Presence of the Inositol 1,4,5-trisphosphate Receptor and Inositol 1,4,5-trisphosphate-mediated Ca^{2+} Release in the Nucleoplasm

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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. Since the report of IP_3-induced Ca^{2+} release from the nucleus (2), 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 (3).

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 (4). 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 and Ca^{2+} storage granules in the nucleoplasm using immunogold EM and HVEM and found the widespread presence of all three isoforms of IP_3Rs (IP_3R-1, -2, and -3) in the nucleoplasm.

2. Results

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_3Rs-labeling gold particles were found in the endoplasmic reticulum (ER), secretory granule membranes, and the nucleus, but not in mitochondria. In the nucleus, the IP_3Rs-labeling gold particles were localized not only in the membrane region but also in the nucleoplasm. The presence of IP_3Rs was also studied using non-neuroendocrine NIH3T3 cells. IP_3Rs-labeling gold particles were found in the ER and the nucleus, but not in mitochondria (Fig. 1B). The IP_3Rs-labeling gold particles were not restricted to the membrane region of the nucleus, but they were localized

in both the heterochromatin and euchromatin regions as well.

To further confirm the specificity of the IP_3R-labeling gold particles, presence of calreticulin, an ER marker protein, in NIH3T3 cells was also tested. As shown in Fig. 1C, 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 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. 1D).

3. Discussion

Despite the known presence of the IP_3Rs in the NE (3), the present EM pictures clearly show that the IP_3Rs are not restricted to the membrane area but are distributed throughout the nucleoplasm. Moreover, all three isoforms of the IP_3Rs were widely localized both the heterochromatin and the euchromatin regions of both neuroendocrine adrenal chromaffin cells and non-neuroendocrine NIH3T3 cells, thus clearly demonstrating the presence of IP_3Rs 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 mM (5) appears to be of direct relevance. 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.

Deducing from the fact that the IP_3R interacts with the Ca^{2+} storage protein chromogranin B, 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. By the immunogold EM and high voltage electron microscopy, 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.

4. References

1. Berridge MJ, Lipp P, and Bootman MD. (2000) Nat. Rev. Mol. Cell Biol. 1, 11-21.
2. Malviya, AN, Rogue P, and Vincendon G. (1990) Proc. Natl. Acad. Sci. USA 87, 9270-9274.
3. Gerasimenko OV, Gerasimenko JV, Tepikin AV, and Petersen OH. (1995) Cell 80, 439-444.
4. D'Santos CS, Clarke JJ, and Divecha N. (1998) Biochim. Biophys. Acta 1436, 201-232.
5. Yoo SH, You SH, Kang MK, Huh YH, Lee CS, and Shim CS. (2002) J. Biol. Chem. 277, 16011-16021.

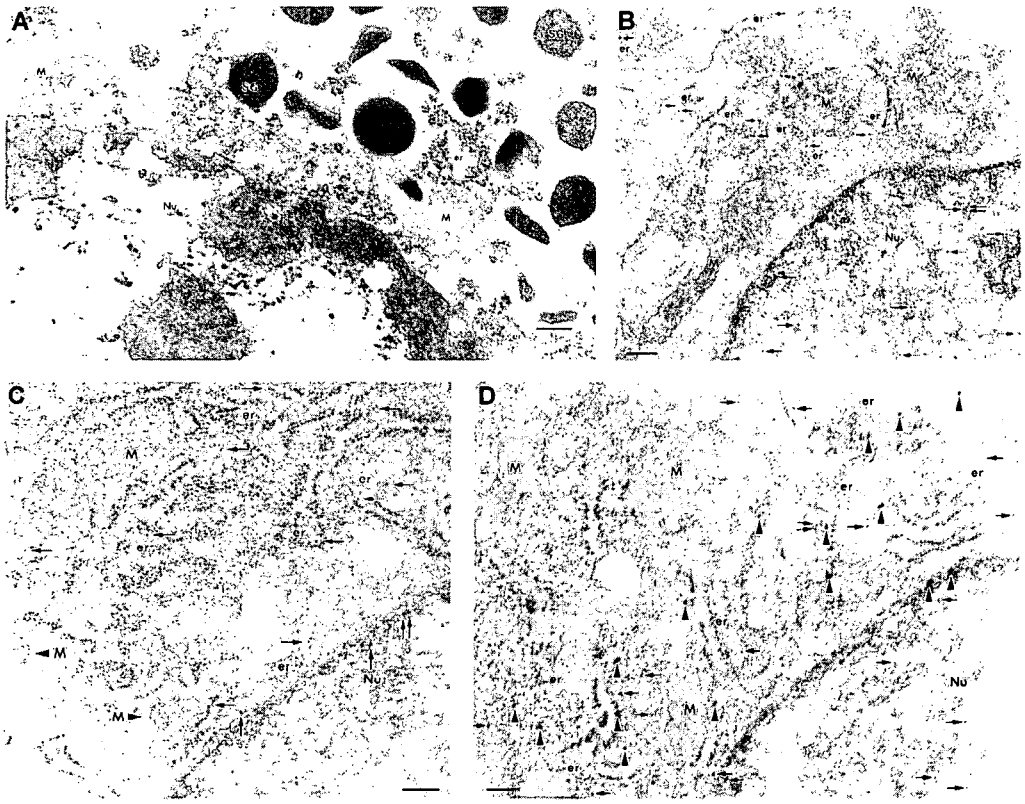

Fig. 1. Immunogold EM showing the localization of the IP_3Rs. A and B: Bovine adrenal medullary chromaffin cells (A) and NIH3T3 cells (B) were immunolabeled for the IP_3Rs (10 nm gold) with affinity-purified IP_3Rs antibodies. C: NIH3T3 cells were immunolabeled for calreticulin (10 nm gold) with the affinity-purified calreticulin antibody. D: NIH3T3 cells were double immunolabeled for calreticulin (15 nm gold) and the IP_3R-1 (10 nm gold). Bar = 200 nm.