activation. Thus, in addition to a  $\text{Ca}^{2+}$  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,  $\text{Ins}(1,4,5)\text{P}_3$ , which is generated in response to cell-surface activation by a variety of agonists [28]. Indeed, an  $\text{Ins}(1,4,5)\text{P}_3$ -sensitive  $\text{Ca}^{2+}$ -release channel, usually referred to as the  $\text{Ins}(1,4,5)\text{P}_3$  receptor, is widely distributed among non-muscle cells [29].

In muscle cells, another  $\text{Ca}^{2+}$ -release channel is found, the ryanodine receptor [30]. This channel becomes  $\text{Ca}^{2+}$ -conductive in response to ryanodine, caffeine and  $\text{Ca}^{2+}$ , 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  $\text{Ins}(1,4,5)\text{P}_3$  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  $\text{Ins}(1,4,5)\text{P}_3$  receptor revealed some areas of high homology between the two proteins. Thus, there seems to be a genetically related family of intracellular  $\text{Ca}^{2+}$  release channels.

## 3. HETEROGENEITY OF $\text{Ca}^{2+}$ STORES

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

$\text{Ca}^{2+}$ -storage proteins and  $\text{Ca}^{2+}$ -release channels, are the basic construction elements of a cell for the assembly of  $\text{Ca}^{2+}$ -storage organelles. However, does the relative simplicity of their construction elements lead to a uniform structure of  $\text{Ca}^{2+}$  stores? Indeed, until some years ago, the well-known role of the sarcoplasmic reticulum as a  $\text{Ca}^{2+}$  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.

(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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  stores stem from the ER, they must somehow be able to lose selectively the protein synthesizing part of the ER without losing the  $\text{Ca}^{2+}$ -storing part. Not surprisingly, studies in these ER-poor cell types have always identified structures other than the ER as  $\text{Ca}^{2+}$ -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  $\text{Ins}(1,4,5)\text{P}_3$ -induced  $\text{Ca}^{2+}$  release or  $\text{Ins}(1,4,5)\text{P}_3$  binding (for review see [5,6]). Again, this does not exclude a role of the ER in  $\text{Ca}^{2+}$  storage, as the ER breaks during homogenization and a subfraction of the ER, involved in  $\text{Ca}^{2+}$  homeostasis, might have been purified. However, the latter results are not compatible with the whole ER being the  $\text{Ca}^{2+}$  store in these cell types.

(iv) Functional studies in various cell types suggest the existence of various intracellular  $\text{Ca}^{2+}$  stores: (a)  $\text{Ins}(1,4,5)\text{P}_3$ -sensitive  $\text{Ca}^{2+}$  stores, i.e.  $\text{Ca}^{2+}$  stores that release  $\text{Ca}^{2+}$  in response to  $\text{Ins}(1,4,5)\text{P}_3$ , presumably via the  $\text{Ins}(1,4,5)\text{P}_3$  receptor; (b)  $\text{Ca}^{2+}$ /caffeine-sensitive  $\text{Ca}^{2+}$  stores, i.e.  $\text{Ca}^{2+}$  stores that release  $\text{Ca}^{2+}$  in response to  $[\text{Ca}^{2+}]_i$  elevations and to caffeine, presumably via the ryanodine receptor; (c)  $\text{Ins}(1,4,5)\text{P}_3$ -insensitive and  $\text{Ca}^{2+}$ -insensitive  $\text{Ca}^{2+}$  stores. No physiological release mechanism for this type of  $\text{Ca}^{2+}$  store is known. In many cases these stores seem to be physically separated, as  $\text{Ins}(1,4,5)\text{P}_3$ -induced  $\text{Ca}^{2+}$  release does not affect the  $\text{Ca}^{2+}$  content of the  $\text{Ca}^{2+}$ -sensitive  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ -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].

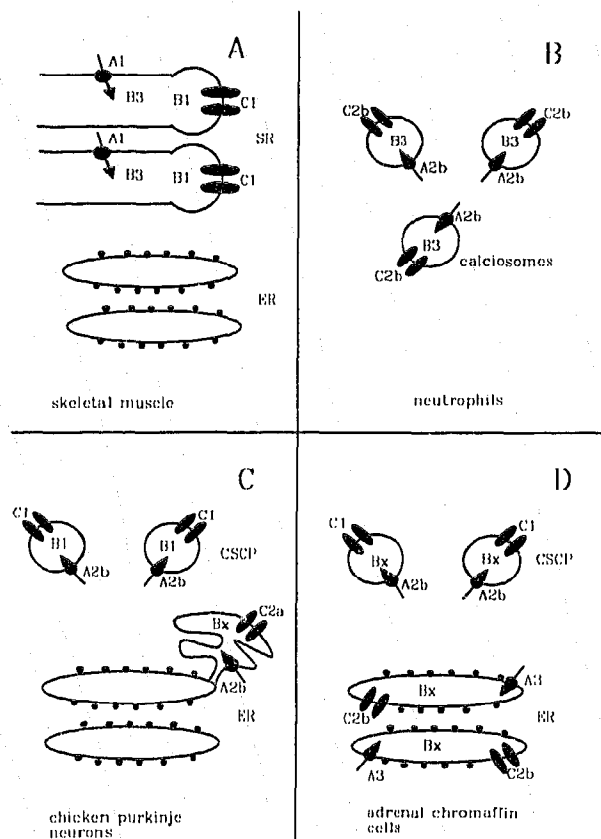


Fig. 1. Models of  $\text{Ca}^{2+}$ -storage organelles and their relationship with the endoplasmic reticulum in various cellular systems. Proteins of  $\text{Ca}^{2+}$ -storage organelles are coded as follows (see also text): fast-type  $\text{Ca}^{2+}$ -ATPase = A1; non-muscle slow-type  $\text{Ca}^{2+}$ -ATPase = A2b; putative 140 kDa  $\text{Ca}^{2+}$  pump of adrenal chromaffin cells = A3; skeletal muscle calsequestrin = B1; calreticulin = B3; unknown = Bx; Ryanodine receptor = C1;  $\text{Ins}(1,4,5)\text{P}_3$  receptor, neuronal form = C2a;  $\text{Ins}(1,4,5)\text{P}_3$  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  $\text{Ins}(1,4,5)\text{P}_3$ -sensitive  $\text{Ca}^{2+}$  store (= calciosomes). In chicken Purkinje neurons (panel C, [42]) smooth-surfaced cisternae of the endoplasmic reticulum might serve as  $\text{Ins}(1,4,5)\text{P}_3$ -sensitive  $\text{Ca}^{2+}$  stores and a calsequestrin containing compartment as  $\text{Ca}^{2+}$ /caffeine-sensitive  $\text{Ca}^{2+}$  pool (CSCP). In adrenal chromaffin cells (panel D, [14]), the entire endoplasmic reticulum, endowed with a putative 140 kDa  $\text{Ca}^{2+}$  pump might serve as  $\text{Ins}(1,4,5)\text{P}_3$ -sensitive  $\text{Ca}^{2+}$  store, while a separate organelle, endowed with the non-muscle slow-type  $\text{Ca}^{2+}$ -ATPase might serve as  $\text{Ca}^{2+}$ /caffeine-sensitive  $\text{Ca}^{2+}$  pool (CSCP).

(vii) Morphological studies on the subcellular distribution of the  $\text{Ins}(1,4,5)\text{P}_3$  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  $\text{Ca}^{2+}$ -storage organelles in different cell types. Although there is no proof for such an extreme individualism of cells with respect to  $\text{Ca}^{2+}$ -storage organelles, this possibility deserves attention. In order to illustrate this putative heterogeneity, I will assign codes to the basic construction elements of  $\text{Ca}^{2+}$ -storage organelles:

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

(B)  $\text{Ca}^{2+}$ -storage proteins: skeletal muscle calsequestrin = B1; cardiac calsequestrin = B2; calreticulin = B3; endoplasmic reticulum protein 94/ERp 99 = B4; unknown = Bx

(C)  $\text{Ca}^{2+}$ -release channels: Ryanodine receptor = C1;  $\text{Ins}(1,4,5)\text{P}_3$  receptor, neuronal form = C2a;  $\text{Ins}(1,4,5)\text{P}_3$  receptor, non-neuronal form = C2b; unknown = Cx

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

(E) Relationship ER/ $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  store. Calciosomes as described in neutrophils and related myeloid cells [36] would be A2b,B3,C2b,E3  $\text{Ca}^{2+}$  stores. The two  $\text{Ca}^{2+}$  stores proposed in adrenal chromaffin cells [14] would be A3,Bx,C2b,E1 ( $\text{Ins}(1,4,5)\text{P}_3$ -sensitive  $\text{Ca}^{2+}$  store) and A2b,Bx,C1,E3 ( $\text{Ca}^{2+}$ /caffeine-sensitive  $\text{Ca}^{2+}$  store). The potential spatial organization of some of these  $\text{Ca}^{2+}$  stores is schematically represented in Fig. 1. (The possibility of a differential spatial organization of  $\text{Ca}^{2+}$ -uptake sites and  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$  homeostasis, our knowledge of the structure of  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$ -storage organelles, will be necessary for the better understanding of the structure of  $\text{Ca}^{2+}$ -storage organelles.

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