Presence of the inositol 1,4,5-triphosphate receptor isoforms in the nucleoplasm

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Abstract Although the inositol 1,4,5-triphosphate (IP_3)-induced nuclear Ca^{2+} release has been shown to play key roles in nuclear functions, the presence of IP_3 receptor (IP_3R)/Ca^{2+} channels in the nucleoplasm has not been found. Recently, the IP_3R/Ca^{2+} channels were reported to exist in the nucleoplasmic reticulum structure, an extension of the nuclear envelope. Here we investigated the potential existence of the IP_3Rs in the nucleoplasm and found the presence of all three IP_3R isoforms in neuroendocrine and non-neuroendocrine cells. The IP_3Rs were widely scattered in the nucleoplasm, localizing in both the heterochromatin and euchromatin regions.

Key words: Inositol 1,4,5-triphosphate receptor; Nucleus; Nucleoplasm; Inositol 1,4,5-triphosphate; Ca^{2+} release

1. Introduction

Despite the critical roles calcium ions play in controlling nuclear functions including chromosome replication and transcription control [1], very little information is available regarding the Ca^{2+} control mechanisms in the nucleus. In a recent study, chromosomes were shown to contain 20–32 mM Ca^{2+}, and the chromosomal Ca^{2+} concentration was shown to fluctuate depending on the chromosome replication state [2]. This indicates that the nucleus not only stores a large amount of Ca^{2+} but also has a high capacity Ca^{2+}-buffering ability.

Underscoring the importance of nuclear Ca^{2+}, inositol 1,4,5-triphosphate (IP_3)-mediated nuclear Ca^{2+} release is known to be essential in the fusion of nuclear vesicles during cell division [3]. However, since the report of IP_3-induced Ca^{2+} release from the nucleus [4], nuclear Ca^{2+} release has until recently been attributed to the IP_3-induced Ca^{2+} release from the nuclear envelope (NE) through the IP_3R/Ca^{2+} channels that exist in the NE [5–7].

Furthermore, some NE membranes have been shown to penetrate into the nucleoplasm, appearing as thin channel-like [8] or reticulum-like [9] structures. This nucleoplasmic reticulum was reported to contain the IP_3 receptor (IP_3R)/Ca^{2+} channels, enabling the nucleoplasmic reticulum to function as an IP_3-sensitive Ca^{2+} store [9]. In view of the presence of IP_3Rs in the NE [5–7], the existence of IP_3Rs in the nucleoplasmic reticulum is not surprising. Moreover, the IP_3-induced Ca^{2+} release from the nucleoplasmic reticulum was proposed to be directly responsible for the IP_3-induced Ca^{2+} mobilization in the nucleoplasm [9].

Nevertheless, there is still a question of how IP_3 produced as a result of agonist application causes a robust release of Ca^{2+} from the nucleoplasmic reticulum deep in the nucleoplasm. The IP_3 that opens the IP_3R/Ca^{2+} channels of the nucleoplasmic reticulum should also open the NE IP_3R/Ca^{2+} channels ahead of the nucleoplasmic reticulum IP_3R/Ca^{2+} channels because the nucleoplasmic reticulum is the extension of the NE into the nucleoplasm [8,9]. Even in the case where IP_3 induces release of Ca^{2+} from the nucleoplasmic reticulum, the question whether this calcium is the source of the robust Ca^{2+} increases in the nucleoplasm still remains because the nucleoplasmic reticulum occupies a very limited area of the total nucleoplasm [8,9].

The possibility of the presence and operation of the IP_3-mediated nuclear Ca^{2+} control mechanism in the nucleoplasm has been implied from the findings that the nucleoplasm contains phosphatidylinositol 4,5-bisphosphate (PIP_2) and phospholipase C activity [10–14]. Considering that the presence of IP_3Rs in the nucleoplasm is a prerequisite for the IP_3-mediated Ca^{2+} release mechanism to operate in the nucleoplasm, we have explored in the present study the possibility of the existence of IP_3Rs in the nucleoplasm and found the widespread presence of all three isoforms of IP_3Rs (IP_3R-1, -2, and -3) in the nucleoplasm.

2. Materials and methods

2.1. Antibodies

IP_3R peptides specific to the terminal 10–13 amino acids of type 1 (HPPHMNVNPQQPA), type 2 (SNTPHENHHMPPA) and type 3 (FVDVQNCMSR) were synthesized with a carboxy-terminal cysteine and anti-rabbit polyclonal antibodies were raised. The polyclonal anti-rabbit antibodies were affinity-purified on each immobilized peptide following the procedure described [15], and the specificity of each antibody was confirmed [16]. Calreticulin antibody was from Calbiochem (USA).

2.2. Immunocytochemical localization of IP_3R-1, -2, and -3 in adrenal chromaffin and NIH3T3 cells

For the immunogold electron microscopic (EM) study of chromaffin cells, tissue samples from bovine adrenal medulla were fixed for 2 h at 4°C in phosphate-buffered saline (PBS) containing 0.1% glutaraldehyde, 4% paraformaldehyde, and 3.5% sucrose. After three washes in PBS, the tissues were postfixed with 1% osmium tetroxide on ice for

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Abbreviations: IP_3R, inositol 1,4,5-triphosphate receptor; NE, nuclear envelope; ER, endoplasmic reticulum

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2 h, washed three times, and stained en bloc with 0.5% uranyl acetate, all in PBS. The tissues were then embedded in Epon 812 after dehydration in an ethanol series. Ultrathin sections were collected on Formvar/carbon-coated nickel grids, which were then floated on drops of freshly prepared 3% sodium metaperiodate [17] for 30 min. The immunogold labeling procedure was modified from Spector et al. [18] and the manufacturer's recommended protocol (British Biocell International, UK). After etching and washing, the grids were placed on 50 μl droplets of solution A (phosphate saline solution, pH 8.2, containing 4% normal goat serum, 1% bovine serum albumin, 0.1% Tween 20, 0.1% sodium azide) for 30 min. Grids were then incubated for 2 h at room temperature in a humidified chamber on 50 μl droplets of each IP_3R isoform-specific antibody appropriately diluted in solution B (solution A but with 1% normal goat serum), followed by rinses in solution B. The grids were reacted with 10 nm gold-conjugated goat anti-rabbit IgG diluted in solution A. Controls for the specificity of each IP_3R isoform-specific immunogold labeling included (1) omitting the primary antibody, (2) replacing the primary antibody with the preimmune serum, and (3) adding the primary antibody in the excess presence of each IP_3R isoform-specific peptide that had been used to raise the antibody. After washes in PBS and deionized water, the grids were stained with uranyl acetate (7 min) and lead citrate (2 min), and were viewed with a Zeiss EM912 electron microscope.

For the immunogold EM study of NIH3T3 cells, NIH3T3 cells that had been grown on culture dishes were rinsed with PBS, followed by fixation in PBS containing 0.1% glutaraldehyde, 4% paraformaldehyde, and 3.5% sucrose for 1 h at 4°C. The cells were then scraped from the culture dish and centrifuged to obtain the cell pellet that was later embedded in 1% agar in PBS. The cell blocks were then washed three times in PBS, followed by postfixation with 1% osmium tetroxide on ice for 2 h. The remaining steps followed the procedure described above for the adrenal chromaffin cells. For the IP_3R-1 and calreticulin double immunogold labeling experiment, the grids that had gone through the IP_3R-1 labeling step with 10 nm particles were reacted once more with the calreticulin antibody labeled with 15 nm gold particles.

3. Results

3.1. IP_3R-1, -2, and -3 in the nucleus of adrenal chromaffin cells

To determine the presence of IP_3Rs in the nucleus, the presence of each isoform of IP_3R in the subcellular organelles of bovine adrenal medullary chromaffin cells was examined using immunogold EM. As shown in Fig. 1A, the IP_3R-1-labeling gold particles were found in the endoplasmic reticulum (ER), secretory granules, and the nucleus, but not in mitochondria. Presence of the IP_3R in the ER [19,20], the nucleus [5–7], and secretory granules [15,21–23] has been reported before. In secretory granules where the membrane structures are visible, the IP_3R-1-labeling gold particles were localized primarily in the membrane regions. However, in the nucleus the IP_3R-1-labeling gold particles were localized not only in the membrane region but also in the nucleoplasm.

The localization of IP_3R-2 was similar to that of IP_3R-1. As shown in Fig. 1B, the IP_3R-2-labeling gold particles were found in the ER, secretory granules, and the nucleus, but not in mitochondria. Again, in secretory granules the IP_3R-2-labeling gold particles were localized primarily in the membrane regions. However, in the nucleus the IP_3R-2-labeling gold particles were localized not only in the membrane region but in the nucleoplasm as well. Likewise, the IP_3R-3-labeling gold particles were also localized in the ER, secretory granules, and the nucleus, but not in mitochondria (Fig. 1C). In secretory granules the IP_3R-3-labeling gold particles were localized primarily in the membrane regions, but in the nucleus they were found in both the membrane and the nucleoplasm. Identical experiments were carried out either in the presence of an excess amount of each IP_3R isoform-specific peptide that had been used to raise the cognate antibody or in the absence of the primary antibody, or with the preimmune serum in place of the primary antibody (Fig. 1D).

Consistent with the absence of IP_3Rs in mitochondria, virtually no IP_3R-labeling gold particles were found in mitochondria except the particles shown as a result of non-specific binding (see Table 1). Nevertheless, despite our present staining method that could have revealed any reticulum-like or channel-like structures, we did not notice any nucleoplasmic reticulum-like or nuclear channel-like structures [8,9] in these pictures.

To compare the relative abundance of the IP_3R isoforms in each organelle, we counted IP_3R isoform-labeling gold particles in each organelle from 15 different images that had been prepared from five to seven different tissue preparations. As shown in Table 1, 23.1 IP_3R-1-labeling gold particles/μm^2 of the ER were found while the number of IP_3R-1-labeling gold particles/μm^2 of the nucleus was 24.9. Even taking 3.2 gold particles found per μm^2 of mitochondria, which is the level of non-specific binding, into consideration, 24.9 IP_3R-1-labeling gold particles found per μm^2 of the nucleus clearly demonstrates the presence of IP_3R-1 in the nucleus. Differentiation of the IP_3R-1-labeling gold particles between the NE and the nucleoplasm indicated that the IP_3R-1 concentration in the NE is higher than that in either the nucleoplasm or the ER (Table 1).

In an identical experiment, the number of IP_3R-2-labeling gold particles found per μm^2 of the nucleus was 29.8 while that of the ER was 26.1, and the number for mitochondria was 1.8. Similar to the results shown with the IP_3R-1, the

Table 1
Distribution of the IP_3R-1, -2, and -3-labeled gold particles in bovine adrenal medullary chromaffin cell

	IP_3R-1		IP_3R-2		IP_3R-3	
	Number of gold particles[a]/area viewed (μm^2)	Gold particles/μm^2	Number of gold particles[b]/area viewed (μm^2)	Gold particles/μm^2	Number of gold particles[c]/area viewed (μm^2)	Gold particles/μm^2
Secretory granule	555/6.05	91.7	519/5.71	90.9	534/5.93	90.1
Nucleus	455/18.25	24.9	505/16.96	29.8	573/21.15	27.1
NE	35/0.97	36.08	39/0.90	43.38	43/1.12	38.36
Nucleoplasm	420/17.28	24.30	466/16.06	29.01	530/20.03	26.46
ER	207/8.96	23.1	185/7.08	26.1	214/8.41	25.5
Mitochondria	11/3.40	3.2	5/2.83	1.8	6/2.87	2.1

[a]Fifteen images from six different tissue preparations were used.
[b]Fifteen images from five different tissue preparations were used.
[c]Fifteen images from seven different tissue preparations were used.

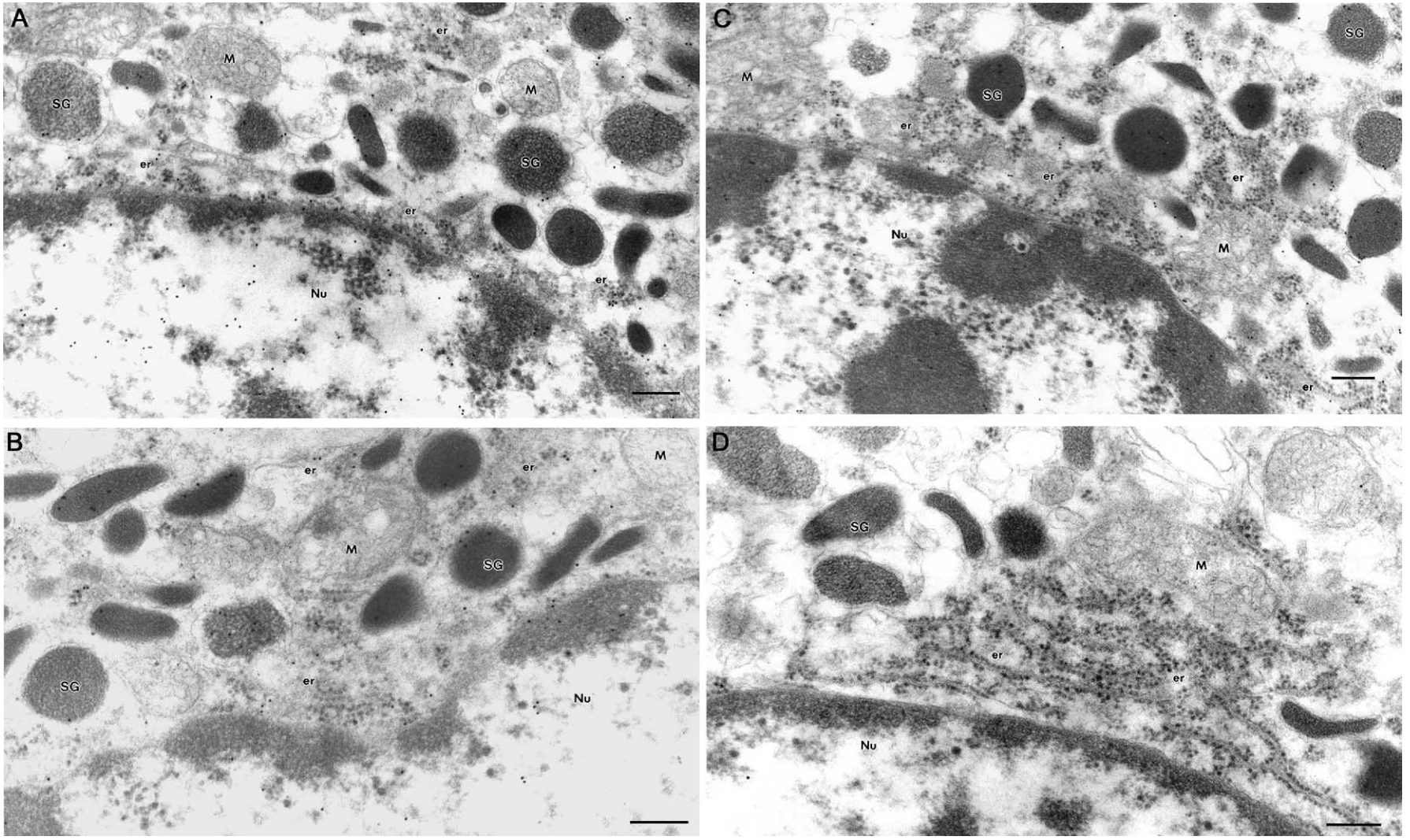

Fig. 1. Immunogold EM showing the localization of the IP_3R-1, -2, and -3 in bovine adrenal medullary chromaffin cells. Bovine adrenal medullary chromaffin cells were immunolabeled for the IP_3R-1 (A), IP_3R-2 (B), and IP_3R-3 (C) (10 nm gold) with affinity-purified isoform-specific IP_3R antibodies. Identical experiments were carried out either in the presence of an excess amount of each IP_3R isoform-specific peptide or in the absence of the primary antibody, or with the preimmune serum in place of the primary antibody (D). The IP_3R isoform-labeling gold particles are localized in the endoplasmic reticulum (er) and the nucleus (Nu), but not in mitochondria (M). The IP_3R isoform-labeling gold particles are localized not only in the membrane region but also in the nucleoplasmic region of the nucleus. Bar = 200 nm.

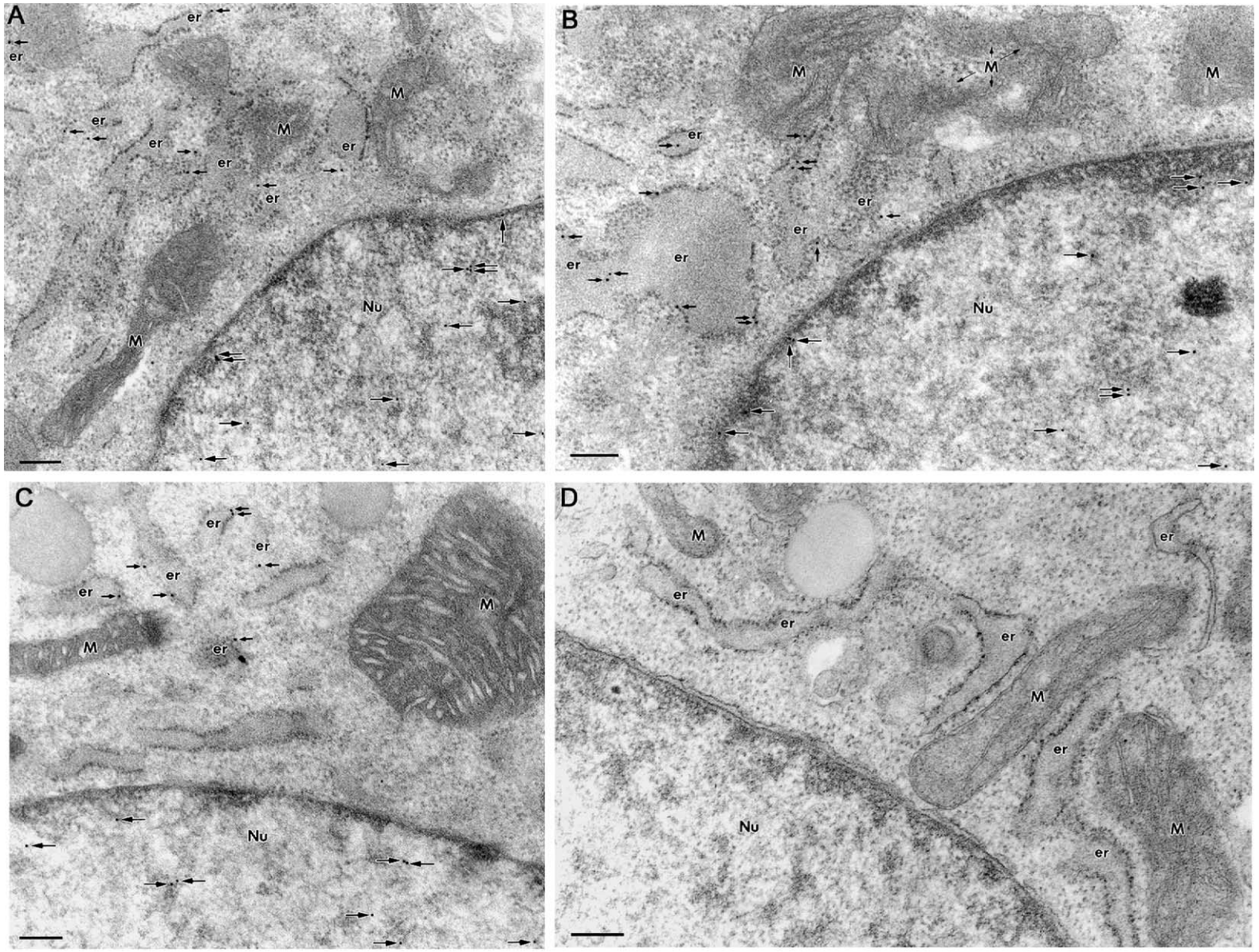

Fig. 2. Immunogold EM showing the localization of the IP_3R-1, -2, and -3 in NIH3T3 cells. Non-neuroendocrine NIH3T3 cells were immunolabeled for the IP_3R-1 (A), IP_3R-2 (B), and IP_3R-3 (C) (10 nm gold) with affinity-purified isoform-specific antibodies. Identical experiments were carried out either in the presence of an excess amount of each IP_3R isoform-specific peptide or in the absence of the primary antibody, or with the preimmune serum in place of the primary antibody (D). The IP_3R isoform-labeling gold particles are localized in the ER and the nucleus (Nu), but not in mitochondria (M). The IP_3R isoform-labeling gold particles (indicated by arrows) are localized not only in the membrane region but also in the nucleoplasmic region of the nucleus. Bar = 200 nm.

Table 2
Distribution of the IP_3R-1, -2, and -3-labeled gold particles in NIH3T3 cell

	IP_3R-1		IP_3R-2		IP_3R-3	
	Number of gold particles[a]/area viewed (μm^2)	Gold particles/μm^2	Number of gold particles[b]/area viewed (μm^2)	Gold particles/μm^2	Number of gold particles[c]/area viewed (μm^2)	Gold particles/μm^2
Nucleus	105/23.49	4.5	124/20.16	6.2	64/17.07	3.8
NE	11/1.24	8.83	13/1.07	12.15	7/0.90	7.74
Nucleoplasm	94/22.24	4.23	111/19.09	5.81	57/16.17	3.53
ER	76/10.19	7.5	65/6.23	10.4	37/6.37	5.8
Mitochondria	6/8.16	0.7	4/5.46	0.7	3/5.59	0.5

[a]Fifteen images from eight different tissue preparations were used.
[b]Thirteen images from seven different tissue preparations were used.
[c]Twelve images from seven different tissue preparations were used.

nuclear membrane had a higher concentration of IP_3R-2 than either the nucleoplasm or the ER. Further, the number of IP_3R-3-labeling gold particles found per μm^2 of the nucleus was 27.1 while those of the ER and mitochondria were 25.5 and 2.1, respectively. Again, the IP_3R-3 concentration in the NE was higher than that of either the nucleoplasm or the ER.

3.2. *IP_3R-1, -2, and -3 in the nucleus of NIH3T3 cells*

The presence of IP_3Rs was also studied using non-neuroendocrine NIH3T3 cells. IP_3R-1-labeling gold particles were found in the ER and the nucleus, but not in mitochondria (Fig. 2A). Similar to the results shown with the neuroendocrine chromaffin cells (Fig. 1A), the IP_3R-1-labeling gold particles were not restricted to the membrane region of the nucleus, but they were localized in the nucleoplasm as well. The IP_3R-1-labeling gold particles in the nucleus were widely localized in both the heterochromatin and euchromatin regions.

Likewise, the IP_3R-2-labeling gold particles were found in the ER and the nucleus, but not in mitochondria (Fig. 2B). The IP_3R-2-labeling gold particles were not restricted to the membrane region of the nucleus, but were localized in the nucleoplasm as well. Similarly, the IP_3R-3-labeling gold particles were found in the ER and the nucleus, but not in mitochondria (Fig. 2C). Again, the IP_3R-3-labeling gold particles were widely localized in the nucleus, including both the heterochromatin and euchromatin regions. Consistent with the absence of IP_3Rs in mitochondria, virtually no IP_3R-labeling gold particles were found in mitochondria of non-neuroendocrine NIH3T3 cells except the particles shown as a result of non-specific binding (see Table 2). Identical experiments were carried out either in the presence of an excess amount of each IP_3R isoform-specific peptide that had been used to raise the cognate antibody or in the absence of the primary antibody, or with the preimmune serum in place of the primary antibody (Fig. 2D).

To compare the relative abundance of the IP_3R isoforms in each organelle, we counted IP_3R isoform-labeling gold particles in each organelle from 12–15 different images prepared from seven or eight different tissue preparations. As shown in Table 2, 7.5 IP_3R-1-labeling gold particles/μm^2 of the ER were found while the number of IP_3R-1-labeling gold particles/μm^2 of the nucleus was 4.5. The number of IP_3R-1-labeling gold particles found per μm^2 of mitochondria was 0.7, showing the level of non-specific binding in this experiment. Hence, 4.5 IP_3R-1-labeling gold particles found per μm^2 of the nucleus clearly demonstrate the presence of IP_3R-1 in the nucleus of NIH3T3 cells, as was the case with adrenal chromaffin cells. Differentiation of the IP_3R-1-labeling gold particles between the NE and the nucleoplasm indicated that the IP_3R-1 concentration in the NE is higher than that in either the nucleoplasm or the ER (Table 2).

Further, the number of IP_3R-2-labeling gold particles found per μm^2 of the nucleus was 6.2 while that of the ER was 10.4. Unlike adrenal chromaffin cells, which appeared to have slightly higher concentrations of IP_3Rs in the nucleus than in the ER, the concentration of IP_3R-2 in NIH3T3 cells was significantly higher in the ER than in the nucleus. However, the IP_3R-2 concentration in the NE is higher than that in the ER.

In an identical experiment, the number of IP_3R-3-labeling gold particles the nucleus was 3.8 while that of the ER was 5.8. Similar to the IP_3R-2, the concentration of IP_3R-3 was significantly higher in the ER than in the nucleus. The number of each IP_3R isoform-labeling gold particles found per μm^2 of mitochondria was 0.5–0.7 (Table 2), indicating the level of non-specific binding in the NIH3T3 cells. Nevertheless, similar to the results shown with the IP_3R-1 and -2, the IP_3R-3 concentration in the NE is higher than that in the ER. Again, nucleoplasmic reticulum-like structures were not observed in the NIH3T3 cells examined.

To validate the specificity of the IP_3R antibodies, the total

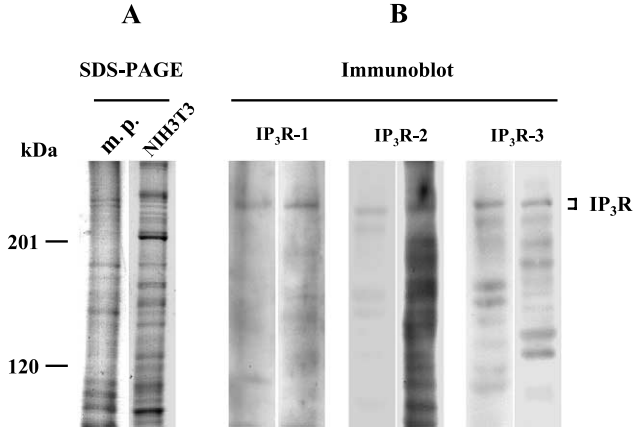

Fig. 3. Immunoblot of the secretory granule integral membrane proteins and NIH3T3 protein extracts with each type-specific IP_3R antibody. Secretory granule integral membrane proteins from bovine adrenal medullary chromaffin cells (m.p.) and the total protein extracts from NIH3T3 cells (NIH3T3) were separated on a 7% SDS-polyacrylamide gel. 10 μg of each proteins was visualized with Coomassie blue staining (A) while 50 μg of granule membrane proteins and 100 μg of NIH3T3 proteins were probed with type-specific IP_3R (IP_3R-1, -2, and -3) antibodies used in the immunogold cytochemical studies (B). A 260–280 kDa membrane protein specifically reacted with each IP_3R antibody.

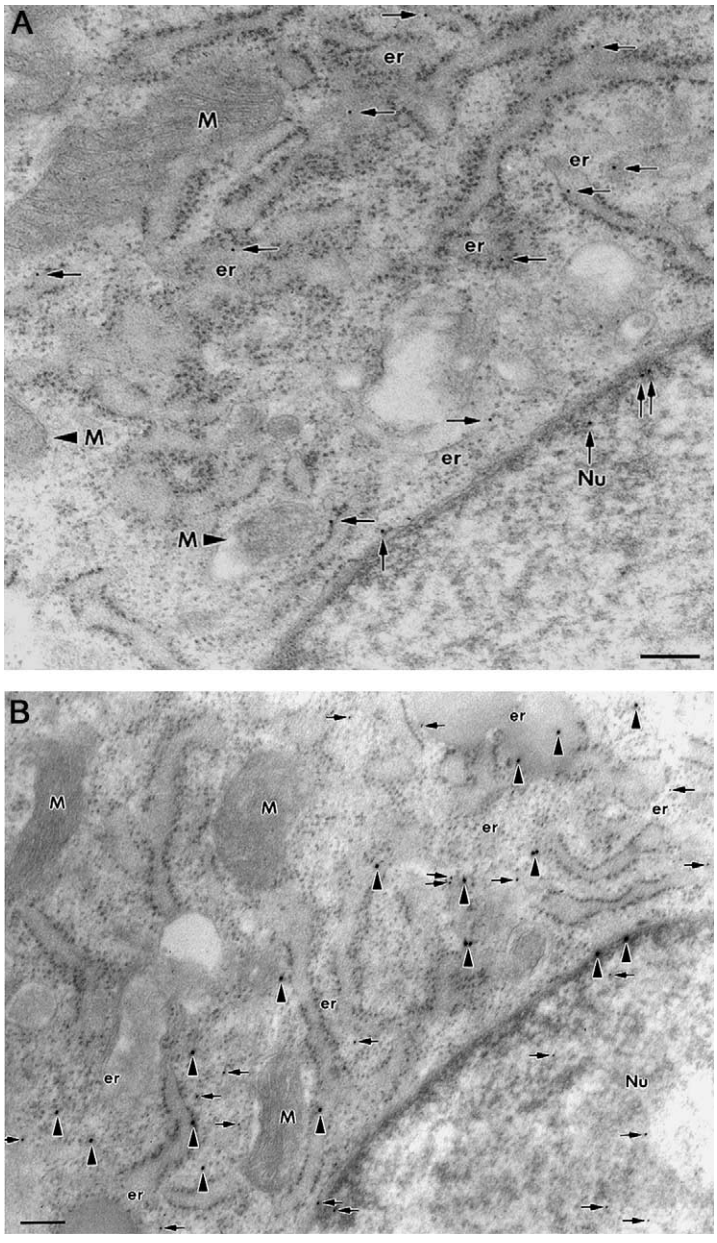

Fig. 4. Immunogold EM showing the localization of calreticulin in NIH3T3 cells. A: NIH3T3 cells were immunolabeled for calreticulin (10 nm gold) with the affinity-purified calreticulin antibody. The calreticulin-labeling gold particles (indicated by arrows) are localized in the ER and the NE, but not in mitochondria (M). Bar = 200 nm. B: NIH3T3 cells were double immunolabeled for calreticulin (15 nm gold) and the IP_3R-1 (10 nm gold). The calreticulin-labeling gold particles (indicated by large arrowheads) are localized in the ER and the NE while the IP_3R-1-labeling gold particles (indicated by arrows) are localized in the ER, NE and the nucleoplasm, but not in mitochondria (M). Bar = 200 nm.

protein extracts of NIH3T3 cells were separated on an sodium dodecyl sulfate (SDS)-polyacrylamide gel and the presence of each IP_3R isoform was examined by immunoblot analysis using each IP_3R-specific antibody. As shown in Fig. 3, each IP_3R isoform-specific antibody reacted with a protein of ~270 kDa in the total NIH3T3 cell extracts. The same antibody also reacted with ~270 kDa proteins of the membrane proteins of bovine chromaffin granules, confirming not only the presence of the IP_3R isoforms in the NIH3T3 cells but also the specificity of each IP_3R isoform-specific antibody. A few smaller bands appear to be fragmented IP_3Rs.

To further confirm the specificity of the IP_3R-labeling gold particles, presence of calreticulin, an ER marker protein [24], in NIH3T3 cells was also tested. As shown in Fig. 4A, calreticulin-labeling gold particles were present in the ER and the NE, but not in the nucleoplasm. Further, in the IP_3R and calreticulin double labeling immunogold experiment, the IP_3R-1-labeling gold particles were localized in the ER, NE and the nucleoplasm, whereas the calreticulin-labeling gold particles were localized in the ER and the NE, but not in the nucleoplasm (Fig. 4B). To compare the relative abundance of calreticulin in each organelle, we counted calreticulin-labeling gold particles in each organelle from 15 different images that had been prepared from five different tissue preparations. The number of calreticulin-labeling gold particles localized per μm^2 of each organelle was 10.1 for the ER, 2.8 for the nucleus, and 0.51 for mitochondria. When the calreticulin-labeling gold particles were differentiated between the NE and the

nucleoplasm, there were 19.0 gold particles/μm^2 of the NE and 0.79 gold particles/μm^2 of the nucleoplasm. The number of gold particles localized per μm^2 of the nucleoplasm was slightly higher than that of mitochondria, the organelle known not to contain calreticulin. Hence, these results clearly indicate the presence of calreticulin in the ER and the NE as expected, but suggest the absence of calreticulin in the nucleoplasm.

4. Discussion

Despite the known presence of the IP_3Rs in the NE [5–7], the present results do not show a preferential localization of the IP_3Rs in the membrane regions of the nucleus (Figs. 1 and 2). In contrast to the notion that the IP_3Rs are limited to the membrane regions of the NE, the EM pictures clearly show that the IP_3Rs are not restricted to the membrane area but are distributed throughout the nucleoplasm. The widespread presence of the IP_3Rs in the nucleus not only indicates that the localization of the IP_3Rs cannot be limited to any channel-like [8] or NE reticulum-like [9] structures that have been shown to occupy only a very limited area in the nucleoplasm, but it underscores the presence of IP_3Rs in the nucleoplasm far beyond any NE extensions that may have penetrated into the nucleoplasm. Moreover, all three isoforms of the IP_3R were widely localized throughout the nucleus, including both the heterochromatin and the euchromatin regions of both neuroendocrine adrenal chromaffin cells and non-neuroendocrine NIH3T3 cells, thus clearly demonstrating the presence of all three IP_3R isoforms in the nucleoplasm.

Although it had previously been reported that some of the NE membranes can penetrate into the nucleoplasm [8] and IP_3Rs are found in the NE extensions [25], we did not notice any invaginated NE membranes in the present study. Since our present staining method [17] should have revealed any NE extensions in the nucleoplasm, the present results indicate the absence of such structures in the pictures viewed. Further, the IP_3Rs were found to be widely scattered in the nucleus (Figs. 1 and 2), not limited to any local areas. Hence the IP_3Rs that had been shown to localize in the nucleoplasm cannot be the ones contributed by the NE membranes that may have penetrated into the nucleoplasm.

In neuroendocrine adrenal chromaffin cells, the number of IP_3R isoform-labeling gold particles per μm^2 of the nucleus was higher than that of the ER in all three IP_3R isoforms (Table 1). However, this was not the case in non-neuroendocrine NIH3T3 cells where the number of IP_3R isoform-labeling gold particles per μm^2 of the nucleus was significantly lower than that of the ER in all three IP_3R isoforms (Table 2). Though the reason for this difference is not apparent, the higher concentrations of the IP_3Rs in the nucleus of neuroendocrine cells than those in the ER might be due to more active nuclear activities, including transcription of many secretion-related genes, in secretory cells compared to non-secretory cells. Since dynamic nuclear activities cannot occur without an active control of nuclear Ca^{2+} concentrations, it may be a natural consequence that neuroendocrine cells have relatively higher concentrations of the IP_3Rs in the nucleus than those in the ER.

Consistent with the present results, the nucleus is known to contain a phosphoinositide signaling system of its own, including molecules such as PIP_2, phospholipase C, IP_3, and diacylglycerol (DAG) [10–14]. The existence of these molecules and the phosphoinositide signaling system indicates in nucleo production of the signaling molecules IP_3 and DAG, which suggests the presence and operation of signaling systems involving these molecules in the nucleus. Further, nuclear IP_3 was shown to release Ca^{2+} in nucleo through the IP_3R/Ca^{2+} channels, thereby stimulating the fusion of nuclear vesicles in NE assembly [3]. Blocking of Ca^{2+} release through the IP_3R/Ca^{2+} channels also inhibited the fusion of nuclear vesicles [3], underscoring the importance of the IP_3-induced Ca^{2+} release in nuclear processes. In this respect, the present results provide the molecular basis for the IP_3-induced Ca^{2+} mobilization in the nucleoplasm.

Moreover, despite the demonstrated presence of all three isoforms of IP_3Rs in the nucleoplasm, the question of where the calcium that can be released through the IP_3R/Ca^{2+} channels is stored in the nucleoplasm still remains. In this regard, the finding that a high capacity, low affinity Ca^{2+} storage protein chromogranin B is present in the nucleus of adrenal chromaffin cells in 20–40 μM [26] appears to be of direct relevance. Chromogranin B was originally found in secretory granules of neuroendocrine cells, and binds 93 mol Ca^{2+}/mol with a K_d of 1.5 mM [27]. From the nuclear concentration and the high capacity Ca^{2+} binding property, chromogranin B is expected to bind a millimolar range of Ca^{2+} in the nucleus, an amount sufficient to control a wide range of nuclear Ca^{2+} concentrations. Nevertheless, neither chromogranin B nor other Ca^{2+} storage proteins have been identified in non-neuroendocrine cells so far, although it is highly probable that other functionally equivalent Ca^{2+} binding proteins play similar roles.

Deducing from the fact that the IP_3R interacts with the Ca^{2+} storage protein chromogranin B [28], it is highly plausible that the nuclear IP_3R/Ca^{2+} channels and Ca^{2+} storage proteins form a complex to store and release nuclear calcium in response to IP_3. Hence, we hypothesize that this complex may consist of the IP_3R/Ca^{2+} channel, Ca^{2+} storage protein, and phospholipids, thus forming a proteolipid Ca^{2+} store complex in a small vesicular structure. In this respect, the released nuclear Ca^{2+} may also be sequestered by this proteolipid complex structure, without invoking the need to be pumped out or be removed through the nuclear pore complex.

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