JCB Mini-Review

Is all of the endoplasmic reticulum created equal? The effects of the heterogeneous distribution of endoplasmic reticulum Ca^{2+}-handling proteins

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The endoplasmic reticulum is a heterogeneous compartment with respect to the distribution of its Ca^{2+}-handling proteins, namely the Ca^{2+}-binding proteins, the Ca^{2+} pumps and the Ca^{2+} release channels. The nonuniform distribution of these proteins may explain the functional heterogeneity of the endoplasmic reticulum, such as the generation of spatially complex Ca^{2+} signals, Ca^{2+} homeostasis, and protein folding and quality control.

Introduction

In the past, the endoplasmic reticulum was viewed as a single, continuous, and homogeneous compartment with a uniform Ca^{2+} store. However, such a simplistic view does not explain the functional heterogeneity of this complex organelle. In particular, the endoplasmic reticulum must provide a pool of rapidly exchanging Ca^{2+} for signal generation, while concurrently maintaining areas within its lumen with stably high Ca^{2+} levels for proper protein folding and processing (Rooney and Meldolesi, 1996). Today it is widely accepted that although the endoplasmic reticulum is physically continuous (i.e., lumenally connected) (Subramanian and Meyer, 1997), it is spatially and functionally heterogeneous (Villa et al., 1993; Meldolesi and Pozzan, 1998; Baumann and Walz, 2001; Blaustein and Golovina, 2001). This heterogeneity may be established by the nonuniform distribution of endoplasmic reticulum Ca^{2+}-handling proteins: (1) the Ca^{2+}-binding proteins, such as calreticulin, calsequestrin, glucose-regulated protein 78 and 94 (Grp78/BiP and Grp94), protein disulfide isomerase (PDI),* and proteins belonging to the PDI-like family, ERp72, ERp57, and the newly identified ERp29 (Sargsyan et al., 2002); (2) the Ca^{2+} uptake channels, sarcoplasmic/endoplasmic reticulum Ca^{2+}-transporting ATPases (SERCAs), and (3) the Ca^{2+} release channels, inositol 1,4,5-trisphosphate ($InsP_3$) receptors and ryanodine receptors. A nonuniform distribution of these Ca^{2+}-handling proteins necessarily divides the endoplasmic reticulum into subdomains, which extend beyond the classical divisions of rough endoplasmic reticulum, smooth endoplasmic reticulum, and nuclear envelope. Unique accumulations of Ca^{2+}-handling proteins in the endoplasmic reticulum may determine special areas of this membrane system, involved in either Ca^{2+} signaling, Ca^{2+} homeostasis, or protein folding and processing.

Ca^{2+} signaling

Spatially and temporally complex Ca^{2+} signals generated by the endoplasmic reticulum underlie a diversity of cellular processes (Berridge et al., 2000; Johnson and Chang, 2000). Such Ca^{2+}-dependent pathways include muscle contraction, secretion, proliferation, apoptosis, cell adhesion, differentiation, motility, cellular metabolism, fertilization, and control of gene expression (Johnson and Chang, 2000). It is an enormous task for the cell to be able to encode information in the form of Ca^{2+} waves and oscillations, such that signal fidelity is maintained and the correct cellular outcome is achieved. The endoplasmic reticulum is able to solve this problem by establishing a nonuniform distribution of its Ca^{2+}-handling proteins, thus creating spatially heterogeneous Ca^{2+} stores within its lumen (Pezzati et al., 1997; Montero et al., 1997; Golovina and Blaustein, 2000; Blaustein and Golovina, 2001). Heterogeneity in endoplasmic reticulum lumenal Ca^{2+} has previously been demonstrated using electron energy loss imaging (Pezzati et al., 1997), and such store heterogeneity is sufficient to generate Ca^{2+} signals with enough complexity to control various Ca^{2+}-dependent cellular processes (Johnson and Chang, 2000). Furthermore, these Ca^{2+} signals may play a critical role not only in the cytoplasm but also in the endoplasmic reticulum lumen (Corbett and Michalak, 2000).

The pancreatic acinar cell is a model cell type used to study the effects of the spatial heterogeneity of Ca^{2+}-handling

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*Abbreviations used in this paper: $InsP_3$, inositol 1,4,5-trisphosphate; PDI, protein disulfide isomerase; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-transporting ATPase.

proteins on Ca^{2+}-dependent events and has been extensively characterized by the group of Petersen (Belan et al., 1996; Petersen et al., 1999) and others (Kasai et al., 1993; Nathanson et al., 1994; Lee et al., 1997a,b; Leite et al., 1999). The pancreatic acinar cell has special Ca^{2+} release sites in its apical region, termed the trigger zone, which are enriched in $InsP_3$ receptors (Nathanson et al., 1994; Lee et al., 1997b; Petersen et al., 1999), whereas its uptake sites, enriched in SERCAs, are located in the basal region (Lee et al., 1997a; Petersen et al., 1999). In pancreatic acinar cells, the focus has been on the distribution of Ca^{2+} pumps and channels and their critical role in the generation of spatially complex Ca^{2+} waves and oscillations. We suggest here, however, that Ca^{2+} signal generation and regulation is an intricate process, which likely also requires input from the endoplasmic reticulum lumenal environment and its Ca^{2+}-binding proteins (Simpson et al., 1997; Johnson and Chang, 2000).

The major Ca^{2+}-binding protein of the endoplasmic reticulum of smooth muscle and nonmuscle cells is calreticulin (Milner et al., 1991), and its homologue in the sarcoplasmic reticulum of striated muscle is calsequestrin. Calreticulin may influence the action of both SERCAs and $InsP_3$ receptors (Corbett and Michalak, 2000), thereby modulating Ca^{2+} uptake and release, respectively, and thus regulating Ca^{2+} signals. In particular, Camacho's group has shown that calreticulin, in the lumen of the endoplasmic reticulum, inhibits Ca^{2+} uptake by the SERCA2b pump, thus inhibiting the continued generation of Ca^{2+} waves (Camacho and Lechleiter, 1995). This may be due to a direct interaction between calreticulin and the lumenal COOH-terminal tail of the SERCA2b isoform. In support of this interaction, calreticulin has been shown to colocalize with SERCA2b (John et al., 1998). In addition, a recent report indicates that a mathematical model, developed from single cell Ca^{2+} dynamics, has predicted an interaction between calreticulin and the SERCA pump (Baker et al., 2002). This model suggests that calreticulin alters the pump's affinity for Ca^{2+}, thus regulating Ca^{2+} oscillations. The effects of calreticulin expression on $InsP_3$ receptor activity may also be direct. Cell subfractionation experiments have revealed that calreticulin copurifies with $InsP_3$ binding sites (Enyedi et al., 1993), whereas double immunolabeling experiments have shown that calreticulin colocalizes with the $InsP_3$ receptor in the acrosome, in the equatorial segment, and in cytosolic vesicles of human spermatozoa (Naaby-Hansen et al., 2001). In summary, although the strategic placement of Ca^{2+} pumps and channels is imperative in generating spatially complex Ca^{2+} signals (Johnson and Chang, 2000), it is the responsibility of Ca^{2+}-binding proteins to provide a releasable pool of Ca^{2+} near the release channels, and to modulate the activity of the pumps and channels to regulate Ca^{2+} waves and oscillations.

The arrangement of Ca^{2+}-handling proteins in the endoplasmic reticulum creates specialized Ca^{2+}-handling subdomains. For example, in astrocytes and oligodendrocytes, Ca^{2+} wave amplification sites exist along the endoplasmic reticulum, which are enriched in calreticulin, SERCAs, and $InsP_3$ receptors and thus exhibit elevated Ca^{2+} release kinetics (Simpson et al., 1997, 1998). This organization between the $InsP_3$ receptor and calreticulin is similar to the specialization seen in the junctional sarcoplasmic reticulum in striated muscle between calsequestrin and the ryanodine receptor (Allen and Katz, 2000; Gatti et al., 2001). Calreticulin is excluded from these junctional areas, and is found in the longitudinal sarcoplasmic reticulum, from where calsequestrin is excluded (Allen and Katz, 2000). The mechanisms underlying the heterogeneous distribution of endoplasmic reticulum Ca^{2+}-binding proteins have only recently been elucidated for calsequestrin. The term condensation is used to refer to the head-to-tail oligomerization of calsequestrin, which is responsible for creating dense cores of the protein that are heterogeneously located throughout the sarcoplasmic reticulum (Gatti et al., 2001). Other endoplasmic/sarcoplasmic reticulum resident proteins may utilize a similar mechanism to establish a nonuniform distribution.

Multifunctionality of the endoplasmic reticulum

The heterogeneous distribution of Ca^{2+}-handling proteins organizes the endoplasmic reticulum into various functional domains, some responsible for Ca^{2+} signaling, some for Ca^{2+} homeostasis, and others for protein folding and quality control. For example, some parts of the endoplasmic reticulum that are enriched in $InsP_3$ receptors, SERCAs, and certain Ca^{2+}-binding proteins may be responsible for rapid Ca^{2+} uptake and release, whereas other regions enriched only in Ca^{2+}-binding proteins may be left to carry out the housekeeping functions of the endoplasmic reticulum (i.e., protein processing), which are also Ca^{2+}-dependent but are shielded from fluctuations in Ca^{2+} concentration (Rooney and Meldolesi, 1996). Therefore, the distribution of resident Ca^{2+}-binding proteins may also be potentially important in protein folding and quality control, as most of these proteins are involved in aspects of protein folding and maturation (i.e., chaperoning), and are enriched in the rough endoplasmic reticulum, the site of protein synthesis (Opas et al., 1991; Baumann and Walz, 2001), but are nonuniformly concentrated within this compartment (Baumann and Walz, 2001). Chaperones are weakly associated with one another and form a matrix in which they become embedded, resulting in their increased local concentration (Baumann and Walz, 2001). Fig. 1 compares the distribution of calreticulin to that of PDI and Grp94 within the endoplasmic reticulum of mouse embryonic fibroblasts. PDI exhibits an overlapping yet distinct distribution with calreticulin, whereas Grp94 exhibits virtually complete overlap with calreticulin (Fig. 1, A and B, respectively). The differential distribution of various lumenal Ca^{2+}-binding proteins may be of great physiological importance for Ca^{2+} homeostasis. Calreticulin and Grp94 are two major Ca^{2+} storage proteins of the endoplasmic reticulum, by virtue of their extremely high Ca^{2+} capacity (Milner et al., 1991; Argon and Simen, 1999), which is not matched by any other endoplasmic reticulum protein. Fluctuations of Ca^{2+} concentration in the lumen of the endoplasmic reticulum, which are ultimately regulated by Ca^{2+}-handling proteins, may have profound effects on the structure and function of integral and lumenal (peripheral) membrane proteins and likely contribute to the functional heterogeneity of the endoplasmic reticulum (Corbett and Michalak, 2000). Furthermore, endoplasmic reticulum

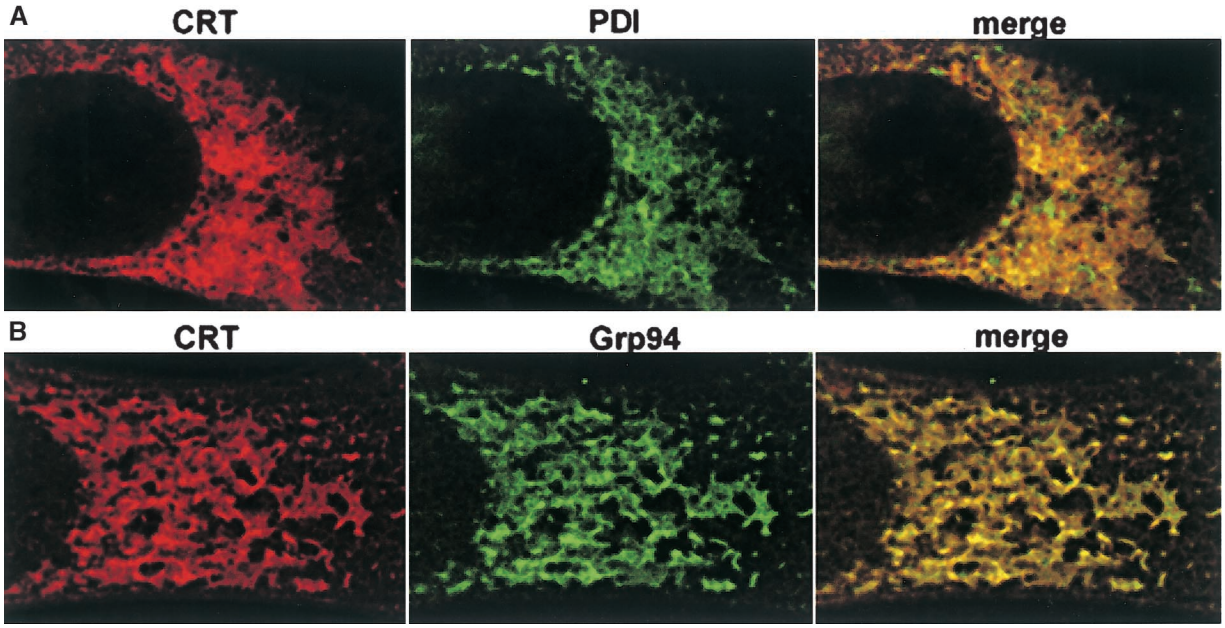

Figure 1. **The distribution of Ca^{2+}-binding proteins within the endoplasmic reticulum of mouse embryonic fibroblasts, in relation to calreticulin (CRT), as shown by double immunofluorescence labeling and visualized by confocal microscopy.** For methods, see Mesaeli et al. (1999). Left to right: CRT distribution (red), PDI, or Grp94 distribution (green). The last column of each row is an overlay of the two previous images; yellow represents areas of overlap. (A) The distributions of CRT and PDI are overlapping yet distinct. Note the green areas indicative of PDI localization only. (B) The distribution of Grp94 shows virtually complete overlap compared with that of CRT.

functions involved with protein processing have been shown to underlie various diseases such as Alzheimer's disease, Parkinson's disease, and α_1-antitrypsin deficiency (Kopito and Ron, 2000; Paschen and Frandsen, 2001; Rutishauser and Spiess, 2002). The unique distributions of chaperones may be significant in such protein folding pathologies.

Additionally, it was recently postulated that the endoplasmic reticulum and sarcoplasmic reticulum may play different roles in cells in which they coexist (Jaconi et al., 2000; Mesaeli et al., 2001). For example, in cardiomyocytes, the sarcoplasmic reticulum is involved in the classical role of excitation–contraction coupling, whereas the endoplasmic reticulum has been suggested to perform housekeeping functions, such as protein turnover (Mesaeli et al., 2001). The heterogeneous distribution of calsequestrin and calreticulin in the heart, along with their respective release channels, ryanodine receptors in the sarcoplasmic reticulum and $InsP_3$ receptors in the endoplasmic reticulum, may be responsible for this duality of function. Studies on calreticulin deficient cardiomyocytes show that these cells exhibit spontaneous contraction, thus suggesting a functional sarcoplasmic reticulum (Mesaeli et al., 1999). However, calreticulin null fibroblasts show impaired Ca^{2+} homeostasis by the endoplasmic reticulum (Nakamura et al., 2001), and this may also be the case for calreticulin-null cardiomyocytes. At the early stages of development, the endoplasmic reticulum may play a critical role in protein and lipid synthesis as well as in the regulation of Ca^{2+}-dependent transcriptional processes. These will play only a minor role in the mature heart. In mature muscle, the sarcoplasmic reticulum becomes responsible for regulating Ca^{2+}uptake and release and excitation–contraction coupling.

Interestingly, heterogeneity in compartmentalization may already be evident during development. In human oocytes, for example, the endoplasmic/sarcoplasmic reticulum–associated Ca^{2+}-binding proteins are nonuniformly distributed

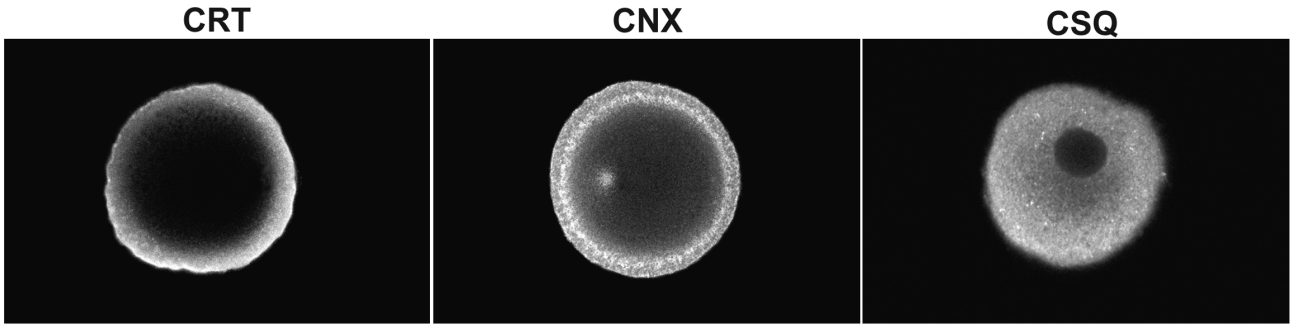

Figure 2. **Human oocytes showing distribution of calreticulin (CRT), calnexin (CNX), and calsequestrin (CSQ).** CRT predominates in the cell cortex; CNX is also in the cell cortex, but in a trilaminar arrangement; and finally, CSQ is found spread throughout the cell.

(Balakier et al., 2002). Calreticulin is predominant in the cell cortex, whereas calsequestrin is found throughout the entire cytoplasm (Fig. 2). Such differential distribution of calreticulin and calsequestrin indicates that oocytes have two distinct Ca^{2+} storage compartments: one enriched in calreticulin (and the $InsP_3$ receptor), and the other in calsequestrin (and the ryanodine receptor). Calsequestrin may localize to certain regions by the condensation mechanism, whereas calreticulin may utilize specific protein–protein interactions to achieve its localization in the endoplasmic reticulum. Interestingly, calnexin, like calreticulin, is also predominant in the cell cortex where it is found in a peculiar trilaminar arrangement (Fig. 2). The differential distribution of these proteins may reflect their functional differences. Calnexin is a chaperone, calsequestrin is a Ca^{2+} storage protein, whereas calreticulin carries out both functions. Thus, certain regions of the endoplasmic reticulum may be involved in intensive protein processing required for oocyte maturation and embryo development, whereas other regions may be involved in Ca^{2+} homeostasis. In conclusion, the spatial heterogeneity of the endoplasmic reticulum may be established early on in development, and this warrants further investigation. Deciphering the organization of Ca^{2+}-handling proteins in the endoplasmic reticulum may hold a clue to our understanding of the generation of the multifunctionality of this membrane system.

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