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# Redundant mechanisms of calcium-induced calcium release underlying calcium waves during fertilization of sea urchin eggs

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in circulating B cells (21), and neural cells expressing p75<sup>NGFR</sup> survive in media containing serum (Fig. 2). The mechanism by which unbound p75<sup>NGFR</sup> or other members of this receptor superfamily lead to neural cell death is unknown. However, the structural and functional relation between p75<sup>NGFR</sup> and TNFR I and II suggests that they may have similar mechanisms of action.

The highest level of expression of p75<sup>NGFR</sup> in the central nervous system occurs in cholinergic neurons of the nucleus basalis of Meynert, the cells most severely affected in Alzheimer's disease. These cells continue to express normal (22) or supra-normal (23) amounts of p75<sup>NGFR</sup> mRNA and protein during the neuronal degeneration associated with Alzheimer's disease. In contrast, cholinergic cells of the brainstem that resemble those of the nucleus basalis morphologically, but do not express p75<sup>NGFR</sup> (24), do not degenerate in Alzheimer's disease (25).

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## Redundant Mechanisms of Calcium-Induced Calcium Release Underlying Calcium Waves During Fertilization of Sea Urchin Eggs

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Propagating Ca<sup>2+</sup> waves are a characteristic feature of Ca<sup>2+</sup>-linked signal transduction pathways. Intracellular Ca<sup>2+</sup> waves are formed by regenerative stimulation of Ca<sup>2+</sup> release from intracellular stores by Ca<sup>2+</sup> itself. Mechanisms that rely on either inositol trisphosphate or ryanodine receptor channels have been proposed to account for Ca<sup>2+</sup> waves in various cell types. Both channel types contributed to the Ca<sup>2+</sup> wave during fertilization of sea urchin eggs. Alternative mechanisms of Ca<sup>2+</sup> release imply redundancy but may also allow for modulation and diversity in the generation of Ca<sup>2+</sup> waves.

Transient increases in the concentration of calcium ions ([Ca<sup>2+</sup>]<sub>i</sub>) act as cell signals. In general, the signal shows spatial and temporal inhomogeneity and takes the form of waves or oscillations within the cell (1).

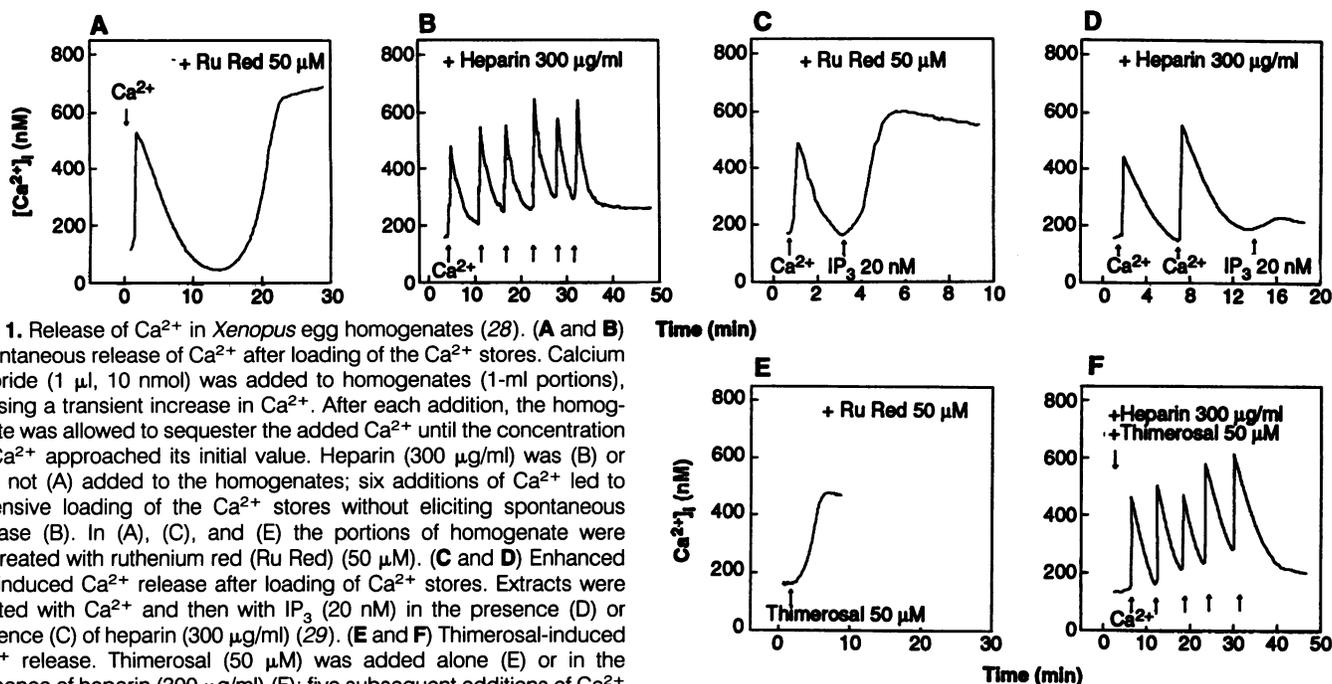
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Several mechanisms have been proposed to account for regenerative Ca<sup>2+</sup> release (2). Release of Ca<sup>2+</sup> from internal stores can be stimulated by an increase in [Ca<sup>2+</sup>]<sub>i</sub>; this process is termed Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (CICR) (3). This Ca<sup>2+</sup> release appears to be mediated by Ca<sup>2+</sup> channels in the endoplasmic reticulum (ER) that are sensitive to cytoplasmic agonists, to [Ca<sup>2+</sup>]<sub>i</sub>, and to the amount of Ca<sup>2+</sup> in the lumen of the ER (4). Two closely related Ca<sup>2+</sup> channels with these properties are the inositol trisphosphate (IP<sub>3</sub>) receptor (IP<sub>3</sub>R) (5) and the



**Fig. 1.** Release of  $Ca^{2+}$  in *Xenopus* egg homogenates (28). (A and B) Spontaneous release of  $Ca^{2+}$  after loading of the  $Ca^{2+}$  stores. Calcium chloride (1  $\mu$ l, 10 nmol) was added to homogenates (1-ml portions), causing a transient increase in  $Ca^{2+}$ . After each addition, the homogenate was allowed to sequester the added  $Ca^{2+}$  until the concentration of  $Ca^{2+}$  approached its initial value. Heparin (300  $\mu$ g/ml) was (B) or was not (A) added to the homogenates; six additions of  $Ca^{2+}$  led to extensive loading of the  $Ca^{2+}$  stores without eliciting spontaneous release (B). In (A), (C), and (E) the portions of homogenate were pretreated with ruthenium red (Ru Red) (50  $\mu$ M). (C and D) Enhanced  $IP_3$ -induced  $Ca^{2+}$  release after loading of  $Ca^{2+}$  stores. Extracts were treated with  $Ca^{2+}$  and then with  $IP_3$  (20 nM) in the presence (D) or absence (C) of heparin (300  $\mu$ g/ml) (29). (E and F) Thimerosal-induced  $Ca^{2+}$  release. Thimerosal (50  $\mu$ M) was added alone (E) or in the presence of heparin (300  $\mu$ g/ml) (F); five subsequent additions of  $Ca^{2+}$  failed to elicit an overload response (F).

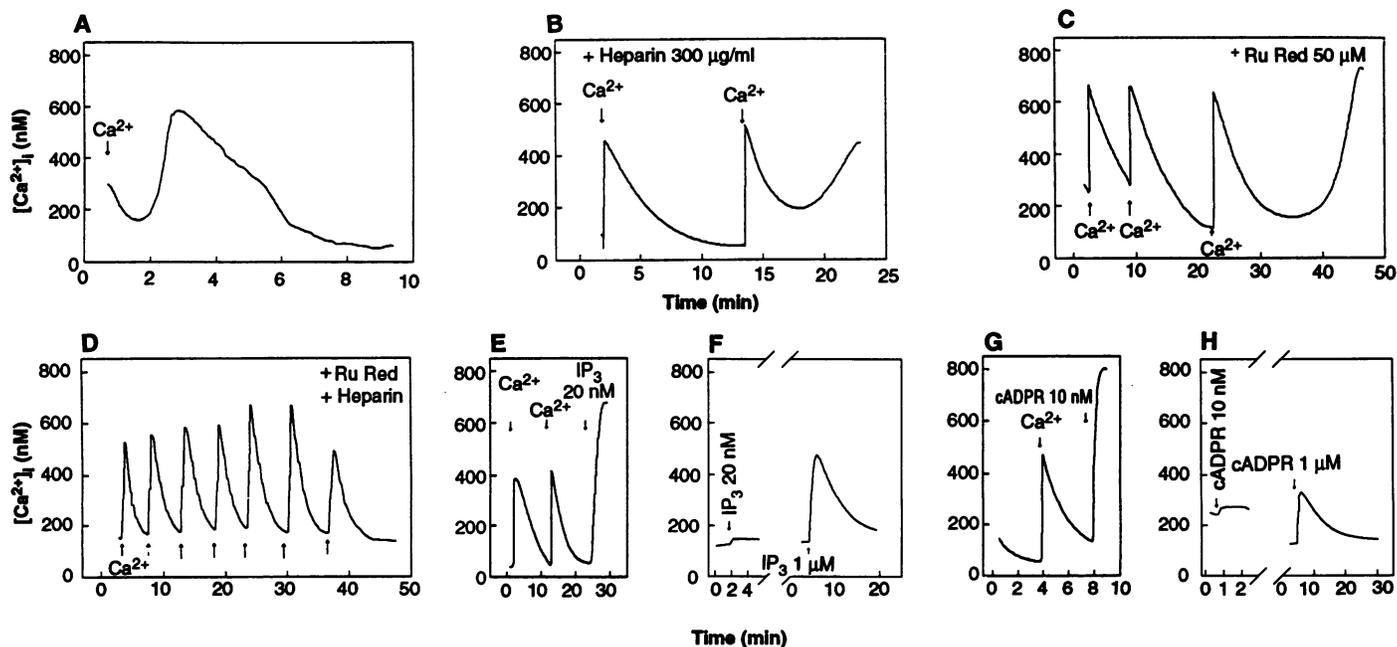
ryanodine receptor (RyR) (6), which appears to be regulated by cyclic adenosine diphosphate-ribose (cADPR) (7, 8).

Activating eggs have prominent spatial and temporal  $Ca^{2+}$  waves (9). We have pharmacologically dissected the waves of  $[Ca^{2+}]_i$  in both intact eggs and homogenates. We found that in frog and sea urchin eggs  $Ca^{2+}$  waves indeed have the same basis

in CICR, but the mechanisms that have been put forward to account individually for  $Ca^{2+}$  wave propagation in various cell types were found to be acting together in sea urchin eggs. Simple egg homogenates sequester  $Ca^{2+}$  into vesicular stores and release it in response to  $Ca^{2+}$ -releasing messengers (7, 10). We used homogenates to define the basic properties of CICR and to

identify the release channel responsible.

Adenosine triphosphate (ATP)-dependent sequestration of added  $Ca^{2+}$  (7) overloaded the  $Ca^{2+}$  stores and led to spontaneous release of  $Ca^{2+}$  in frog egg homogenates after sequestration (Fig. 1A). Frog egg homogenates treated in this way were 50 times as sensitive to  $IP_3$  as untreated homogenates (Fig. 1, C and D) because



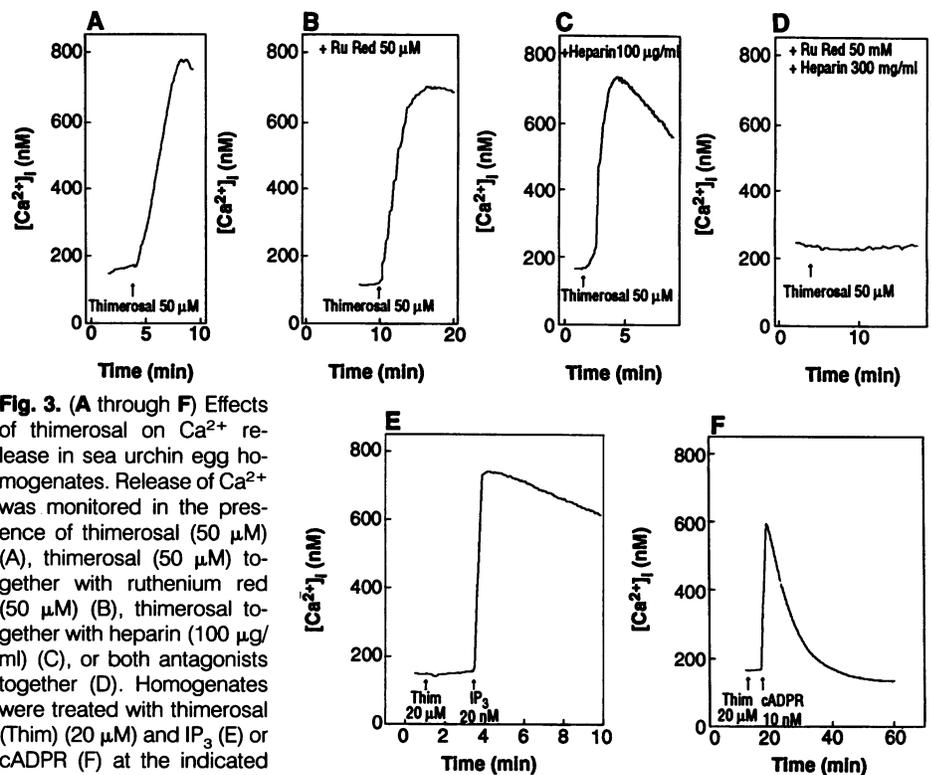
**Fig. 2.** Release of  $Ca^{2+}$  in sea urchin egg homogenates. (A through D) Spontaneous  $Ca^{2+}$  release after the addition of  $Ca^{2+}$ . Portions of  $Ca^{2+}$  were added to a sea urchin egg homogenate (as in Fig. 1) without other additions (A) or in the presence of heparin (300  $\mu$ g/ml) (B), or ruthenium red (50  $\mu$ M) (C), or both agents together (D). (E through H) After  $Ca^{2+}$

addition, 20 nM  $IP_3$  (E) or 10 nM cADPR (H) was added. Homogenates without any added  $Ca^{2+}$  were also exposed to  $IP_3$  (F and G) or to cADPR (H) at the indicated concentrations. Methods were similar to those described in (7, 10).

maximal  $\text{Ca}^{2+}$  release in untreated homogenates required  $1.1 \pm 0.2 \mu\text{M}$  (mean  $\pm$  SEM,  $n = 4$ )  $\text{IP}_3$ . The spontaneous release may result from the sensitization of the preparation to  $\text{IP}_3$  in the homogenate (11) because spontaneous release is blocked by the  $\text{IP}_3\text{R}$  antagonist heparin (Fig. 1B). The  $\text{IP}_3\text{R}$  is also sensitized by treatment with oxidizing sulfhydryl reagents (12, 13). Addition of the sulfhydryl reagent thimerosal to a frog egg homogenate led to a large but initially slow  $\text{Ca}^{2+}$  release, the kinetics of which were similar to those of the  $\text{Ca}^{2+}$  release prompted by the addition of  $\text{Ca}^{2+}$  (Fig. 1E). The effect of thimerosal was also enhanced by  $\text{Ca}^{2+}$  loading (14). This effect appeared to result from sensitization of  $\text{IP}_3\text{R}$  because thimerosal caused no  $\text{Ca}^{2+}$  release in the presence of heparin, even after repeated additions of  $\text{Ca}^{2+}$  (Fig. 1F).

We also added the RyR agonists cADPR, caffeine, and ryanodine to frog egg homogenates. They did not cause  $\text{Ca}^{2+}$  release, nor did pretreatment of homogenate portions with the RyR antagonist ruthenium red (14) affect CICR (Fig. 1, A, C, and E). Egg homogenates therefore resemble intact frog oocytes in showing regenerative  $\text{Ca}^{2+}$  release through  $\text{IP}_3\text{R}$  (15).

Sea urchin egg homogenates behaved differently. They too showed spontaneous release of  $\text{Ca}^{2+}$  and sensitization to  $\text{IP}_3$  after  $\text{Ca}^{2+}$  loading (Fig. 2, A and E), but the spontaneous release was not abolished by heparin (Fig. 2B). This spontaneous release might occur through RyR (16); caffeine and ryanodine caused  $\text{Ca}^{2+}$  release in sea urchin eggs (7, 17) and in homogenates (7), whereas ruthenium red blocked caffeine-induced  $\text{Ca}^{2+}$  release. Spontaneous release after addition of  $\text{Ca}^{2+}$  was detected in the presence of ruthenium red (Fig. 2C). However, spontaneous release was abolished if both ruthenium red and heparin were present (Fig. 2D), implying that both RyR and  $\text{IP}_3\text{R}$  contribute to CICR in sea urchin egg homogenates. Loading stores in the sea urchin egg homogenate with  $\text{Ca}^{2+}$  sensitized  $\text{IP}_3\text{R}$  to  $\text{IP}_3$  and RyR to cADPR by a factor of 50 or more (Fig. 2, E through H). A similar sensitization of RyR occurred with caffeine, whereby loading stores with  $\text{Ca}^{2+}$  allowed a maximal  $\text{Ca}^{2+}$  release at 0.2 to 0.5 mM caffeine (14), whereas 10 mM caffeine gave a maximal response in untreated homogenates (Table 1) (7). It has been suggested that thimerosal acts solely on  $\text{IP}_3\text{R}$  (12) and also solely on RyR (18). Thimerosal caused release of  $\text{Ca}^{2+}$  in sea urchin egg homogenates (Fig. 3A). However, in the sea urchin egg homogenates, neither ruthenium red nor heparin alone prevented thimerosal's effects (Fig. 3, B and C). Together, ruthenium red and heparin did inhibit  $\text{Ca}^{2+}$  release in response to thimerosal (Fig. 3D). Thimerosal also



**Fig. 3.** (A through F) Effects of thimerosal on  $\text{Ca}^{2+}$  release in sea urchin egg homogenates. Release of  $\text{Ca}^{2+}$  was monitored in the presence of thimerosal ( $50 \mu\text{M}$ ) (A), thimerosal ( $50 \mu\text{M}$ ) together with ruthenium red ( $50 \mu\text{M}$ ) (B), thimerosal together with heparin ( $100 \mu\text{g/ml}$ ) (C), or both antagonists together (D). Homogenates were treated with thimerosal (Thim) ( $20 \mu\text{M}$ ) and  $\text{IP}_3$  (E) or cADPR (F) at the indicated concentrations.

**Table 1.** The inhibition of  $\text{Ca}^{2+}$  release in sea urchin eggs and egg homogenates measured with fura 2 in response to the addition or microinjection of  $\text{IP}_3$ , cADPR, caffeine, or ryanodine (to the final concentration shown) was measured as described (7, 32). The  $\text{Ca}^{2+}$  transients are quantified as the peak height attained (nM). Mean and SEM are shown, with the number of measurements in parentheses.

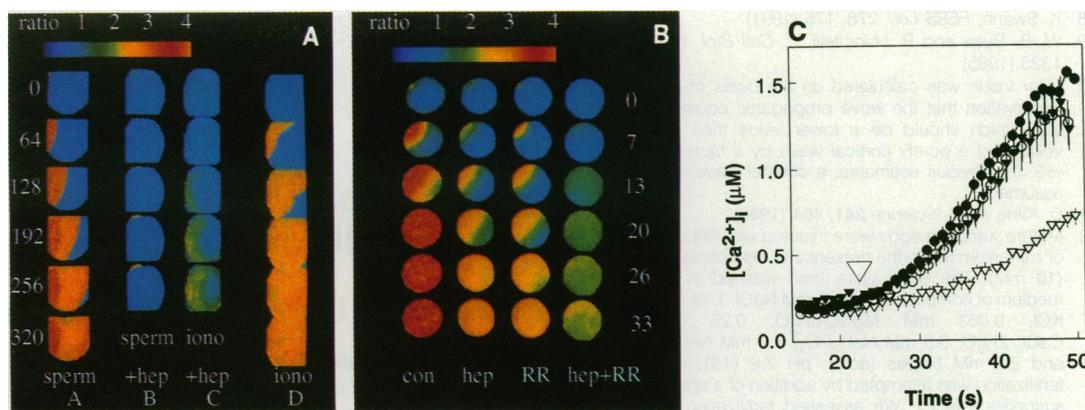
Calcium-releasing agent	Heparin ( $300 \mu\text{g/ml}$ )	Ruthenium red ( $50 \mu\text{M}$ )
<i>Homogenates</i>		
$\text{IP}_3$ ( $1 \mu\text{M}$ )	$25 \pm 8$ (4)	$510 \pm 38$ (4)
cADPR ( $0.1 \mu\text{M}$ )	$905 \pm 58$ (4)	$53 \pm 21$ (4)
Caffeine ( $10 \text{ mM}$ )	$726 \pm 103$ (4)	$15 \pm 7$ (4)
Ryanodine ( $600 \mu\text{M}$ )	$569 \pm 95$ (4)	$25 \pm 9$ (4)
<i>Eggs</i>		
$\text{IP}_3$ ( $20 \text{ nM}$ )	$190 \pm 30$ (3)	$1290 \pm 320$ (5)
cADPR ( $40 \text{ nM}$ )	$1030 \pm 180$ (3)	$250 \pm 40$ (4)

sensitized the homogenates to both  $\text{IP}_3$  (Fig. 3E) and cADPR (Fig. 3F). Thus, the effects of thimerosal and  $\text{Ca}^{2+}$  loading are similar, and thimerosal appears to affect both RyR and  $\text{IP}_3\text{R}$  (13). The sea urchin egg appears to have both a functional  $\text{IP}_3\text{R}$  and a functional RyR, whereas the frog egg has only a functional  $\text{IP}_3\text{R}$ .

In the frog egg  $\text{Ca}^{2+}$  waves can be induced by pricking the egg or by local application of a  $\text{Ca}^{2+}$  ionophore (19). The response to a  $\text{Ca}^{2+}$  ionophore indicates that a CICR mechanism is involved. We measured the  $\text{Ca}^{2+}$  waves after local application of the  $\text{Ca}^{2+}$  ionophore ionomycin (Fig. 4A, column D). Because we used a  $\text{Ca}^{2+}$ -sensitive fluorescent dye and a confocal fluorescent microscope, we were able to visualize the

$\text{Ca}^{2+}$  wave in an equatorial section through the egg. The wave crossed the cytoplasm and was not confined to the cortex. The wavefront was roughly spherical and traveled at  $5 \mu\text{m/s}$  (20). The  $[\text{Ca}^{2+}]_i$  was relatively uniform across the cytoplasm behind the wave. The  $\text{Ca}^{2+}$  wave at fertilization had very similar characteristics (Fig. 4A, column A). The wave started at the presumed site of sperm-egg interaction (sperm were added locally at the 10 o'clock position or upper left portion of the egg) and crossed the egg over a period of 5 to 6 min. Inseminated eggs that had been microinjected with heparin showed only a local increase in  $[\text{Ca}^{2+}]_i$ , presumably at the site of sperm-egg interaction, indicating that heparin had completely blocked the regenerative wave mechanism

**Fig. 4.** (A) Images of  $Ca^{2+}$  waves in *Xenopus* eggs at fertilization and after parthenogenic activation (30). Confocal images of cytoplasmic  $Ca^{2+}$ , with the  $Ca^{2+}$ -sensitive dye calcium green. High  $Ca^{2+}$  concentrations are shown in red, and resting  $Ca^{2+}$  concentrations in blue. Successive images shown were recorded 64 s apart. The egg diameter is 1 mm, and the image in each frame represents 0.7 mm. The number column is time (in seconds). Column A:  $Ca^{2+}$  wave at fertilization. Column B: Insemination after microinjection of heparin (300  $\mu$ g/ml). After 15 min, ionomycin was applied to the same egg at the 9 o'clock position (column C). Column D: Image of a different egg, to which ionomycin was applied at the 10 o'clock position. (B) Corresponding  $Ca^{2+}$  waves at fertilization in sea urchin eggs (31, 32). The images shown were recorded 6.6 s apart. The egg is 100  $\mu$ m in diameter. Column 1: The fertilization wave started at the site of sperm entry (10 o'clock position) in all columns and crosses the egg over a period of 20 s. Column 2: Egg microinjected with heparin (300  $\mu$ g/ml) and then fertilized. Eggs were microinjected with ruthenium red alone (RR) (50  $\mu$ M) (column 3) or with ruthenium red and heparin (300



$\mu$ g/ml) (column 4) and then fertilized. (C) Measurement of  $[Ca^{2+}]_i$  in single sea urchin eggs at fertilization with fura 2. Control fertilization (●); heparin (300  $\mu$ g/ml) (▼); 50  $\mu$ M ruthenium red (○); heparin (300  $\mu$ g/ml) + 50  $\mu$ M ruthenium red (▽). The error bars represent the standard error of the mean of five experiments. The large inverted triangle indicates the time at which an increase in  $[Ca^{2+}]_i$  was first detected. Because there were variable latencies for the initiation of responses, the traces were superimposed for comparison with the initiation of  $Ca^{2+}$  transients occurring at 22 s.

(Fig. 4A, column B). When ionomycin was applied locally with a micropipette to the same egg, we saw a larger, local increase in  $[Ca^{2+}]_i$  but no  $Ca^{2+}$  wave (Fig. 4A, column C). Heparin at a concentration of 100  $\mu$ g/ml halved the wave velocity.

We verified our measurements of  $Ca^{2+}$  concentration by scoring egg activation with contraction of the pigment as a criterion (21). Heparin caused 50% inhibition of egg activation at concentrations of 100 to 200  $\mu$ g/ml. Egg activation was effectively blocked by a heparin concentration of 300  $\mu$ g/ml with 4 out of 35 eggs activated upon insemination but was unaffected by ruthenium red at concentrations of up to 50  $\mu$ M with 25 out of 29 eggs activated upon insemination (22). These data support our conclusion that the  $Ca^{2+}$  wave in frog eggs is governed by  $IP_3R$ .

The sea urchin egg is much smaller than the frog egg, but the fertilization  $Ca^{2+}$  wave travels at a similar velocity (23) (Fig. 4B, column 1). It passes as a spherical wavefront through an equatorial section. The region behind the wavefront showed no inhomogeneities in  $Ca^{2+}$  concentration, either in the egg nucleus (the egg nucleus is present in the optical section of Fig. 4B, column 1, for example) or in subcortical regions, where it has been suggested that  $RyR$  may be relatively abundant (24). All compartments appeared to change uniformly. Microinjection of either heparin or ruthenium red reduced the amplitude and propagation velocity slightly but did not prevent the wave from crossing the egg (Fig. 4B, columns 2 and 3). Microinjection of both antagonists together led to a loss of the  $Ca^{2+}$  wave (Fig. 4B, column 4). Be-

cause of the failure of the mechanical block to polyspermy in the absence of the  $Ca^{2+}$  wave, eggs were polyspermic under these conditions.

The specificity of  $Ca^{2+}$  release agonists and antagonists in sea urchin eggs and homogenates is shown in Table 1. Release of  $Ca^{2+}$  by  $RyR$  agonists was blocked selectively by ruthenium red, whereas heparin selectively blocked release by  $IP_3$ . This selectivity was preserved between homogenates and eggs. Because calibration of absolute  $Ca^{2+}$  concentrations is difficult with the use of the single-wavelength confocal imaging technique, we confirmed our results with whole cell fluorescence with the  $Ca^{2+}$ -sensitive ratio-dye fura 2. The  $Ca^{2+}$  wave is represented by the rising phase of the whole cell signal, which was affected only by the simultaneous presence of heparin and ruthenium red (Fig. 4C).

The outcome of these experiments indicates that frog eggs have a single CICR mechanism that uses  $IP_3R$ , whereas in sea urchin eggs both  $IP_3R$  and  $RyR$  contribute to the formation of  $Ca^{2+}$  waves. The coexistence of the two types of  $Ca^{2+}$  release channel was a prediction of the two-store model for  $Ca^{2+}$  oscillations (2) that led to the search for  $RyR$  in nonmuscle cells. In the two-store model, CICR is mediated only by  $RyR$ . Our results indicate that CICR can occur through either release channel in the same cell.

This redundancy may have advantages. Redundant  $Ca^{2+}$  release mechanisms allow separate modulation of the two channels by different agonists (25). The two channel types have different thresholds for  $Ca^{2+}$ -dependent activation and inactivation (26). Differential

coexpression and colocalization of  $IP_3R$  and  $RyR$  (27) will generate a large number of different  $Ca^{2+}$  signaling phenotypes.

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22. Mature *Xenopus* eggs were injected with heparin or ruthenium red in the presence of chlorbutanol (10 mM). The eggs were then washed in F1 medium of composition: 41.25 mM NaCl, 1.75 mM KCl, 0.063 mM  $MgCl_2 \cdot 6H_2O$ , 0.25 mM  $CaCl_2 \cdot 2H_2O$ , 0.5 mM  $NaH_2PO_4$ , 1.9 mM NaOH, and 2.5 mM Hepes (acid); pH 7.8 (19), and fertilization was attempted by addition of a sperm suspension in F1. We assessed fertilization by viewing the formation of a fertilization envelope and the contraction of the pigmented hemisphere toward the animal pole (21).
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28. *Xenopus* egg homogenates were prepared as described for sea urchin eggs (7) but with the following modifications: We removed egg jelly by swirling the eggs in F1 cysteine medium (19), and the eggs were washed in  $Ca^{2+}$ -free F1 containing 5 mM EGTA. The eggs were homogenized in a 70% intracellular medium (IM) solution (7). The resulting 20% homogenates were incubated with an ATP-regenerating system, mitochondrial inhibitors, and the fluorescent dye fura 2 (7). Portions (1 ml) were transferred to a cuvette, and fluorescence was monitored at 510 nm, with excitation at 340 and 380 nm, in a Hitachi fluorimeter at 22°C. The ratio of the signals at the two excitation wavelengths was used to determine the concentration of free  $Ca^{2+}$ , as described in (32). Calibrations were done in the presence and absence of heparin or ruthenium red. Each experiment in Figs. 1 to 4 is representative of at least three determinations that gave similar results.
29. The effect is not trivially explained as a consequence of the amount of  $Ca^{2+}$  in the store because this would affect the amount of  $Ca^{2+}$  available for release but not an increased sensitivity to maximal  $Ca^{2+}$  release by an agonist (11).
30. One of three experiments is shown in each case (each was performed at 20°C). Mature *Xenopus* eggs were obtained as described in (19). Eggs were stored in modified Barth's solution (MBS) medium containing the anesthetic chlorbutanol (88 mM NaCl, 1 mM KCl, 2.4 mM  $NaHCO_3$ , 0.8 mM  $MgSO_4$ , 0.5 mM  $NaH_2PO_4$ , 15 mM tris acid, and 10 mM chlorbutanol; pH 7.6) to reduce hydration of the jelly coat and to prevent egg activation during microinjection. Eggs were microinjected with  $Ca^{2+}$  indicator (calcium green: Molecular Probes, Junction City, OR). Just before insemination, eggs were transferred to the low-salt medium, F1. Images were obtained and processed with a Leica CLSM (Leica Lasertechnik, Heidelberg, Germany) microscope with 480-nm excitation and emission filters and a  $\times 5$  objective. To obtain ratio images that eliminated dye concentration artifacts, we divided the experimental images pixel by pixel by a reference image obtained at the beginning of the experiment.
31. Eggs were obtained, microinjected, and fertilized, and the fura 2 signal was calibrated as described in (32). The confocal images are representative of at least five experiments for each condition. *Lytechinus pictus* eggs were maintained at 16°C.
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## Calcium Mobilization by Dual Receptors During Fertilization of Sea Urchin Eggs

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Fertilization is accompanied by a transient increase in the concentration of intracellular  $Ca^{2+}$ , which serves as a signal for initiating development. Some of the  $Ca^{2+}$  appears to be released from intracellular stores by the binding of inositol trisphosphate ( $IP_3$ ) to its receptor. However, in sea urchin eggs, other mechanisms appear to participate. Cyclic adenosine diphosphate-ribose (cADPR), a naturally occurring metabolite of nicotinamide adenine dinucleotide, is as potent as  $IP_3$  in mobilizing  $Ca^{2+}$  in sea urchin eggs. Experiments with antagonists of the cADPR and  $IP_3$  receptors revealed that both  $Ca^{2+}$  mobilizing systems were activated during fertilization. Blockage of either of the systems alone was not sufficient to prevent the sperm-induced  $Ca^{2+}$  transient. This study provides direct evidence for a physiological role of cADPR in the  $Ca^{2+}$  signaling process.

Mobilization of intracellular  $Ca^{2+}$  occurs in a wide variety of cellular processes and is mediated by at least two major mechanisms. One messenger molecule that links surface receptor activation to the release of  $Ca^{2+}$  from internal stores is  $IP_3$  (1). Another major mechanism is  $Ca^{2+}$ -induced  $Ca^{2+}$  release (CICR), which is well characterized in muscle (2, 3) and may function in mechanisms of  $Ca^{2+}$  oscillation and  $Ca^{2+}$  wave propagation (4, 5). Cyclic ADP-ribose (cADPR) is a cyclic metabolite (6) synthesized by a ubiquitous enzyme, ADP-ribosyl cyclase (7, 8), that utilizes nicotinamide adenine dinucleotide ( $NAD^+$ ) as substrate. The cADPR molecule occurs naturally in mammalian tissues (9) and is as potent as  $IP_3$  in mobilizing  $Ca^{2+}$  in sea urchin eggs (10) and vertebrate cells (11, 12). Some evidence indicates that cADPR may be an endogenous regulator of the CICR process (13–15).

The similarity between ligand binding to surface receptors and the sperm-egg interaction during fertilization has led to the proposal that  $Ca^{2+}$  mobilization associated with fertilization is similarly mediated by  $IP_3$ . Indeed, phosphoinositide metabolism increased during fertilization coincident with the increase in the concentration of intracellular  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ) and the cortical exocytotic reaction (16, 17). Microinjection of  $IP_3$  into eggs activates a transient increase in  $[Ca^{2+}]_i$  similar to that which occurs after fertilization (18). In hamster eggs, injection of antibody to the  $IP_3$  receptor can block changes in  $[Ca^{2+}]_i$  induced by  $IP_3$  or fertilization (19). However, in sea

urchin eggs, heparin (an antagonist for the  $IP_3$  receptor) only delays the onset of the transient increase in  $[Ca^{2+}]_i$  but does not prevent it, suggesting that other mechanisms for mobilizing  $Ca^{2+}$  may exist (20, 21).

We investigated the effects of heparin on the  $IP_3$ -induced  $Ca^{2+}$  mobilization in intact sea urchin eggs and homogenates. In egg homogenates,  $Ca^{2+}$  release induced by  $IP_3$  (1  $\mu M$ ) was completely blocked by heparin (0.2 mg/ml) (Fig. 1) (10). However, heparin did not affect  $Ca^{2+}$  release induced by cADPR (Fig. 1). We used the cortical exocytotic reaction in intact eggs as an index for  $Ca^{2+}$  mobilization to determine the effective concentration of heparin needed to block  $IP_3$ -induced  $Ca^{2+}$  mobilization (22). Microinjection of  $IP_3$  (0.15  $\mu M$ , intracellular concentration) into *Lytechinus pictus* eggs induced the cortical reaction in 100% of the injected eggs (10). If eggs were first microinjected with heparin [H5765 (Sigma)] at a final intracellular concentration of 1.9 mg/ml and subsequently injected with 0.12 to 0.24  $\mu M$   $IP_3$ , seven out of seven of the injected eggs did not show a cortical reaction. Increasing the concentration of  $IP_3$  to 0.72  $\mu M$  partially overcame the block by heparin, with two eggs giving a full cortical reaction and two eggs out of a total of eight eggs injected showing a partial cortical reaction (50%). To block 0.72  $\mu M$   $IP_3$  from inducing the cortical reaction, the heparin concentration had to be increased to 4.7 mg/ml (six out of six eggs were blocked). Thus, the blockage by heparin is competitive in nature (20, 21). The higher effective concentration of heparin in intact eggs as compared with homogenates is likely to result

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