Cell, Vol. 80, 439–444, February 10, 1995, Copyright © 1995 by Cell Press

ATP-Dependent Accumulation and Inositol Trisphosphate- or Cyclic ADP-Ribose-Mediated Release of Ca^{2+} from the Nuclear Envelope

Oleg V. Gerasimenko, Julia V. Gerasimenko, Alexei V. Tepikin, and Ole H. Petersen
Medical Research Council Secretory Control Research Group
Physiological Laboratory
University of Liverpool
Liverpool L69 3BX
England

Summary

Uptake and release of Ca^{2+} from isolated liver nuclei were studied with fluorescent probes. We show with the help of digital imaging and confocal microscopy that the Ca^{2+}-sensitive fluorescent probe Fura 2 is concentrated in or around the nuclear envelope and that the distribution of Fura 2 fluorescence is similar to that of an endoplasmic reticulum marker. The previously demonstrated ATP-dependent uptake of Ca^{2+} into isolated nuclei and release of the accumulated Ca^{2+} by inositol 1,4,5-trisphosphate (IP_3) are therefore due to transport of Ca^{2+} into and out of the nuclear envelope and not the nucleoplasm. Dextrans labeled with fluorescent Ca^{2+} indicators (calcium-Green 1 and Fura 2) are distributed uniformly in the nucleoplasm and can be used to show that changes in the external Ca^{2+} concentration produce rapid changes in the nucleoplasmic Ca^{2+} concentration. Nevertheless, IP_3 and cyclic ADP-ribose evoke transient intranuclear Ca^{2+} elevations. The release from the Ca^{2+} stores in or around the nuclear envelope appears to be directed into the nucleoplasm from where it can diffuse out through the permeable nuclear pore complexes.

Introduction

Ca^{2+} is important for the control of fundamental nuclear processes (Tombes et al., 1992). Recently, it has been shown that nuclear vesicle fusion requires Ca^{2+} mobilization (Sullivan et al., 1993). The nucleus contains an endoplasmic reticulum (ER)-type Ca^{2+} pump (Lanini et al., 1992), functional receptors for the Ca^{2+}-releasing messenger inositol 1,4,5-trisphosphate (IP_3) (Nicotera et al., 1990; Malviya et al., 1990; Kume et al., 1993), and mechanisms for IP_3 production (Divecha et al., 1993).

Although the nuclear membrane is considered to be very permeable to solutes with a molecular mass of up to 10–20 kDa (Lang et al., 1986), differences between the Ca^{2+} concentration in the nucleoplasm and the cytosol have been observed, particularly after stimulation (Berridge, 1993). However, recent studies suggest that at least some of the earlier results were due to artifacts (Gillot and Whitaker, 1993; Al-Mohanna et al., 1994). Measurements of the nuclear Ca^{2+} concentration with specifically targeted recombinant aequorin indicate that, in general, cytosolic and nucleoplasmic Ca^{2+} concentrations equilibrate rapidly (Brini et al., 1993), but other workers have proposed that the nucleus is largely insulated from large cytosolic Ca^{2+} changes (Al-Mohanna et al., 1994).

On the basis of work on isolated liver nuclei, it has been concluded that there is ATP-dependent Ca^{2+} uptake into and IP_3-evoked release of Ca^{2+} from the intranuclear space (Nicotera et al., 1989, 1990). The most direct measurements of the nucleoplasmic Ca^{2+} concentration in intact cells (Brini et al., 1993) indicates that the nuclear envelope is very permeable to Ca^{2+}. It is now difficult to understand how the mechanisms previously proposed for nuclear Ca^{2+} homeostasis, based on work on isolated nuclei (Nicotera et al., 1989, 1990, 1992), can operate. We have therefore investigated Ca^{2+} transport and the fluorescent Ca^{2+}-sensitive dye distributions in isolated liver nuclei using digital imaging and confocal microscopy.

Our data show that when isolated nuclei are exposed to the cell-permeable fluorescent dye Fura 2 acetoxymethylester (Fura 2–AM), the Fura 2 indicator is concentrated in or around the nuclear envelope with a very low concentration in the nucleoplasm. The distribution is virtually identical to that of the ER marker 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6(3)$). It is therefore clear that the Ca^{2+} accumulation measured is due to uptake into a peripheral envelope of stores with ER properties and not into the nucleoplasm. We have furthermore shown that when the nucleoplasmic Ca^{2+} concentration is measured using dextrans attached to fluorescent Ca^{2+}-sensitive dyes, changes in the external Ca^{2+} concentration produce similar rapid changes in the nucleoplasm, indicating a high degree of permeability across the nuclear envelope. Both the Ca^{2+}-releasing messengers IP_3 and cyclic ADP-ribose (cADPr) evoke release of accumulated Ca^{2+} directed into the nucleoplasm from where it can diffuse out through the nuclear pores (nuclear pore complex).

Results

Distribution of Fluorescent Dyes in Isolated Nuclei

The distribution of the fluorescence of Fura 2 loaded in its AM form into an isolated nucleus is shown in Figure 1A. The intensity of fluorescence, measured at the excitation wavelength of 360 nm (the isobestic point of Fura 2) by the imaging system, is low in the central area of the projection of the nucleus and relatively high in the periphery of the nucleus ($n > 100$). The distribution of the fluorescence of Fura 2 resembles the fluorescence of the ER marker $DiOC_6(3)$ (Figure 1B) ($n = 7$). This similarity in the patterns of distributions indicates that Fura 2 is concentrated in areas of the ER (and the nuclear envelope) that remain connected to the nuclei after the isolation procedure, but not in the nucleoplasm. In further experiments, the rim distributions of Fura 2 (Figure 2A) ($n = 9$) and $DiOC_6(3)$ ($n = 8$) (Figure 2B) were verified using a confocal microscope.

Fluorescent dextrans were added to the external solution. Dextrans (MW 70,000) labeled with fluorescent Ca^{2+}

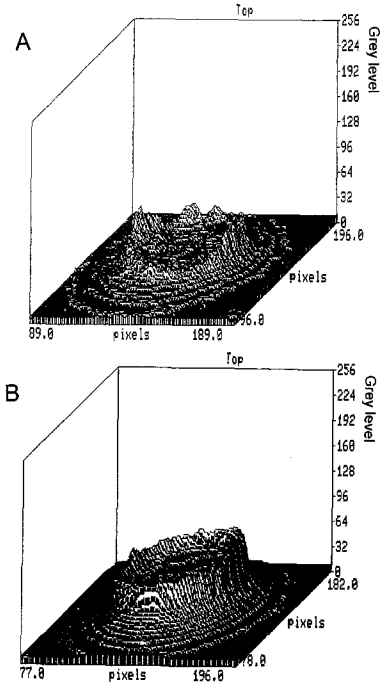

Figure 1. Both the Ca^{2+}-Sensitive Fluorescent Probe Fura 2 and the Fluorescent ER Marker $DiOC_6(3)$ Are Concentrated in the Nuclear Envelope

Distributions of the fluorescence of Fura 2 (loaded in AM form) and $DiOC_6(3)$ in single isolated liver nuclei measured with an imaging system; 1 μm corresponds to six pixels.

(A) Nucleus incubated with Fura 2–AM (excitation, 360 nm; emission, 515 nm).

(B) Nucleus stained with $DiOC_6(3)$ (excitation, 480 nm; emission, >520 nm).

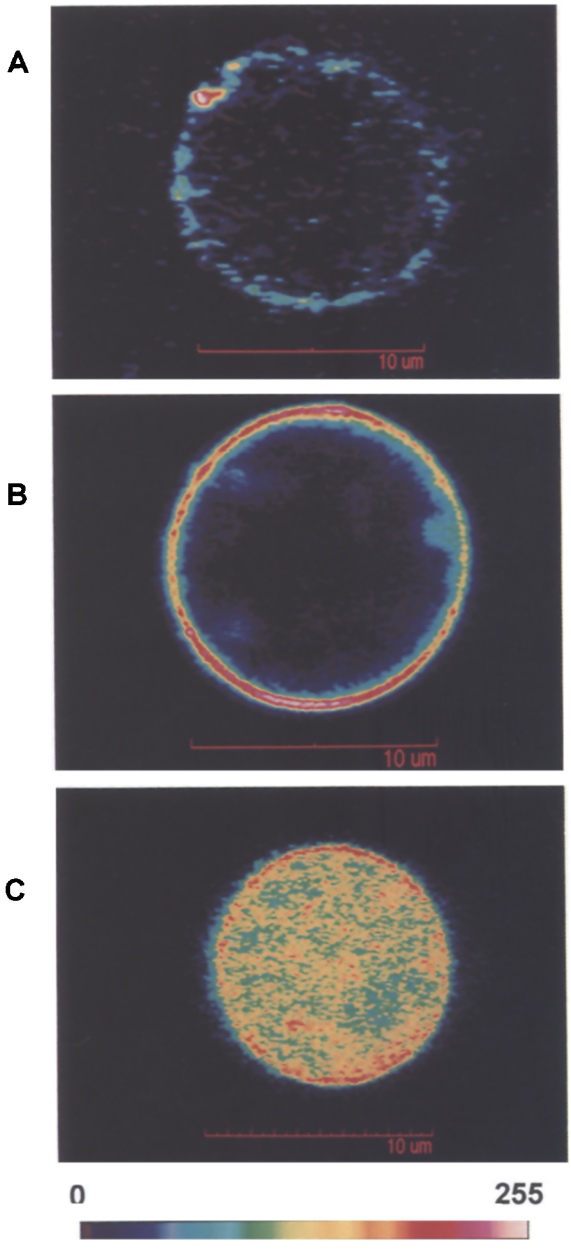

Figure 2. Confocal Microscopy Shows Ring-like Distributions of Fura 2 and $DiOC_6(3)$ at the Periphery of Nucleus in Contrast with Uniform Nucleoplasmic Distribution of Dextran-Linked Fura 2

Color-coded confocal pictures of the distributions of the fluorescence of dyes loaded into isolated nuclei.

(A) Fluorescence of Fura 2–AM-loaded nucleus (excitation, 363.8 nm; emission, >400 nm).

(B) Fluorescence of $DiOC_6(3)$-loaded nucleus (excitation, 480 nm; emission, >520 nm).

(C) Fluorescence of Fura 2–dextran-incubated nucleus (excitation, 363.8 nm; emission, >400 nm).

indicators (calcium–Green 1 and Fura 2) accumulate in the nuclei and remain there for a long time (tens of minutes) after removal of these substances from the external solution. The intensity of fluorescence of a nucleus loaded with Fura 2–dextran was highest in the central part and lowest in the periphery when recorded with the imaging system (Figure 3A) ($n > 100$). A similar type of distribution was found for calcium–Green 1–dextran ($n > 50$). The distribution of the intensity of fluorescence of the dextran-bound calcium indicators in nuclei appears to be similar to that of the DNA–RNA marker ethidium bromide (Figure 3B) ($n = 8$). This bell-shaped distribution of the fluorescent dextrans indicates accumulation of these molecules in the internal areas of the nucleus (nucleoplasm). In further experiments, the uniform distribution of the dextran-bound fluorescent indicators throughout the nucleus was verified using a confocal microscope (see Figure 2C) ($n = 9$).

Ca^{2+} Transport into and out of Isolated Nuclei

Changes of the external Ca^{2+} concentration ($<10^{-8}$ M to 1 mM) did not affect the fluorescence of Fura 2–loaded (in AM form) nuclei ($n = 7$). However, addition of ionomycin to an external solution with a high Ca^{2+} concentration (1 mM) resulted in saturation of the Fura 2 ratio signal, and a subsequent wash with the Ca^{2+} chelator EGTA in the presence of ionomycin induced recovery of the signal followed by a continuous slow decline of the nuclear Ca^{2+} concentration (Figure 4A) ($n = 10$). In another series of experiments, we found ATP-dependent accumulation of Ca^{2+} into the nuclei (Figure 4B) ($n = 12$).

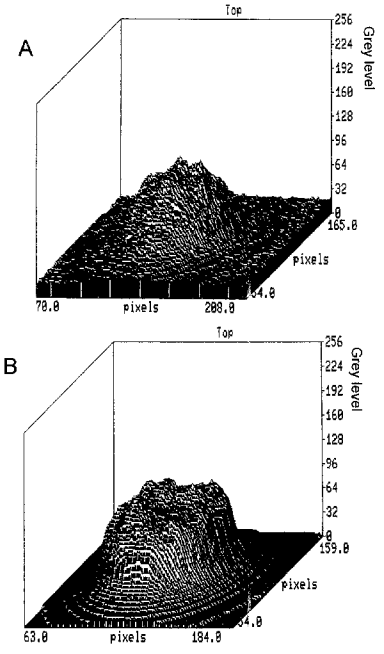

Figure 3. Dextran-Linked Fura 2 and the DNA–RNA Marker Ethidium Bromide Are Distributed throughout the Nucleoplasmic Space

Distributions of the fluorescence of Fura 2–dextran and ethidium bromide in single isolated liver nuclei measured with an imaging system; 1 μm corresponds to six pixels.

(A) Nucleus stained with Fura 2–dextran (excitation, 360 nm; emission, 515 nm).

(B) Nucleus stained with ethidium bromide (excitation, 360 nm; emission, >520 nm).

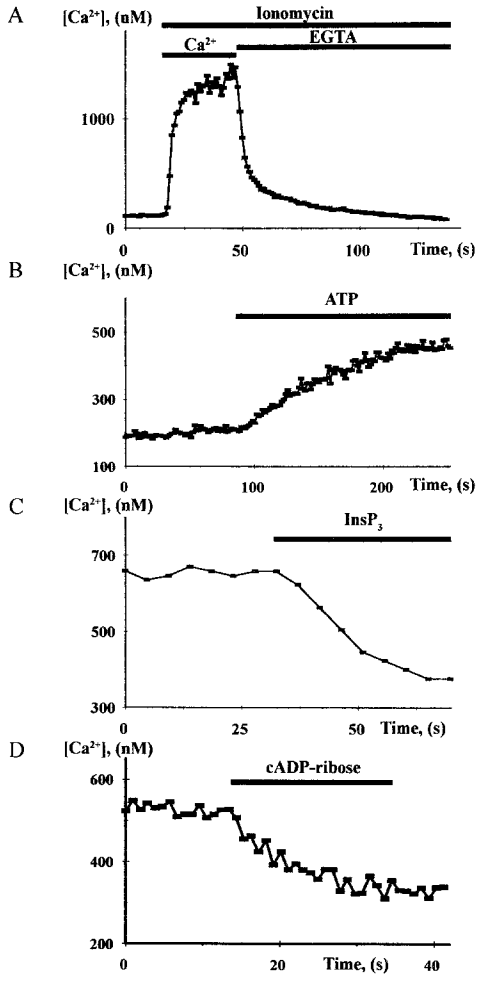

Figure 4. ATP-Dependent Ca^{2+} Uptake and IP_3 as Well as cADPr-Evoked Ca^{2+} Release from Nuclear Envelope

Influence of changing the external calcium concentrations and of applying ATP, IP_3, and cADPr on the free Ca^{2+} concentration in the nuclear envelope. Nuclei loaded with Fura 2–AM (A–C) or calcium–Green 1–AM (D).

(A) Subsequent applications of high Ca^{2+} (1 mM) and EGTA (2 mM) to a single isolated nucleus in the presence of 20 μM ionomycin.

(B) Application of 1 mM ATP to a nucleus in standard solution containing 100 nM free Ca^{2+}.

(C) Nucleus incubated with 200 nM free Ca^{2+} in standard solution in the presence of 1 mM ATP for 2 min was washed with standard solution (without Ca^{2+} and ATP), and 10 μM IP_3 was applied.

(D) Nucleus incubated with 200 nM free Ca^{2+} in standard solution in the presence of 1 mM ATP for 2 min was washed with standard solution (without Ca^{2+} and ATP), and 10 μM cADPr was applied.

Accumulated Ca^{2+} could be released by IP_3, but this effect was difficult to detect in an ATP-containing solution. After removal of ATP from the external milieu, IP_3 was able to produce a marked decrease in the nuclear Ca^{2+} concentration (Figure 4C) (n = 7). We confirmed results obtained earlier (Nicotera et al., 1989, 1990; Hechtenberg and Beyersmann, 1993) in experiments with suspensions of isolated liver nuclei, showing the existence of ATP-dependent accumulation of Ca^{2+} as well as IP_3- and calmidazolium-induced Ca^{2+} release. However, in our experiments we were able to control the distribution of the Ca^{2+} indicator, and we found that all these effects occur in isolated nuclei, with a rim distribution of Fura 2. Ca^{2+} accumulation and release therefore occur into and from stores around the nuclear envelope.

Since there is now evidence for a Ca^{2+}-releasing messenger role for cADPr in some systems (Lee et al., 1989; Hua et al., 1994; Thorn et al., 1994), we also investigated the effect of this agent. Figure 4D shows that 10 μM cADPr evoked a clear loss of Ca^{2+} from the nuclear envelope (n = 6).

The accumulation of dextrans labeled with Ca^{2+}-sensitive indicators in the internal areas of the nuclei (see Figures 2C and 3) gave us a unique opportunity to monitor Ca^{2+} changes in the nucleoplasm. Changes of the Ca^{2+} concentration in the external solution resulted in rapid changes of the fluorescence of the dextran-bound Ca^{2+} indicators. An increase of the external Ca^{2+} concentration to 1 mM caused saturation of the indicators in the nucleoplasm, whereas a subsequent application of EGTA (2 mM) resulted in reversal of the signal, indicating that Ca^{2+} was being removed from the nucleoplasm (Figure 5A) (n = 10). These rapid changes of fluorescence of the dextran-bound indicators strongly suggest that the nuclear envelope is easily permeable to Ca^{2+}. However, stimulation of nuclei that accumulated Ca^{2+} in the presence of ATP with IP_3 (n = 9) (Figure 5B) and cADPr (Figure 5C) (n = 7) induced

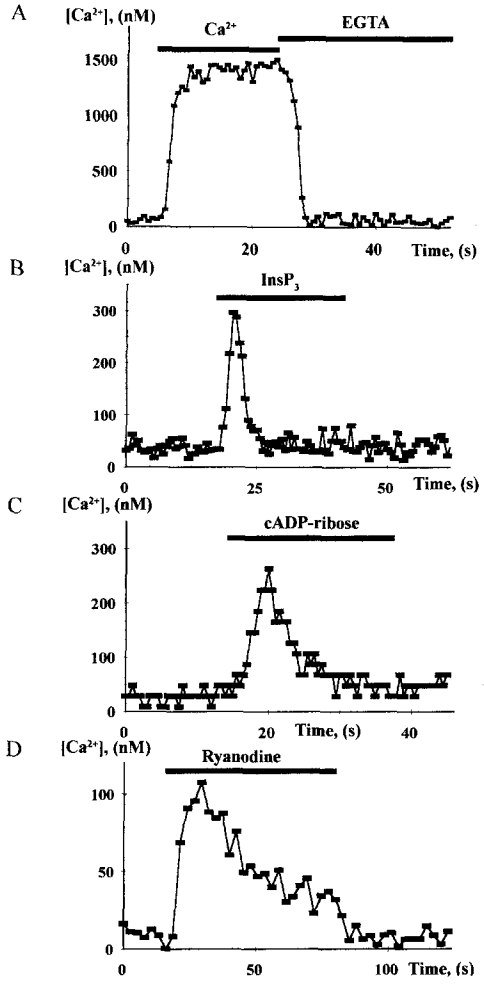

Figure 5. Ca^{2+} Moves Easily across the Nuclear Envelope, and IP_3, cADPr, and Ryanodine Cause Ca^{2+} Release Directed into the Nucleoplasmic Space

Influence of different Ca^{2+} concentrations and IP_3, cADPr, and ryanodine on the free Ca^{2+} concentration in the nucleoplasm. Nuclei loaded by Fura 2–dextran (A–C) or calcium–Green 1–dextran (D).

(A) Subsequent applications of high Ca^{2+} (1 mM) and EGTA (2 mM) to a single isolated nucleus.

(B) Nucleus loaded with Ca^{2+} in the presence of ATP was washed with standard solution (without Ca^{2+} and ATP), and 10 μM IP_3 was applied.

(C) Nucleus loaded with Ca^{2+} in the presence of ATP was washed with standard solution (without Ca^{2+} and ATP), and 10 μM cADPr was applied.

(D) Nucleus loaded with Ca^{2+} in the presence of ATP was washed with standard solution (without Ca^{2+} and ATP), and 1 μM ryanodine was applied.

a rapid transient elevation of the intranuclear Ca^{2+} concentration measured with the dextran-bound Ca^{2+} indicators. The effect of cADPr indicates the presence of functional ryanodine receptors (Meszaros et al., 1993), and we therefore also tested the actions of ryanodine. Ryanodine itself evoked a small but clear rise in the nucleoplasmic Ca^{2+} concentration (Figure 5D) (n = 15). This effect was transient but with a rather longer duration than those induced by IP_3 and cADPr. The dextran-bound Ca^{2+} indicators that accumulate inside the nucleus appear to record Ca^{2+} release from the nuclear envelope, and this release is directed into the nucleoplasm. The transient nature of these responses (Figure 5) is probably due to leakage of Ca^{2+} from the nucleoplasm through the nuclear pore complexes.

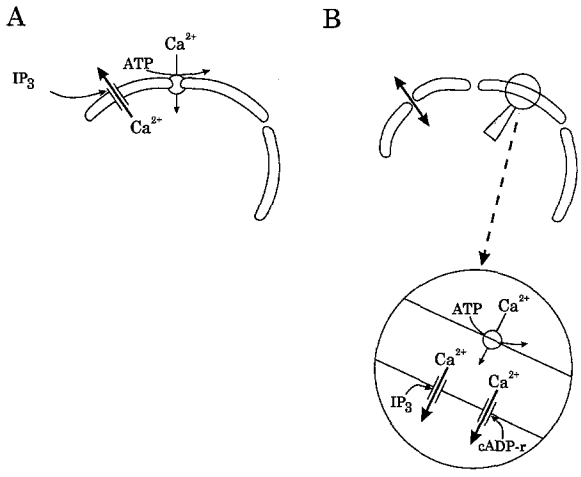

Figure 6. Nuclear Ca^{2+} Transport Models

Schematic diagrams of nuclear Ca^{2+} transport pathways according to earlier results and conclusions from Nicotera et al. (1992) (A) and according to the results presented in this paper (B).

Discussion

Figure 6 summarizes previous conclusions (Nicotera et al., 1989, 1990, 1992) as well as the main conclusions from the results presented in this paper concerning Ca^{2+} transport into and out of isolated nuclei. Although we have reproduced the principal results from the studies of Nicotera et al. (1989, 1990), namely the ATP-dependent Ca^{2+} uptake into isolated nuclei and the IP_3-evoked release of the accumulated Ca^{2+}, we have now shown that these Ca^{2+} transports do not occur into and out of the nucleoplasmic space, but into and out of the nuclear envelope.

The nuclear envelope is a highly dynamic and complex structure (Newport and Forbes, 1987). The outer membrane has ER characteristics and has a different protein composition from the inner membrane. The space between the outer and inner nuclear membranes (the lumen of the nuclear envelope) is an extension of the ER lumen. Our studies do not allow us to reach definitive conclusions about the precise localizations of the Ca^{2+} ATPase and Ca^{2+} release channels. They could in principle be situated on the inner or outer nuclear membranes (or both) or perhaps even on ER extensions of the nuclear envelope that adhere to the nucleus after its isolation. In Figure 6 we have indicated the presence of the two types of Ca^{2+} release channels on the inner nuclear membrane to highlight the finding that IP_3 as well as cADPr can evoke transient rises in the intranucleoplasmic Ca^{2+} concentration, suggesting that the Ca^{2+} release is primarily directed toward the inner nuclear space. We cannot exclude the possibility of Ca^{2+} release from ER membranes into a peripheral space that is close to the nuclear pores and continuous with the intra-

nuclear space, but it should be noted that the high local Ca^{2+} concentrations that can be found in microdomains close to the mouth of IP_3-sensitive channels in intact cells (Rizzuto et al., 1993) depend on the relatively low mobility of Ca^{2+} in the cytosol (Kasai and Petersen, 1994). If there are functional IP_3 receptors on the outside of isolated nuclei placed in a physiological solution, then we would not expect the restrictions to Ca^{2+} diffusion seen in the cytosol (Kasai and Petersen, 1994) to be present, and therefore high local Ca^{2+} concentrations are unlikely. In our experiments the presence of 100 μM high affinity Ca^{2+} chelator EGTA in the bath solution makes it much more unlikely that sufficiently high local Ca^{2+} concentrations could be generated on the outside of the nucleus to influence intranucleoplasmic Ca^{2+} levels, even if IP_3-activated Ca^{2+} release channels were present on the outer surface. Lanini et al. (1992) have previously suggested that Ca^{2+} ATPase molecules are present on both outer and inner nuclear membranes. Our results clearly indicate the existence of Ca^{2+} pumps capable of transporting Ca^{2+} from the environment of the nucleus into the lumen of the nuclear envelope and its associated ER.

Imaging studies on intact cells have shown that in some cases there are very specific subcellular distributions of Ca^{2+} release channels (Burgoyne et al., 1989; Thorn et al., 1993; Kasai et al., 1993; Petersen et al., 1994). A detailed study of specialized subcellular Ca^{2+} release sites requires experiments on isolated cell components. The studies of Nicotera et al. (1990) and Malviya et al. (1990) showed that isolated liver nuclei possess IP_3-sensitive Ca^{2+} release channels. Recently, there has been growing interest in the possibility that cADPr (Lee et al., 1989) may also function as an intracellular Ca^{2+}-releasing messenger. cADPr has been shown directly to regulate single nonskeletal-type ryanodine receptor Ca^{2+} release channels (Meszaros et al., 1993), and recent results in pancreatic acinar cells and neurons indicate that cADPr may be a physiologically important modulator of ryanodine receptors (Hua et al., 1994; Thorn et al., 1994). The results presented in this paper demonstrate directly the presence of functional cADPr and ryanodine receptors in the nuclear envelope. Both the cADPr and IP_3 receptors in the nucleus may contribute to the formation of the regular Ca^{2+} waves sweeping through the hepatocytes in response to agonist stimulation (Thomas et al., 1991).

Experimental Procedures

Single isolated nuclei were obtained from mouse liver by homogenization in sucrose-containing media and by centrifugation using the method of Nicotera et al. (1989) with some modifications; the last high speed centrifugation was omitted, and the final pellet of nuclei was resuspended in standard solution (125 mM KCl, 2 mM K_2HPO_4, 50 mM HEPES, 4 mM $MgCl_2$, 0.1 mM EGTA [pH 7.0]) with 1 mM ATP added.

Isolated nuclei were loaded with Fura 2 by incubation with a Fura 2–AM (20 μM)-containing solution for 45 min at 4°C. Loading of nuclei with fluorescent dextrans (Fura 2–dextran or calcium–Green 1–dextran) was performed by incubation for 10 min at 4°C. Both fluorescent-labeled dextrans were applied at a concentration of 20 μM. Loaded nuclei were washed by centrifugation (1000 × g) for 1 min.

Samples of nuclei loaded with Fura 2 or dextrans labeled with Ca^{2+} indicators were placed in an experimental chamber with a perfusion system and washed by perfusion with standard solution for a few minutes before the beginning of each experiment. Experiments were performed at room temperature (21°C–23°C) on single isolated nuclei. Ethidium bromide staining was used at the end of the experiments.

Staining with ethidium bromide (5 μg/ml) or $DiOC_6(3)$ (2.5 μg/ml) was performed by addition of these dyes to the bath solution for 5 min and subsequent washing with the standard solution. $CaCl_2$ was added to the standard solution to achieve the necessary concentrations of Ca^{2+} in the external solutions. The calcium concentrations in the solutions were regularly checked using fluorescence measurements with Fura 2. Standard Ca^{2+}–EGTA buffers (Molecular Probes) were used for calibration.

Monitoring of the free Ca^{2+} concentrations and the distributions of the fluorescence of the different dyes in the isolated single nuclei was performed using an imaging system from Applied Imaging. Color-coded pictures of the distribution of the fluorescence of Fura 2, Fura 2–dextran, and $DiOC_6(3)$ in isolated nuclei were obtained using the laser scanning confocal microscope ODESSEY (Noran Instruments) with objective Fluor 100×, NA 1.3 oil (Nikon), and a slit of 25 μm.

Fura 2–pentapotassium salt, Fura 2–AM, Fura 2–dextran, calcium–Green 1–AM, calcium–Green 1–dextran, ethidium bromide, and $DIOC_6(3)$ were purchased from Molecular Probes; calmidazolium, IP_3 ionomycin, and ATP from Sigma; cADPr from Amersham; and ryanodine from Calbiochem.

Received August 1, 1994; revised November 3, 1994.

References

Al-Mohanna, F. A., Caddy, K. W. T., and Bolsover, S. R. (1994). The nucleus is insulated from large cytosolic calcium ion changes. Nature *367*, 745–750.

Berridge, M. J. (1993). Inositol trisphosphate and calcium signalling. Nature *361*, 315–325.

Brini, M., Murgia, M., Pasti, L., Picard, D., Pozzan, T., and Rizzuto, R. (1993). Nuclear Ca^{2+} concentration measured with specifically targeted recombinant aequorin. EMBO J. *12*, 4813–4819.

Burgoyne, R. D., Cheek, T. R., Morgan, A., O'Sullivan, A. J., Moreton, R. B., Berridge, M. J., Mata, A. M., Colyer, J., Lee, A. G., and East, J. M. (1989). Distribution of two distinct Ca^{2+}–ATPase-like proteins and their relationship to the agonist-sensitive calcium store in bovine adrenal chromaffin cells. Nature *342*, 72–74.

Divecha, N., Banfic, H., and Irvine, R. F. (1993). Inositides and the nucleus and inositides in the nucleus. Cell *74*, 405–407.

Gillot, I., and Whitaker, M. (1993). Imaging calcium waves in eggs and embryos. J. Exp. Biol. *184*, 213–219.

Hua, S.-Y., Tokimasa, T., Takasawa, S., Furuya, Y., Nohmi, M., Okamoto, H., and Kuba, K. (1994). Cyclic ADP-ribose modulates Ca^{2+} release channels for activation by physiological Ca^{2+} entry in bullfrog sympathetic neurons. Neuron *12*, 1073–1079.

Hechtenberg, S., and Beyersmann, D. (1993). Differential control of free calcium and free zinc levels in isolated bovine liver nuclei. Biochem. J. *289*, 757–760.

Kasai, H., and Petersen, O. H. (1994). Spatial dynamics of second messengers: IP_3 and cAMP as long-range and associative messengers. Trends Neurosci. *17*, 95–101.

Kasai, H., Li, Y. X., and Miyashati, Y. (1993). Subcellular distribution of Ca^{2+} release channels underlying Ca^{2+} waves and oscillations in exocrine pancreas. Cell *74*, 669–677.

Kume, S., Muto, A., Aruya, J., Nakagawa, T., Michikawa, T., Furuiche, T., Nakade, S., Okano, H., and Mikoshiba, K. (1993). The Xenopus IP_3 receptor: structure, function, and localization in oocytes and eggs. Cell *73*, 555–570.

Lang, I., Schulz, M., and Peters, R. (1986). Molecular mobility and nucleocytoplasmic flux in hepatoma cells. J. Cell Biol. *102*, 1183–1190.

Lanini, L., Bachs, O., and Carafoli, E. (1992). The calcium pump of the liver nuclear membrane is identical to that of endoplasmic reticulum. J. Biol. Chem. *267*, 11548–11552.

Lee, H. C., Walseth, T. F., Bratt, G. T., Hayes, R. N., and Clapper,

D. L. (1989). Structural determination of a cyclic metabolite of NAD^+ with intracellular Ca^{2+} mobilizing activity. J. Biol. Chem. *264*, 1608–1615.

Malviya, A. N., Rogue, P., and Vincendon, G. (1990). Stereospecific inositol 1,4,5-[^{32}P] trisphosphate binding to isolated rat liver nuclei: evidence for inositol trisphosphate receptor–mediated calcium release from the nucleus. Proc. Natl. Acad. Sci. USA *87*, 9270–9274.

Meszaros, L. G., Bak, J., and Chu, A. (1993). Cyclic ADP-ribose as an endogenous regulator of the non-skeletal type ryanodine receptor Ca^{2+} channel. Nature *364*, 76–79.

Newport, J., and Forbes, D. J. (1987). The nucleus: structure, function and dynamics. Annu. Rev. Biochem. *56*, 535–565.

Nicotera, P., McConkey, D. J., Jones, D. P., and Orrenius, S. (1989). ATP-stimulates Ca^{2+} uptake and increases the free Ca^{2+} concentration in isolated rat liver nuclei. Proc. Natl. Acad. Sci. USA *86*, 453–457.

Nicotera, P., Orrenius, S., Nilsson, T., and Berggren, P.-O. (1990). An inositol 1,4,5-trisphosphate-sensitive Ca^{2+} pool in liver nuclei. Proc. Natl. Acad. Sci. USA *87*, 6858–6862.

Nicotera, P., Bellomo, G., and Orrenius, S. (1992). Calcium-mediated mechanisms in chemically induced cell death. Annu. Rev. Pharmacol. Toxicol. *32*, 449–470.

Petersen, O. H., Petersen, C. C. H., and Kasai, H. (1994). Calcium and hormone action. Annu. Rev. Physiol. *56*, 297–319.

Rizzuto, R., Brini, M., Murgia, M., and Pozzan, T. (1993). Microdomains with high Ca^{2+} close to IP_3-sensitive channels that are sensed by neighboring mitochondria. Science *262*, 744–747.

Sullivan, K. M. C., Busa, W. B., and Wilson, K. L. (1993). Calcium mobilization is required for nuclear vesicle fusion in vitro: implications for membrane traffic and IP_3 receptor function. Cell *73*, 1411–1422.

Thomas, A. P., Renard, D. C., and Rooney, T. A. (1991). Spatial and temporal organization of calcium signalling in hepatocytes. Cell Calcium *12*, 111–126.

Thorn, P., Lawrie, A. M., Smith, P. M., Gallacher, D. V., and Petersen, O. H. (1993). Local and global cytosolic Ca^{2+} oscillations in exocrine cells evoked by agonists and inositol trisphosphate. Cell *74*, 661–668.

Thorn, P., Gerasimenko, O., and Petersen, O. H. (1994). Cyclic ADP-ribose regulation of ryanodine receptors involved in agonist evoked cytosolic Ca^{2+} oscillations in pancreatic acinar cells. EMBO J. *13*, 2038–2043.

Tombes, R. M., Simerly, C., Borisy, G. G., and Schatten, G. (1992). Meiosis, egg activation and nuclear breakdown are differentially reliant on Ca^{2+}, whereas germinal vesicle breakdown is Ca^{2+}-independent in the mouse oocyte. J. Cell Biol. *117*, 799–811.