DEVELOPMENTAL BIOLOGY 161, 466-476 (1994)

Presence of Inositol 1,4,5-Trisphosphate Receptor, Calreticulin, and Calsequestrin in Eggs of Sea Urchins and *Xenopus laevis*

JAN B. PARYS,*[,1] SANDRA M. MCPHERSON,† LORI MATHEWS,† KEVIN P. CAMPBELL,* AND FRANK J. LONGO†

*Howard Hughes Medical Institute and Department of Physiology and Biophysics and †Department of Anatomy, University of Iowa College of Medicine, Iowa City, Iowa 52242

Accepted October 1, 1993

The presence of inositol 1,4,5-trisphosphate receptor ($InsP_3R$), calreticulin, and calsequestrin was demonstrated in eggs of sea urchins (*Lytechinus pictus, Lytechinus variegatus,* and *Strongylocentroutus purpuratus*) and *Xenopus laevis*. Binding of inositol 1,4,5-trisphosphate ($InsP_3$) to microsomes of *L. pictus* eggs was inhibited by heparin and NaCl. An affinity-purified antibody against the C-terminal of the type I $InsP_3R$, which recognizes $InsP_3R$ isoforms of rabbit brain (273 kDa) and *Xenopus* oocytes and eggs (256 kDa), reacted with a 373-kDa protein in sea urchin eggs. The 373-kDa protein was tentatively identified as the sea urchin egg $InsP_3R$. Observations with fluorescence microscopy indicated that the $InsP_3R$ is present throughout the cytoplasm of sea urchin eggs in a pattern consistent with the distribution of endoplasmic reticulum. Small differences in the relative amount of reaction deposits in cortex vs subcortex were noted among the species of sea urchins examined. Reaction product was also localized to the periphery of female pronuclei in eggs of all three sea urchins. $InsP_3R$ reactivity was present in the perinuclear region, along the periphery of the germinal vesicle, and throughout the animal and vegetal hemispheres of *Xenopus* oocytes. A similar cytoplasmic staining pattern was also observed in eggs, although islands of reactivity, much larger than those in oocytes, were present in the animal hemisphere of eggs. Calreticulin and calsequestrin in sea urchin eggs had the same molecular mass as in rabbit brain (56 and 60 kDa, respectively), but differed from those present in *Xenopus* oocytes/eggs (61 and 57 kDa, respectively). The distribution of calreticulin and calsequestrin in both sea urchin and *Xenopus* oocytes and eggs was similar to that observed for the $InsP_3R$. These results are discussed in relation to previous studies of Ca^{2+} regulation during egg development and fertilization and suggest that in the oocytes and eggs of the species examined, $InsP_3$-sensitive Ca^{2+} stores play an important role in the regulation of cellular Ca^{2+}.

INTRODUCTION

Activation of deuterostome eggs is dependent on a transient increase in intracellular Ca^{2+}, which is initiated at the site of sperm-egg fusion and is then propagated through the egg as a Ca^{2+} wave (Jaffe, 1983; Busa, 1990). Although various activators, including $InsP_3$,[2] cGMP, cADP-ribose, and Ca^{2+} itself, have been proposed as initiators of the Ca^{2+} signal, the exact mechanisms whereby the Ca^{2+} wave is initiated and propagated are not yet known (Busa, 1990; Whitaker and Crossley, 1990; Nuccitelli, 1991; Whalley *et al.*, 1992).

In somatic cells, two mechanisms involved in the elevation of intracellular Ca^{2+} have been described: $InsP_3$-induced Ca^{2+} release (IICR) and Ca^{2+}-induced Ca^{2+} release (CICR) (Berridge and Irvine, 1989). While the former system is mediated by the $InsP_3R$ (Ferris *et al.*, 1989), the latter was generally thought to be regulated by the ryanodine receptor (Imagawa *et al.*, 1987; McPherson *et al.*, 1991). Recent evidence indicates that the $InsP_3R$ can also induce CICR (see Berridge, 1993).

A substantial literature exists concerning the effects of $InsP_3$ in oocytes and eggs of different species, and recently the presence of an $InsP_3R$ was demonstrated in fertilized hamster eggs (Miyazaki *et al.*, 1992) and in *Xenopus laevis* oocytes and eggs (Parys *et al.*, 1992; Kume *et al.*, 1993). The *Xenopus* oocyte/egg $InsP_3R$ has been purified and shown to represent a different isoform of the type I $InsP_3R$ (Parys *et al.*, 1992). Sequence analysis demonstrated that the *Xenopus* oocyte/egg $InsP_3R$ shares 90% identity at the amino acid level with the mouse type I $InsP_3R$ (Kume *et al.*, 1993). No evidence was

[1] Present address: Laboratorium voor Fysiologie, KULeuven, B-3000 Leuven, Belgium.

[2] Abbreviations used: $InsP_3$, D-*myo*-inositol 1,4,5-trisphosphate; $InsP_3R$, $InsP_3$ receptor; AH, animal hemisphere; VH, vegetal hemisphere; IICR, $InsP_3$-induced Ca^{2+} release; CICR, Ca^{2+}-induced Ca^{2+} release; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; ASW, artificial seawater; CaFASW, Ca^{2+}-free artificial seawater; CIB, cortical isolation buffer; BSA, bovine serum albumin.

0012-1606/94 $6.00

found for a ryanodine receptor in *Xenopus* eggs and oocytes (Parys *et al.*, 1992). Recent investigations indicate that Ca^{2+} waves in *Xenopus* oocytes and hamster eggs, which have been described as resulting from CICR, are due to a Ca^{2+}-modulated serial Ca^{2+} release of $InsP_3$-sensitive Ca^{2+} stores (Lechleiter and Clapham, 1992; DeLisle and Welsh, 1992; Miyazaki *et al.*, 1992; Parys *et al.*, 1992). In sea urchin eggs the presence of $InsP_3$-sensitive Ca^{2+} stores is well established (Whitaker and Irvine, 1984; Clapper and Lee, 1985; Turner *et al.*, 1986; Oberdorf *et al.*, 1986; Swann and Whitaker, 1986) but recent evidence also indicates the presence of $InsP_3$-insensitive Ca^{2+} stores and of a ryanodine receptor-like protein (Rakow and Shen, 1990; Fujiwara *et al.*, 1990; Galione *et al.*, 1991; McPherson *et al.*, 1992; Buck *et al.*, 1992).

The exact roles of the $InsP_3R$ and $InsP_3$-sensitive stores in eggs have not yet been delineated. We, therefore, investigated the presence and localization of the $InsP_3R$ in eggs of two organisms commonly employed in developmental biology studies, sea urchins (*Lytechinus pictus*, *Lytechinus variegatus*, and *Strongylocentrotus purpuratus*) and the South African clawed toad, *Xenopus laevis*. In addition, the presence and localization of two Ca^{2+} binding proteins, calreticulin and calsequestrin, were determined. Calreticulin, widely distributed in muscle and nonmuscle tissues (Treves *et al.*, 1990; Milner *et al.*, 1991; Tharin *et al.*, 1992), is believed to be associated with $InsP_3$-sensitive Ca^{2+} stores (Krause *et al.*, 1990; Van Delden *et al.*, 1992). Calsequestrin in chicken cerebellum is also associated with $InsP_3$-sensitive stores (Volpe *et al.*, 1990). Using biochemical and microscopic immunomethods, we show that the $InsP_3R$ is present throughout the cytoplasm of sea urchin and *Xenopus* eggs and oocytes, in a pattern consistent with the distribution of endoplasmic reticulum. Calreticulin and calsequestrin have fluorescent localization patterns similar to that of the $InsP_3R$. These results indicate important roles for the $InsP_3R$ and for $InsP_3$-sensitive Ca^{2+} stores during gamete development and fertilization.

MATERIALS AND METHODS

Preparation and analysis of membrane fractions. Eggs and pellets of isolated cortices of sea urchins, *L. pictus*, *L. variegatus*, and *S. purpuratus*, were prepared as described (McPherson *et al.*, 1992). The supernatant, obtained after isolation of cortices, contained most of the cytoplasm and was brought to pH 7.25 by addition of maleic acid. Tris-HCl (pH 7.25) and sucrose were added to final concentrations of 20 and 100 m*M*, respectively. After pelleting a fraction enriched in yolk bodies (4500*g*, 15 min), microsomes were recovered by high-speed centrifugation (142,000*g*, 35 min). Full-grown (stage VI) oocytes were procured from ovaries of *X. laevis;* unfertilized, mature eggs were obtained from gravid females stimulated to ovulate by an injection of human chorionic gonadotropin (Parys *et al.*, 1992). Membranes (yolk bodies, melanosomes, and microsomes) from oocytes and eggs of *Xenopus* (Parys *et al.*, 1992), rabbit light sarcoplasmic reticulum (Campbell *et al.*, 1980), and rabbit whole brain or rabbit cerebellar microsomes (McPherson and Campbell, 1990) were obtained as described and suspended in end medium (20 m*M* Tris-HCl, pH 7.25, 300 m*M* sucrose, 0.8 m*M* benzamidine; 0.2 m*M* PMSF). Protein was determined by a modified Lowry procedure (Peterson, 1977). Measurement of [^{3}H]$InsP_3$ binding activity was performed according to Parys *et al.* (1992).

Antibodies. Antibodies against the $InsP_3R$ (Parys *et al.*, 1992) and chicken cardiac calsequestrin (Jorgensen and Campbell, 1984) were prepared as described. Pure recombinant calreticulin and an antibody against calreticulin were a gift from Dr. R. Clark (University of Iowa). Except where indicated, all antibodies used were affinity-purified as described previously (Parys *et al.*, 1992).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. Samples were analyzed on 3–12% linear gradient SDS-PAGE (Laemmli, 1970). Proteins were transferred to nitrocellulose (Towbin *et al.*, 1979) followed by overnight incubation with antibodies and visualized using peroxidase-conjugated secondary antibodies.

Immunofluorescence microscopy. Sea urchin eggs were fixed in 3% paraformaldehyde/0.1% glutaraldehyde in artificial seawater (ASW), in Ca^{2+}-free artificial seawater (CaFASW; McPherson *et al.*, 1992), or in cortical isolation buffer (CIB; 0.4 *M* mannitol, 0.2 *M* KCl, 50 m*M* Hepes, 50 m*M* Pipes, 2.5 m*M* $MgCl_2$, 20 m*M* EGTA, pH 6.8). *Xenopus* oocytes and eggs were fixed in 3% paraformaldehyde/0.1% glutaraldehyde in 50 m*M* sodium phosphate buffer, pH 7.4, amphibian Ringers (73 m*M* NaCl, 2 m*M* KCl, 25 m*M* $NaHCO_3$), or CIB. Fixation was for 2 hr at 4°C, followed by an overnight wash in the corresponding buffer. Fixed specimens were embedded in gelatin as described by Henson *et al.* (1989) or in Tissue-Tek (Miles Inc., Elkhart, IN), frozen in liquid nitrogen, and cryosectioned.

Sections were blocked with 0.2% bovine serum albumin (BSA) in PBS (137 m*M* NaCl, 3 m*M* KCl, 6 m*M* $NaHPO_4$, 1.5 m*M* KH_2PO_4, pH 7.4; 15 min) and incubated for 30 min at room temperature in the affinity-purified antibodies listed above. Cryosections 5 μm thick were incubated with affinity-purified antibodies diluted 1:2 to 1:10 with 0.2% BSA in PBS. Specimens were washed twice in PBS, followed by a 30-min incubation in FITC-conjugated secondary antibody. After two 5-min washes in PBS, the specimens were mounted in 0.1%

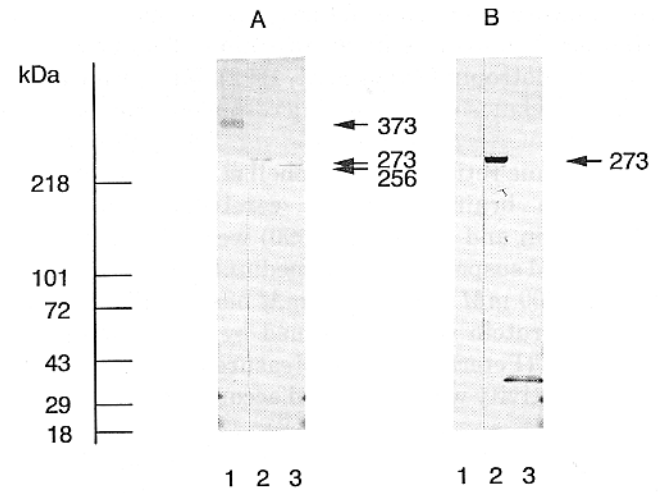

FIG. 1. Immunoblot of $InsP_3R$ molecules using Rbt52, an affinity-purified antibody directed against the C-terminal of the type I $InsP_3R$ (A), or Goat44, an affinity-purified polyclonal antibody against rabbit brain $InsP_3R$ (B). Samples: *L. pictus* cortical membranes, 100 μg (1); rabbit cerebellar membranes, 50 μg (2); and *Xenopus* oocyte microsomes, 200 μg (3). The molecular masses of the $InsP_3R$ molecules are indicated.

p-phenylenediamine in 90% glycerol, examined with an inverted microscope (Nikon Inc., Garden City, NJ) equipped with fluorescence optics, and photographed with TMAX 400 film (Eastman Kodak Co., Rochester, NJ). To demonstrate antibody specificity, specimens were incubated in (1) secondary antibody without a prior treatment with primary antibody or (2) immunodepleted solutions (McPherson *et al.*, 1992). The affinity-purified antibody against the C-terminal of the type I $InsP_3R$ was diluted 1:4 with 0.2% BSA in PBS and then incubated with the C-terminal peptide. After a 2-hr incubation, cryosections were reacted first with the processed fluid and then with the secondary antibody as described above.

RESULTS

Structural and biochemical analyses of the sea urchin egg $InsP_3R$. Specific $InsP_3$ binding activity of *L. pictus* microsomes was 50 ± 21 fmole/mg protein (n = 3) and 95% inhibited by heparin (50 μg/ml). The high binding activity and heparin sensitivity strongly suggested the presence of an $InsP_3R$, similar to $InsP_3R$ molecules from higher organisms. This was confirmed in Western blots, as an antibody against the C-terminal of the type I $InsP_3R$ (Rbt52) recognized a single protein band in *L. pictus* membrane preparations (Fig. 1a). The protein band had a distinctly higher molecular mass (373 ± 7 kDa, n = 6) than the $InsP_3R$ from mammalian brain or from *Xenopus* oocytes and eggs (Fig. 1a). An affinity-purified polyclonal antibody directed against pure rabbit brain $InsP_3R$ (Goat44) did not recognize any protein on immunoblots of *L. pictus* membranes (Fig. 1b). This was not surprising as the antibody did not recognize the $InsP_3R$ from *Xenopus* oocytes. In *Xenopus* oocytes (but not in eggs; data not shown) a 33-kDa protein was specifically labeled by the Goat44 antibody; its relation to the $InsP_3R$ is unknown.

Isolation of the $InsP_3R$ from sea urchin eggs was attempted; however, the scheme used for purification of the $InsP_3R$ from mammalian brain and from amphibian oocytes and eggs (Parys *et al.*, 1992) resulted in a rapid decay of [^{3}H]$InsP_3$ binding activity in sea urchin egg preparations. Binding of $InsP_3$ to *L. pictus* egg microsomes was found to be highly salt-sensitive (Fig. 2). Sodium chloride, even at concentrations as low as 100 m*M*, inhibited $InsP_3$ binding. $InsP_3$ binding to rabbit brain microsomes, which was used as a control, was much less sensitive to NaCl. KCl only slightly influenced $InsP_3$ binding in both *L. pictus* egg and rabbit brain microsomes (Fig. 2). Since the decay of binding activity during purification could be due to the strong inhibiting effects of Na^+, we attempted to purify the *L. pictus* $InsP_3R$ under conditions where Na^+ was replaced by K^+. Decay of binding activity was, however, still observed, suggesting that during solubilization and purification a co-factor or a subunit necessary for $InsP_3$ binding is lost. These findings are consistent with observations by Lee (1991), who described the lability of $InsP_3$-sensitive mechanisms in sea urchin eggs. In comparison to *L. pictus* preparations, lower binding activities were obtained from membranes isolated from *L. variegatus* and *S. purpuratus* (data not shown). We do not know if this difference in binding activity reflects a greater lability of the

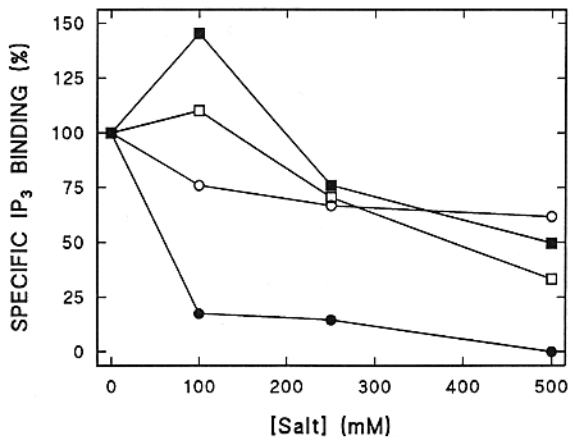

FIG. 2. The effect of varying NaCl and KCl concentrations on $InsP_3$ binding activity in microsomes isolated from *L. pictus* eggs (closed symbols) or from rabbit brain (open symbols). Binding of [^{3}H]$InsP_3$ was measured as previously described (Parys *et al.*, 1992) except for the addition of NaCl (circles) or KCl (squares). Each point is the mean of at least two independent experiments, each done in triplicate.

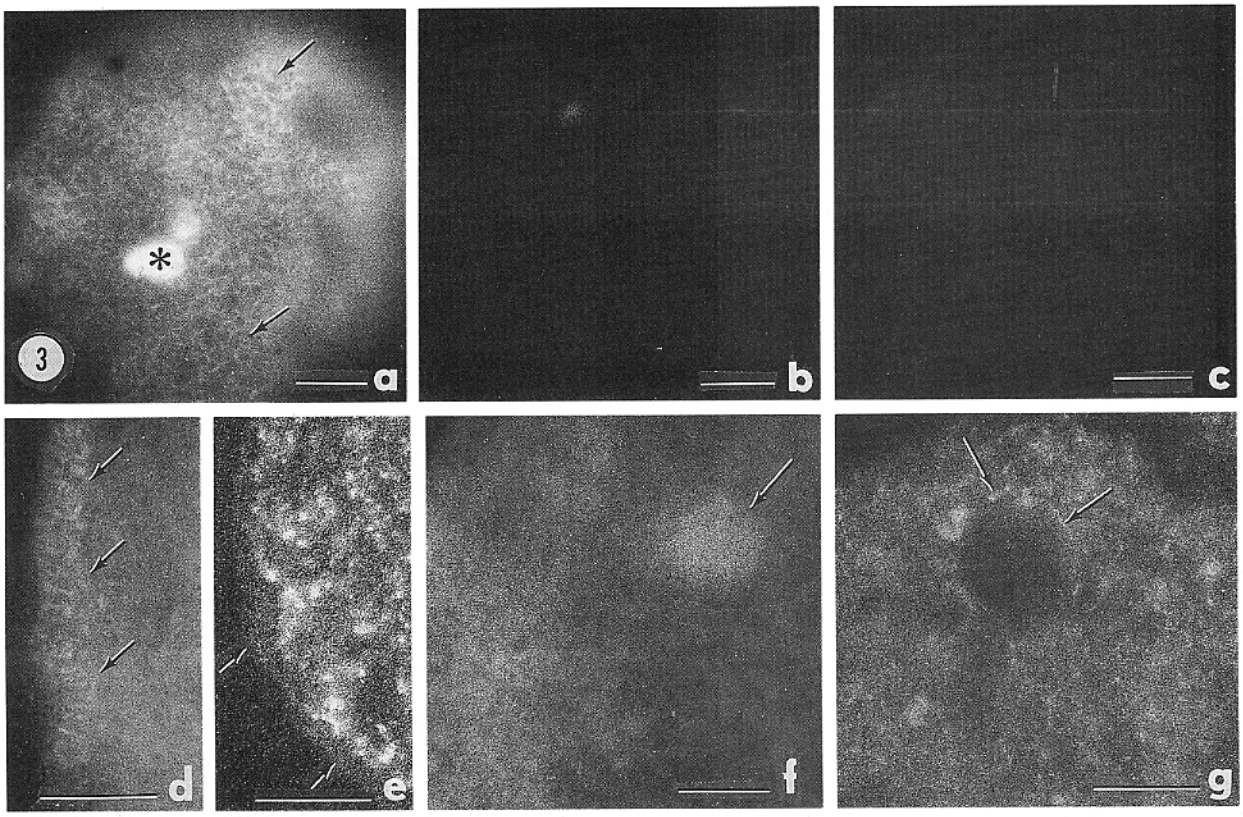

FIG. 3. Immunofluorescent preparations demonstrating the localization of the $InsP_3R$, using affinity purified Rbt52 antibody, in eggs of the sea urchins *S. purpuratus* (a–d and f) and *L. pictus* (e and g). Similar immunofluorescent patterns were also observed in *L. variegatus*. (a) In *S. purpuratus* a reticular staining pattern (arrows) is distributed throughout the egg cytoplasm. (b and c) Control sections of *S. purpuratus* eggs stained only with secondary antibody (b) or with immunodepleted antibody solution (c). (d and e) In *S. purpuratus* (d) cortical staining (arrows) is frequently more intense than that within the subcortex, while in *L. pictus* (e) cortical staining (arrows) is not as bright as that in the subcortex. (f and g) *S. purpuratus* (f) and *L. pictus* (g) eggs which demonstrate $InsP_3R$ reactivity in conjunction with the female pronucleus (arrows). The section in (f) depicts a "surface view" of one pole of the female pronucleus while that in (g) is cut through the organelle and demonstrates reaction product along its perimeter. *, Stained debris. Scale bar, 10 μm.

receptor, differences between species, or environmental factors, such as seasonal variations, that may affect egg development.

Immunolocalization of the InsP$_3$R. Overall, similar staining patterns, employing affinity-purified Rbt52 antibody, were observed in eggs of the three species of sea urchins used in this study; similarities and differences in staining are presented as appropriate. Stained cryosections of all three species contained reaction deposits that were present throughout the entire egg cytoplasm (Figs. 3a–3e), consistent with the distribution of cortical and subcortical endoplasmic reticulum (Terasaki and Jaffe, 1991). A reticular pattern of staining was clearly recognizable in *S. purpuratus* (Fig. 3a), whereas in *L. pictus* and *L. variegatus* reaction deposits were of varying sizes and, overall, formed a less organized network (Fig. 3e). The intensity of cortical and subcortical staining was similar with small variations among species. In *S. purpuratus*, cortical reactivity was often slightly more intense than that present in the subcortex (Fig. 3d), whereas in *L. pictus* cortical staining was often less than that present in the subcortex (Fig. 3e). In all three species, but particularly in *L. pictus* and *S. purpuratus*, antibody staining was also localized to the periphery of the female pronucleus (Figs. 3f and 3g). Cryosections incubated with only the secondary antibody or with immunodepleted solutions were negative (Figs. 3b and 3c).

Reactivity of *Xenopus* oocytes to Rbt52 anti-$InsP_3R$ was distributed throughout the cytoplasm of the animal hemisphere (AH) and vegetal hemisphere (VH) (Fig. 4). Staining in the oocyte AH consisted of interconnected, linear areas that outlined relatively small, and mostly irregular, nonstaining regions (Fig. 4a). The oocyte AH was, for the most part, uniformly stained, although reactivity within its cortex was frequently obscured by pigment granules (Fig. 4a). In contrast, staining in the VH consisted of a relatively larger network that, in general, filled the interstices between the large nonstaining yolk bodies characterizing this region of the oocyte (Fig. 4b).

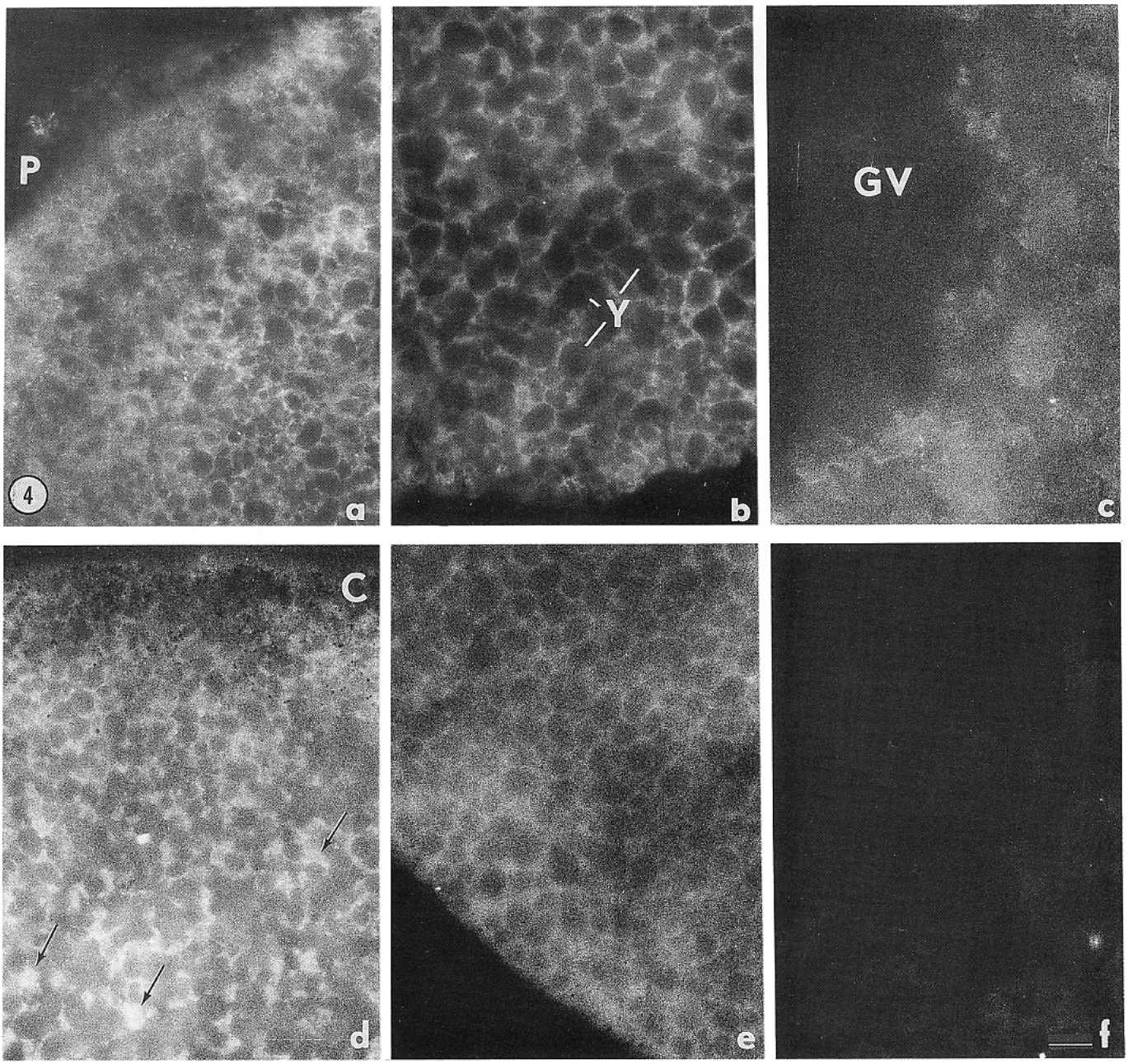

FIG. 4. *Xenopus* oocyte (a–c) and egg (d–f) reacted with affinity-purified Rbt52 antibody to the C-terminal of the $InsP_3R$. Reaction product, distributed throughout the cytoplasm, is present within the AH (a and d) and VH (b and e) of eggs and oocytes. (a) AH of an oocyte showing a reticular staining pattern circumscribing relatively small, nonstaining spaces. The dense pigment layer (P) of the AH partially obscures staining within the cortex. (b) In the VH reactivity is present in regions between yolk bodies (Y) and forms a reticular pattern larger than that present in the AH. (c) Perinuclear reactivity and staining associated with the germinal vesicle (GV). (d) Reactivity in the AH of eggs forms a reticular pattern consisting of relatively large islands (arrows) of staining. In addition to pigment granules, staining is apparent within the cortex (C). (e) Reticular staining pattern characteristic of the VH of eggs. (f) Control section of an egg stained with only secondary antibody. Scale bar, 10 μm.

Major differences in staining intensity of the cortex, subcortex, and interior deep cytoplasm of the VH were not readily apparent (Fig. 4b). Reaction deposits were also present in the perinuclear cytoplasm and associated with the periphery of the germinal vesicle (Fig. 4c). Overall, the staining pattern in eggs was similar to that observed in oocytes, i.e., it consisted of a reticulum that was present throughout the entire egg cytoplasm (Figs. 4d and 4e), corresponding to the cytosolic spaces between yolk bodies. The reticulum in the AH, however,

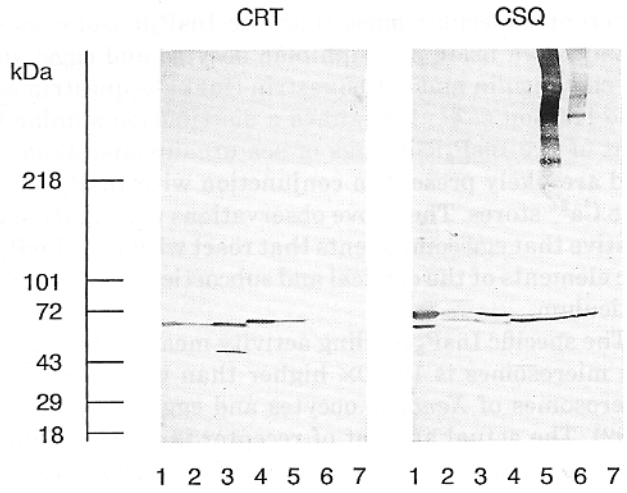

FIG. 5. Detection of calreticulin and calsequestrin on immunoblots of sea urchin and *Xenopus* specimens. Samples: rabbit light sarcoplasmic reticulum, 40 μg (1); *L. pictus* cortical membranes, 200 μg (2); *L. pictus* microsomes, 200 μg (3); *Xenopus* oocyte microsomes, 200 μg (4); *Xenopus* egg microsomes, 200 μg (5); *Xenopus* egg melanosomes, 200 μg (6); and *Xenopus* egg yolk bodies, 200 μg (7). After SDS-PAGE and immunoblotting, staining was with affinity-purified antibody against calreticulin (left) or with antiserum against chicken cardiac calsequestrin (right).

possessed relatively large islands of intense staining (Fig. 4d). Differences in the staining of the VH of eggs and oocytes were not apparent (Figs. 4b and 4e). Control sections in which the primary antibody was omitted were negative (Fig. 4f).

Presence of Ca^{2+} binding proteins. An affinity-purified antibody against calreticulin (Fig. 5, left) recognized a protein in sea urchin eggs with the same molecular mass (56 ± 0.4 kDa, $n = 5$) as mammalian muscle calreticulin (56 ± 0.5 kDa, $n = 4$). Calreticulin in *Xenopus* oocytes and eggs had a somewhat higher molecular mass (61 ± 1 kDa, $n = 5$). To identify calsequestrin, we employed an antiserum against chicken cardiac calsequestrin (Jorgensen and Campbell, 1984) which was previously used to identify a calsequestrin-like protein in sea urchin eggs (Oberdorf *et al.*, 1988) and *Xenopus* oocytes (Parys *et al.*, 1992). As is apparent from data in Fig. 5 (right), this antiserum recognized two proteins, calsequestrin itself and also calreticulin, to which it demonstrated a weak interaction. Calsequestrin of sea urchin eggs had a molecular mass (61 ± 1 kDa, $n = 5$) similar or equal to mammalian muscle calsequestrin (60 ± 1 kDa, $n = 4$). In *Xenopus* oocytes and eggs, calsequestrin had a smaller molecular mass (57 ± 1 kDa, $n = 5$). Both calreticulin and calsequestrin showed a similar distribution in fractionated preparations. In sea urchin eggs, microsomes were enriched in both proteins when compared to isolated cortices (Fig. 5, lanes 2 and 3). In *Xenopus*, calreticulin and calsequestrin were also present in the microsomal fraction with negligible amounts in melanosomes or yolk bodies (Fig. 5, lanes 4–7). In addition, *Xenopus* oocytes contained more of the Ca^{2+} binding proteins than eggs (Fig. 5, lanes 4 and 5).

Cross-reactivity of the anti-calsequestrin antibodies with calreticulin is a widely observed problem which has led to confusion in the past (discussed in Michalak *et al.*, 1992). Therefore, we affinity-purified the antiserum to chicken cardiac calsequestrin using purified rabbit skeletal muscle calsequestrin. The rationale was to obtain antibodies recognizing only evolutionarily conserved regions. The resulting antibody reacted with calsequestrin from various sources, including rabbit and *Xenopus* skeletal muscle, but not oocytes and eggs (data not shown). Presumably, the oocyte and egg calsequestrin shares a certain homology with chicken cardiac calsequestrin, but little or none with skeletal muscle calsequestrin.

Localization of calreticulin and calsequestrin. Calreticulin staining was comparable in the three species of sea urchins employed here and the pattern of localization was similar to that seen with affinity-purified antibody to the $InsP_3R$ (Fig. 6). Staining consisted of particulate fluorescent deposits, which were present throughout the egg cytoplasm (Fig. 6a). Control preparations were negative (data not shown). In *Xenopus* oocytes and eggs localization of affinity-purified calreticulin antibody was also similar to that seen with affinity-purified antibody to the $InsP_3R$, i.e., fluorescent staining was present in the interstices between yolk bodies in both the AH (Fig. 6b) and the VH (data not shown). Control preparations of *Xenopus* oocytes and eggs stained only with the second antibody were negative (data not shown).

Immunolocalization of calsequestrin in sea urchin eggs confirmed the results of Henson *et al.* (1989). Eggs from all three species of sea urchins demonstrated a reticulum consisting of fine particulate staining throughout the cytoplasm, similar to the pattern seen with antibody to the $InsP_3R$. In *Xenopus* oocytes and eggs, there was an intense reactivity to the anti-calsequestrin antibody, which was localized to the interstices between the yolk bodies (data not shown).

DISCUSSION

Although functional effects of $InsP_3$ in sea urchin eggs have been described (Whitaker and Irvine, 1984; Clapper and Lee, 1985; Turner *et al.*, 1986; Oberdorf *et al.*, 1986; Swann and Whitaker, 1986), the presence and localization of an $InsP_3R$ similar to that of higher organisms (Furuichi *et al.*, 1989) have heretofore, not been determined. This is particularly important in view of the potential role of the $InsP_3R$ in the propagation of Ca^{2+} waves in oocytes and eggs (Lechleiter and Clapham,

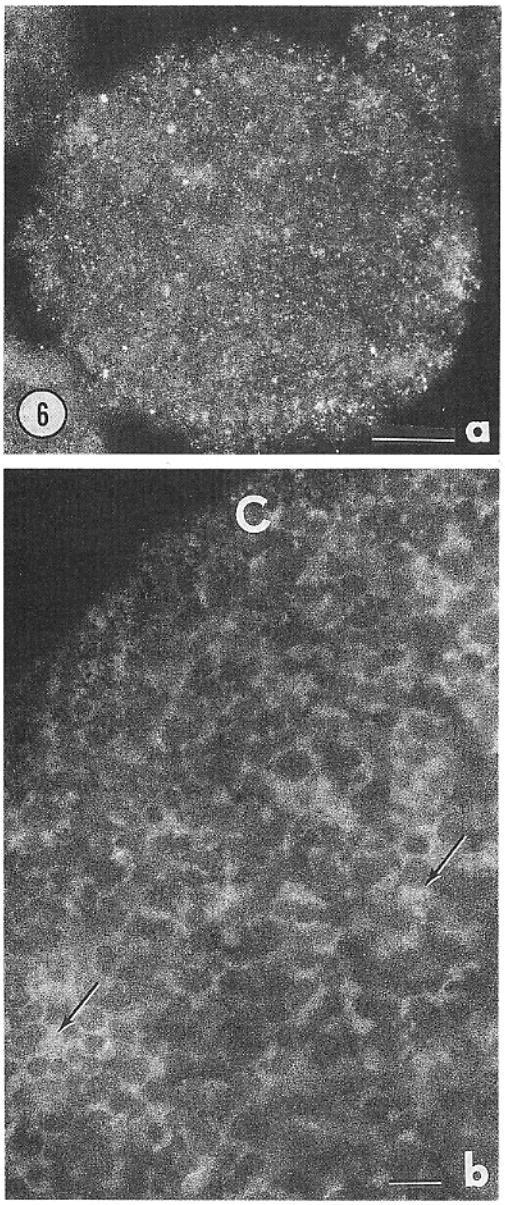

FIG. 6. Localization of calreticulin in *L. pictus* and *Xenopus* eggs. (a) Using an affinity-purified antibody against calreticulin, reaction deposits are distributed throughout the egg cytoplasm of *L. pictus*. Similar localization patterns are found in *L. variegatus* and *S. purpuratus* eggs. (b) Reactivity in the AH of *Xenopus* eggs forms a pattern similar to that seen with affinity-purified Rbt52 antibody in which relatively large islands (arrows) of staining are present. C, cortex. Scale bar, 10 μm.

1992; DeLisle and Welsh, 1992; Miyazaki *et al.*, 1992; Parys *et al.*, 1992). Results of the present study indicate that: (1) microsomes from sea urchin eggs display a heparin-sensitive $InsP_3$ binding activity; (2) an affinity-purified antibody against the C-terminal of the type I $InsP_3R$ recognizes a single protein band on immunoblots of sea urchin egg membranes; this protein band has a different molecular mass than the $InsP_3R$ isoforms of mammalian brain or amphibian oocytes and eggs; and (3) calreticulin and calsequestrin (for calsequestrin see also Henson *et al.*, 1989) have a distribution similar to that of the $InsP_3R$ in eggs of sea urchins and *Xenopus* and are likely present in conjunction with $InsP_3$-sensitive Ca^{2+} stores. The above observations are highly suggestive that egg components that react with anti-$InsP_3R$ are elements of the cortical and subcortical endoplasmic reticulum.

The specific $InsP_3$ binding activity measured in *L. pictus* microsomes is 10–20× higher than that present in microsomes of *Xenopus* oocytes and eggs (Parys *et al.*, 1992). The actual amount of receptor is, however, difficult to estimate, since aggregation of membrane vesicles (Parys *et al.*, 1992), or inhibition of activity, e.g., by Na^+, can lead to underestimation. The inhibition of binding by heparin is an important, distinguishing property common to the $InsP_3R$ from various tissues, including those from mammalian brain (Supattapone *et al.*, 1988), smooth muscle (Mourey *et al.*, 1990), and *Xenopus* oocytes (Parys *et al.*, 1992; see also DeLisle and Welsh, 1992).

In *Strongylocentrotus droebachiensis* both isolated cortices and microsomes sequester Ca^{2+} in an ATP-dependent manner, but $InsP_3$-induced Ca^{2+} release is predominant in the former (Oberdorf *et al.*, 1986). Similarly, $InsP_3$-induced Ca^{2+} release was measured in the cortical fraction of *Arbacia punctulata* (Terasaki and Sardet, 1991). In *L. pictus*, however, $InsP_3$-induced Ca^{2+} release was observed in a membrane fraction enriched in glucose-6-phosphatase, a marker for the endoplasmic reticulum (Clapper and Lee, 1985). These differences may reflect species variations, but as discussed by Oberdorf *et al.* (1986), they may be a manifestation of methodological differences, and in particular of the salt composition of the buffers used. Oberdorf *et al.*'s (1986) hypothesis, that a component necessary for $InsP_3$-induced Ca^{2+} release is lost in high-salt conditions, is in excellent agreement with our observations that NaCl inhibits $InsP_3$ binding in sea urchin egg microsomes.

An affinity-purified antibody against the C-terminal of the type I $InsP_3R$ recognized only one protein on immunoblots from membrane fractions isolated from different species. In rabbit brain and *Xenopus* oocytes, proteins of 273 and 256 kDa were shown to represent two different $InsP_3R$ molecules (Parys *et al.*, 1992). The lower molecular mass of the *Xenopus* oocyte/egg $InsP_3R$ is predominantly due to the absence of the S I and S II splice regions (Kume *et al.*, 1993). It should be pointed out that the above-mentioned molecular mass values are based on the mobility of the proteins on SDS–polyacrylamide gels. However, higher values have been obtained from sequence data of $InsP_3R$ cloned from differ-

ent sources (307 kDa, Südhof *et al.*, 1991, Kume *et al.*, 1993; 313 kDa, Furuichi *et al.*, 1989; 319 kDa, Yoshikawa *et al.*, 1992), indicating anomalous migration of the receptor during electrophoresis. It is, therefore, reasonable to suggest that the protein observed with a molecular mass of 373 kDa in sea urchin membranes represents an $InsP_3R$ molecule. The difference in molecular mass, the extreme salt sensitivity of the $InsP_3R$ binding site, the ineffectiveness of established purification procedures to successfully purify the sea urchin receptor, and the inability of the Goat44 polyclonal antibody to recognize the molecule suggest that a different isoform is present in sea urchin eggs. These distinctions, which may be due to differences in amino acid sequence and/or to post-translational modifications, are not surprising. Investigations with mammalian tissues have demonstrated the existence of several $InsP_3R$ isoforms, which are the products of at least three genes (Furuichi *et al.*, 1989; Südhof *et al.*, 1991; Ross *et al.*, 1992), and of alternative splicing mechanisms (Mignery *et al.*, 1990; Danoff *et al.*, 1991; Nakagawa *et al.*, 1991). Therefore, it is not unexpected that in evolutionarily distant phyla, other types of $InsP_3R$ could evolve (see Yoshikawa *et al.*, 1992). It is interesting to note that the ryanodine receptor in sea urchin eggs (McPherson *et al.*, 1992) also has a molecular mass different from that of its mammalian, avian, amphibian, and piscine counterparts (Olivares *et al.*, 1991), but is similar to that of the $InsP_3R$ described in the present study.

The localization of the $InsP_3R$ is essentially similar in the three species of sea urchins examined here, i.e., other than in the cortex, it is uniformly distributed throughout the egg cytoplasm. Such a distribution is consistent with experiments demonstrating that Ca^{2+} release is initiated at the site of sperm–egg interaction and propagates as a wave through the egg cytoplasm (Eisen *et al.*, 1984; Eisen and Reynolds, 1984; Miyazaki *et al.*, 1986; Hafner *et al.*, 1988; Hamaguchi and Hamaguchi, 1990; Stricker *et al.*, 1992). Variation in the intensity of cortical staining for $InsP_3R$ in sea urchins may be related to a variety of factors, including the actual concentration of receptor within the cortex, the amount of cortical endoplasmic reticulum, the presence of cortical granules of different sizes and distribution, etc. Changes in each of these parameters would yield lesser or greater staining intensities.

Staining associated with the periphery of the female pronucleus in sea urchin eggs is intriguing as a number of laboratories have demonstrated that as the cytoplasmic Ca^{2+} wave passes this organelle, there is a significant intranuclear Ca^{2+} increase that is equal to and/or usually higher than that which occurs in the adjacent cytoplasm (Stricker *et al.*, 1990; Hamaguchi and Hamaguchi, 1990; Stricker *et al.*, 1992; Shen and Buck, 1993). We have not determined whether $InsP_3R$ reactivity associated with the female pronucleus is present in the nuclear envelope and/or in the perinuclear endoplasmic reticulum, but there is evidence for the presence of an $InsP_3R$ in nuclear membranes of somatic cells (Otsu *et al.*, 1990; Walton *et al.*, 1991; Bachs *et al.*, 1992; Matter *et al.*, 1993). In this connection it is important to call attention to studies demonstrating that Ca^{2+} fluxes may be necessary for the activation of nuclear processes at and following fertilization (Whitaker and Patel, 1990; Tombes *et al.*, 1992).

Recent investigations have demonstrated that in addition to IICR, sea urchin eggs contain a CICR system, mediated by a ryanodine receptor (Rakow and Shen, 1990; Fujiwara *et al.*, 1990; McPherson *et al.*, 1992; Buck *et al.*, 1992). In eggs of the three sea urchin species studied here, the ryanodine receptor is located exclusively within the cortical endoplasmic reticulum (McPherson *et al.*, 1992), in sharp contrast to the fairly uniform distribution of the $InsP_3R$. The relationship of ryanodine and $InsP_3$ receptors to each other, physically and functionally, i.e., as Ca^{2+} channels located in the endoplasmic reticulum, and the timing and levels of their activity at egg activation, has yet to be determined. Interestingly, recent observations (Shen and Buck, 1993) of the spatiotemporal release of Ca^{2+} in sea urchin eggs induced by $InsP_3$, ryanodine, and cADP ribose are consistent with the localizations of $InsP_3$ and ryanodine receptors described here and by McPherson *et al.* (1992), respectively.

The endoplasmic reticulum of *Xenopus* eggs and oocytes has been shown to be an interconnected network distributed within the cortex and interstices between yolk bodies that occupy the AH and VH (Gardiner and Grey, 1983; Campanella *et al.*, 1984; Andreucetti *et al.*, 1984). The observations presented here demonstrate a fluorescent staining pattern consistent with such a network. These results are in agreement with recent investigations of Kume *et al.* (1993) in *Xenopus* oocytes and eggs. Parys *et al.* (1992), using the same affinity-purified antibody employed here, reported that the $InsP_3R$ localized to the cortex and perinuclear cytoplasm, with virtually no staining in the remaining cytoplasm of *Xenopus* oocytes. This staining pattern was generated by less than optimal methods of specimen preparation and was not a result of differences in antibody specificity, as suggested by Kume *et al.* (1993). Although the overall staining patterns here are similar to those described by Kume *et al.* (1993), differences in relative intensity of the subcortex and deeper regions of the cytoplasm in the two studies are apparent and may be related to individual variations in specimens, as observed by Kume *et al.* (1993), as well as technical manipulations (e.g., egg procurement). The distribution of $InsP_3R$ throughout the

egg as reported here is in agreement with data on stratified eggs (Han and Nuccitelli, 1990) and demonstrations that $InsP_3$-sensitive stores are present in *Xenopus* eggs to a depth of at least 180 μm from the cell surface (Larabell and Nuccitelli, 1992). Additionally, the present study, as well as that of Kume *et al.* (1993), provides a spatial context for the generation of waves of Ca^{2+} release observed in *Xenopus* (Lechleiter *et al.*, 1991a,b; Lechleiter and Clapham, 1992; DeLisle and Welsh, 1992) and potential insights into how the development of a capacity to undergo an effective cortical granule reaction and Ca^{2+} release might be realized during oocyte maturation (Charbonneau and Grey, 1984; Chiba *et al.*, 1990; Fujiwara *et al.*, 1993).

Observed differences in the molecular masses of calreticulin and calsequestrin among the different tissues investigated may be due to variations in amino acid sequence, glycosylation, or phosphorylation (Milner *et al.*, 1992; Michalak *et al.*, 1992). While calsequestrin seems confined (at least in chordates) to muscle (MacLennan *et al.*, 1983) or (avian) brain (Volpe *et al.*, 1990), calreticulin has been found in all eukaryotic cells investigated thus far (Michalak *et al.*, 1992). In sea urchin eggs, only calsequestrin has been described (Oberdorf *et al.*, 1988; Henson *et al.*, 1989) and shown to have biochemical properties similar to cardiac calsequestrin (Lebeche and Kaminer, 1992). The localization of calsequestrin in the present study is similar to that described by Henson *et al.* (1989, 1990), who postulated that it may be involved in the regulation of Ca^{2+} at egg activation. That both calsequestrin and calreticulin are present in sea urchin and *Xenopus* eggs and demonstrate a pattern of localization similar to that of the $InsP_3R$ suggest that all three proteins, in conjunction with the ryanodine receptor in sea urchin eggs, may be functionally interconnected and involved in the initiation and propagation of Ca^{2+} waves at egg activation (see also Shen and Buck, 1993).

We acknowledge Dr. R. Clark (University of Iowa) for his generous gifts of calreticulin and calreticulin antiserum, Mr. S. Kahl (University of Iowa) for his gift of purified skeletal muscle calsequestrin, and Ms. E. Jacobs for her secretarial assistance and typing of the manuscript. K.P.C. is an Investigator of the Howard Hughes Medical Institute.

REFERENCES

Andreuccetti, P., Denis-Donini, S., Burrini, A. G., and Campanella, C. (1984). Calcium ultrastructural localization in *Xenopus laevis* eggs following activation by pricking or by calcium ionophore A23187. *J. Exp. Zool.* **229,** 295–308.

Bachs, O., Agell, N., and Carafoli, E. (1992). Calcium and calmodulin function in the cell nucleus. *Biochim. Biophys. Acta* **1113,** 259–270.

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

Berridge, M. J., and Irvine, R. F. (1989). Inositol phosphates and cell signalling. *Nature* **341,** 197–205.

Buck, W. R., Rakow, T. L., and Shen, S. S. (1992). Synergistic release of calcium in sea urchin eggs by caffeine and ryanodine. *Exp. Cell Res.* **202,** 59–66.

Busa, W. B., (1990). Involvement of calcium and inositol phosphates in amphibian egg activation. *J. Reprod. Fertil. Suppl.* **42,** 155–161.

Campanella, C., Andreuccetti, P., Taddei, C., and Talevi, R. (1984). The modifications of cortical endoplamic reticulum during *in vitro* maturation of *Xenopus laevis* oocytes and its involvement in cortical granule exocytosis. *J. Exp. Zool.* **229,** 283–293.

Campbell, K. P., Franzini-Armstrong, C., and Shamoo, A. E. (1980). Further characterization of light and heavy sarcoplasmic reticulum vesicles. Identification of the 'sarcoplamic reticulum feet' associated with heavy sarcoplasmic reticulum vesicles. *Biochim. Biophys. Acta* **602,** 97–116.

Charbonneau, M., and Grey, R. D. (1984). The onset of activation responsiveness during maturation coincides with the formation of the cortical endoplasmic reticulum in oocytes of *Xenopus laevis*. *Dev. Biol.* **102,** 90–97.

Chiba, K., Kado, R. T., and Jaffe, L. A. (1990). Development of calcium release mechanisms during starfish oocyte maturation. *Dev. Biol.* **140,** 300–306.

Clapper, P. L., and Lee, H. C. (1985). Inositol trisphosphate induces calcium release from non-mitochondrial stores in sea urchin egg homogenates. *J. Biol. Chem.* **260,** 13947–13954.

Danoff, S. K., Ferris, C. D., Donath, C., Fischer, G. A., Munemitsu, S., Ullrich, A., Snyder, S. H., and Ross, C. A. (1991). Inositol 1,4,5-trisphosphate receptors: Distinct neuronal and nonneuronal forms derived by alternative splicing differ in phosphorylation. *Proc. Natl. Acad. Sci. USA* **88,** 2951–2955.

DeLisle, S., and Welsh, M. J. (1992). Inositol trisphosphate is required for the propagation of calcium waves in *Xenopus* oocytes. *J. Biol. Chem.* **267,** 7963–7966.

Eisen, A., Kiehart, D. P., Wieland, J., and Reynolds, G. T. (1984). Temporal sequence and spatial distribution of early events of fertilization in single sea urchin eggs. *J. Cell Biol.* **99,** 1647–1654.

Eisen, A., and Reynolds, G. T. (1984). Calcium transients during early development in single starfish (*Asterias forbesi*) oocytes. *J. Cell Biol.* **99,** 1878–1882.

Ferris, C. D., Huganir, R. L., Supattapone, S., and Snyder, S. H. (1989). Purified inositol 1,4,5-trisphosphate receptor mediates calcium flux in reconstituted lipid vesicles. *Nature* **342,** 87–89.

Fujiwara, A., Taguchi, K., and Yasumasu, I. (1990). Fertilization membrane formation in sea urchin eggs induced by drugs known to cause Ca^{2+} release from isolated sarcoplasmic reticulum. *Dev. Growth Differ.* **32,** 303–314.

Fujiwara, T., Nakada, K., Shirakawa, H., and Miyazaki, S. (1993). Development of inositol trisphosphate-induced calcium release mechanism during maturation of hamster oocytes. *Dev. Biol.* **156,** 69–79.

Furuichi, T., Yoshikawa, W., Miyawaki, A., Wada, K., Maeda, N., and Mikoshiba, K. (1989). Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P_{400}. *Nature* **342,** 28–32.

Galione, A., Lee, H. C., and Busa, W. B. (1991). Ca^{2+}-induced Ca^{2+} release in sea urchin egg homogenates: Modulation by cyclic ADP-ribose. *Science* **253,** 1143–1146.

Gardiner, M. D., and Grey, R. D. (1983). Membrane junctions in *Xenopus* eggs: Their distribution suggests a role in calcium regulation. *J. Cell Biol.* **96,** 1159–1163.

Hafner, M., Petzelt, C., Nobiling, R., Pawley, J. B., Kramp, D., and Schatten, G. (1988). Wave of free calcium at fertilization in the sea urchin egg visualized with fura-2. *Cell Motil. Cytoskeleton* **9,** 271–277.

Hamaguchi, Y., and Hamaguchi, M. S. (1990). Simultaneous investigation of intracellular Ca^{2+} increase and morphological events upon fertilization in the sand dollar egg. *Cell Struct. Funct.* **15,** 159–162.

Han, J.-K., and Nuccitelli, R. (1990). Inositol 1,4,5-trisphosphate-induced calcium release in the organelle layers of the stratified, intact egg of *Xenopus laevis*. *J. Cell Biol.* **110,** 1103-1110.

Henson, J. H., Begg, D. A., Beaulieu, S. M., Fishkind, D. J., Bonder, E. M., Terasaki, M., Lebeche, D., and Kaminer, B. (1989). A calsequestrin-like protein in the endoplasmic reticulum of the sea urchin: Localization and dynamics in the egg and first cell cycle embryo. *Cell Biol.* **109,** 149-161.

Henson, J. H., Beaulieu, S. M., Kaminer, B., and Begg, D. A. (1990). Differentiation of a calsequestrin-containing endoplasmic reticulum during sea urchin oogenesis. *Dev. Biol.* **142,** 255-269.

Imagawa, T., Smith, J. S., Coronado, R., and Campbell, K. P. (1987). Purified ryanodine receptor from skeletal muscle sarcoplasmic reticulum is the Ca^{2+}-permeable pore of the calcium release channel. *J. Biol. Chem.* **262,** 16636-16643.

Jaffe, L. F. (1983). Sources of calcium in egg activation: A review and hypothesis. *Dev. Biol.* **99,** 265-276.

Jorgensen, A. O., and Campbell, K. P. (1984). Evidence for the presence of calsequestrin in two structurally different regions of myocardial sarcoplasmic reticulum. *J. Cell Biol.* **98,** 1597-1602.

Krause, K.-H., Simmerman, H. K. B., Jones, L. R., and Campbell, K. P. (1990). Sequence similarity of calreticulin with a Ca^{2+}-binding protein that co-purifies with an Ins(1,4,5)P_3-sensitive Ca^{2+} store in HL-60 cells. *Biochem. J.* **270,** 545-548.

Kume, S., Muto, A., Aruga, J., Nakagawa, T., Michikawa, T., Furuichi, 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.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680-685.

Larabell, C., and Nuccitelli, R. (1992). Inositol lipid hydrolysis contributes to the Ca^{2+} wave in the activating egg of *Xenopus laevis*. *Dev. Biol.* **153,** 347-355.

Lebeche, D., and Kaminer, B. (1992). Characterization of a calsequestrin-like protein from sea-urchin eggs. *Biochem. J.* **287,** 741-747.

Lechleiter, J. D., and Clapham, D. E. (1992). Molecular mechanisms of intracellular calcium excitability in *X. laevis* oocytes. *Cell* **69,** 283-294.

Lechleiter, J., Girard, S., Peralta, E., and Clapham, D. (1991a). Spiral calcium wave propagation and annihilation in *Xenopus laevis* oocytes. *Science* **252,** 123-126.

Lechleiter, J., Girard, S., Clapham, D., and Peralta, E. (1991b). Subcellular patterns of calcium release determined by G protein-specific residues of muscarinic receptors. *Nature* **350,** 505-508.

Lee, H. C. (1991). Specific binding of cyclic ADP-ribose to calcium-storing microsomes from sea urchin eggs. *J. Biol. Chem.* **266,** 2276-2281.

Luttmer, S. J., and Longo, J. F. (1985). Ultrastructural and morphometric observations of cortical endoplasmic reticulum in *Arbacia, Spisula* and mouse eggs. *Dev. Growth Differ.* **27,** 349-359.

MacLennan, D. H., Campbell, K. P., and Reithmeier, R. A. F. (1983). Calsequestrin. *In* "Calcium and Cell Function" (A. Martonosi, Ed.), Vol. 4, pp. 151-173. Academic Press, New York.

Matter, N., Ritz, M. F., Freyermuth, S., Rogue, P., and Malviya, A. M. (1993). Stimulation of nuclear protein kinase C leads to phosphorylation of nuclear inositol 1,4,5-trisphosphate receptor and accelerated calcium release by inositol 1,4,5-trisphosphate from isolated rat liver nuclei. *J. Biol. Chem.* **268,** 732-736.

McPherson, P. S., and Campbell, K. P. (1990). Solubilization and biochemical characterization of the high affinity [^{3}H]ryanodine receptor from rabbit brain membranes. *J. Biol. Chem.* **265,** 18454-18460.

McPherson, P. S., Kim, Y.-K., Valdivia, H., Knudson, C. M., Franzini-Armstrong, C., Coronado, R., and Campbell, K. P. (1991). The brain ryanodine receptor: A caffeine sensitive Ca^{2+} release channel. *Neuron* **7,** 17-25.

McPherson, S. M., McPherson, P. S., Mathews, L., Campbell, K. P., and Longo, F. J. (1992). Cortical localization of a calcium release channel in sea urchin eggs. *J. Cell Biol.* **116,** 1111-1121.

Michalak, M., Milner, R. E., Burns, K., and Opas, M. (1992). Calreticulin. *Biochem. J.* **285,** 681-692.

Mignery, G. A., Newton, C. L., Archer, B. T., III, and Südhof, T. C. (1990). Structure and expression of the rat inositol 1,4,5-trisphosphate receptor. *J. Biol. Chem.* **265,** 12679-12685.

Milner, R. E., Baksh, S., Shemanko, C., Carpenter, M. R., Smillie, L., Vance, J. E., Opas, M., and Michalak, M. (1991). Calreticulin, and not calsequestrin, is the major calcium binding protein of smooth muscle sarcoplasmic reticulum and liver endoplasmic reticulum. *J. Biol. Chem.* **266,** 7155-7165.

Milner, R. E., Famulski, K. S., and Michalak, M. (1992). Calcium binding proteins in the sarcoplasmic/endoplasmic reticulum of muscle and nonmuscle cells. *Mol. Cell. Biochem.* **112,** 1-13.

Miyazaki, S., Hashimoto, N., Yoshimoto, Y., Kisimoto, T., Igusa, Y., and Hiramoto, Y. (1986). Temporal and spatial dynamics of the periodic increase in intracellular free calcium at fertilization of golden hamster eggs. *Dev. Biol.* **118,** 259-267.

Miyazaki, S., Yuzaki, M., Nakada, K., Shirakawa, H., Nakanishi, S., Nakade, S., and Mikoshiba, K. (1992). Block of Ca^{2+} wave and Ca^{2+} oscillation by antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster eggs. *Science* **257,** 251-255.

Mourey, R. J., Verma, A., Supattapone, S., and Snyder, S. H. (1990). Purification and characterization of the inositol 1,4,5-trisphosphate receptor protein from rat vas deferens. *Biochem. J.* **272,** 383-389.

Nakagawa, T., Okano, H., Furuichi, T., Aruga, J., and Mikoshiba, K. (1991). The subtypes of the mouse inositol 1,4,5-trisphosphate receptor are expressed in a tissue-specific and developmentally specific manner. *Proc. Natl. Acad. Sci. USA* **88,** 6244-6248.

Nuccitelli, R. (1991). How do sperm activate eggs? *Curr. Top. Dev. Biol.* **25,** 1-16.

Oberdorf, J. A., Head, J. F., and Kaminer, B. (1986). Calcium uptake and release by isolated cortices and microsomes from the unfertilized egg of the sea urchin *Strongylocentrotus droebachiensis*. *J. Cell Biol.* **102,** 2205-2210.

Oberdorf, J. A., Lebeche, D., Head, J. F., and Kaminer, B. (1988). Identification of a calsequestrin-like protein from sea urchin eggs. *J. Biol. Chem.* **263,** 6806-6809.

Olivares, E. B., Tanksley, S. J., Airey, J. A., Beck, C. F., Ouyang, Y., Deerinck, T. J., Ellisman, M. H., and Sutko, J. L. (1991). Nonmammalian vertebrate skeletal muscles express two triad junctional foot protein isoforms. *Biophys. J.* **59,** 1153-1163.

Otsu, H., Yamamoto, A., Maeda, N., Mikoshiba, K., and Tashiro, Y. (1990). Immunogold localization of inositol 1,4,5-trisphosphate ($InsP_3$) receptor in mouse cerebellar Purkinje cells using three monoclonal antibodies. *Cell Struct. Funct.* **15,** 163-173.

Parys, J. B., Sernett, S. W., DeLisle, S., Snyder, P. M., Welsh, M. J., and Campbell, K. P. (1992). Isolation, characterization, and localization of the inositol 1,4,5-trisphosphate receptor protein in *Xenopus laevis* oocytes. *J. Biol. Chem.* **267,** 18776-18782.

Peterson, G. L. (1977). A simplification of the protein assay method of Lowry *et al.* which is more generally applicable. *Anal. Biochem.* **83,** 346-356.

Rakow, T. L., and Shen, S. S. (1990). Multiple stores of calcium are released in the sea urchin egg during fertilization. *Proc. Natl. Acad. Sci. USA* **85,** 9285-9289.

Ross, C. A., Danoff, S. K., Schell, M. J., Snyder, S. H., and Ullrich, A. (1992). Three additional inositol 1,4,5-trisphosphate receptors: Molecular cloning and differential localization in brain and peripheral tissues. *Proc. Natl. Acad. Sci. USA* **89,** 4265-4269.

Sardet, C. (1984). The ultrastructure of the sea urchin egg cortex isolated before and after fertilization. *Dev. Biol.* **105,** 196-210.

Shen, S. S., and Buck, W. R. (1993). Sources of calcium in sea urchin eggs during the fertilization response. *Dev. Biol.* **157,** 157.

Stricker, S. A., Paddock, S., and Schatten, G. (1990). Laser scanning confocal microscopy of living sea urchin embryos: 3-D reconstructions and calcium ion imaging. *J. Cell Biol.* **111,** 113a.

Stricker, S. A., Centonze, V. E., Paddock, S. W., and Schatten, G. (1992). Confocal microscopy of fertilization-induced calcium dynamics in sea urchin eggs. *Dev. Biol.* **149,** 370-380.

Südhof, T. C., Newton, C. I., Archer, B. T., III, Ushkaryov, Y. A., and Mignery, G. A. (1991). Structure of a novel $InsP_3$ receptor. *EMBO J.* **10,** 3199-3206.

Supattapone, S., Worley, P. F., Baraban, J. M., and Snyder, S. H. (1988). Solubilization, purification, and characterization of an inositol trisphosphate receptor. *J. Biol. Chem.* **263,** 1530-1534.

Swann, K., and Whitaker, M. (1986). The part played by inositol trisphosphate and calcium in the propagation of the fertilization wave in sea urchin eggs. *J. Cell Biol.* **103,** 2333-2342.

Terasaki, M., and Jaffe, L. (1991). Organization of the sea urchin endoplasmic reticulum and its reorganization at fertilization. *J. Cell Biol.* **114,** 929-940.

Terasaki, M., and Sardet, C. (1991). Demonstration of calcium uptake and release by sea urchin cortical endoplasmic reticulum. *J. Cell Biol.* **115,** 1031-1037.

Tharin, S., Dziak, E., Michalak, M., and Opas, M. (1992). Widespread tissue distribution of rabbit calreticulin, a non-muscle functional analogue of calsequestrin. *Cell Tissue Res.* **269,** 29-37.

Tombes, R. M., Simerly, C., Borisy, G. G., and Schatten, G. (1992). Meiosis, egg activation, and nuclear envelope 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.

Towbin, H., Staehelin, T., and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76,** 4350-4354.

Treves, S., De Mattei, M., Lanfredi, M., Villa, A., Green, N. M., MacLennan, D. H., Meldolesi, J., and Pozzan, T. (1990). Calreticulin is a candidate for a calsequestrin-like function in Ca^{2+}-storage compartments (calciosomes) of liver and brain. *Biochem. J.* **271,** 473-480.

Turner, P. R., Jaffe, L. A., and Fein, A. (1986). Regulation of cortical vesicle exocytosis in sea urchin eggs by inositol 1,4,5-trisphosphate and GTP-binding protein. *J. Cell Biol.* **102,** 70-76.

Van Delden, C., Favre, C., Spät, A., Cerny, E., Krause, K.-H., and Lew, D. P. (1992). Purification of an inositol 1,4,5-trisphosphate-binding calreticulin-containing intracellular compartment of HL-60 cells. *Biochem. J.* **281,** 651-656.

Volpe, P., Alderson-Lang, B. H., Madeddu, L., Damiani, E., Collins, J. H., and Margreth, A. (1990). Calsequestrin, a component of the inositol 1,4,5-trisphosphate-sensitive Ca^{2+} store of chicken cerebellum. *Neuron* **5,** 713-721.

Walton, P. D., Airey, J. A., Sutko, J. L., Beck, C. F., Mignery, G. A., Südhof, T. C., Deerinck, T. J., and Ellisman, M. H. (1991). Ryanodine and inositol trisphosphate receptors coexist in avian cerebellar Purkinje neurons. *J. Cell Biol.* **1113,** 1145-1157.

Whalley, T., McDougall, A., Crossley, I., Swann, K., and Whitaker, M. (1992). Internal calcium release and activation of sea urchin eggs by cGMP are independent of the phosphoinositide signalling pathway. *Mol. Biol. Cell* **3,** 373-383.

Whitaker, M., and Crossley, I. (1990). How does a sperm activate a sea urchin egg? *NATO ASI Ser. H* **45,** 433-443.

Whitaker, M., and Irvine, R. F. (1984). Inositol 1,4,5-trisphosphate microinjection activates sea urchin eggs. *Nature* **312,** 636-639.

Whitaker, M., and Patel, R. (1990). Calcium and cell cycle control. *Development* **108,** 525-542.

Yoshikawa, S., Tanimura, T., Miyawaki, A., Nakamura, M., Yuzaki, M., Furuichi, T., and Mikoshiba, K. (1992). Molecular cloning and characterization of the inositol 1,4,5-trisphosphate receptor in *Drosophila melanogaster. J. Biol. Chem.* **267,** 16613-16619.