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[REVIEW]

Mechanisms of Egg Activation and Polyspermy Block in Amphibians and Comparative Aspects with Fertilization in Other Vertebrates

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ABSTRACT—For precise temporal activation of the egg during amphibian fertilization, the sperm must provide a signal for egg activation at the time of membrane binding or fusion between sperm and eggs. A fertilizing sperm causes a Ca^{2+} wave which is both necessary and sufficient for egg activation at amphibian fertilization. The Ca^{2+} wave seems to be mediated by IP3-receptors on the endoplasmic reticulum and by IP3 produced by hydrolysis of PLC activated by a Src-related protein tyrosine kinase (Xyk) in *Xenopus* eggs. We have proposed three different hypotheses for initiation of egg activation in amphibian eggs: the Ca^{2+}-influx model, the membrane receptor model, and the soluble factor model. The membrane receptor model and the soluble factor model seems to be applied to the monospermic *Xenopus* fertilization and the physiologically polyspermic *Cynops* fertilization, respectively. The Ca^{2+} wave at egg activation induces a positive fertilization potential which prevents entry of a second sperm in fertilization of monospermic species. In physiologically polyspermic urodele eggs, several sperm enter the egg at normal fertilization, but only one sperm nucleus with a centrosome participates in the embryonic development. The degeneration of accessory sperm nuclei is closely involved in differential distributions of both γ-tubulin and cyclin B in the egg cytoplasm, which causes developing a larger sperm aster and earlier entry into M phase in a zygote nucleus, respectively. We have discussed the molecular mechanisms of egg activation and polyspermy blocks in amphibians and make some comparisons with other vertebrates, such as fishes and mammals.

INTRODUCTION

Fertilization brings about at least three distinct reactions: restoration of the diploid configuration with mixing of the male and female genomes, introduction of the centriole necessary for cell division into the egg in most vertebrates, and activation of the process of cell division for development. Precise temporal activation of the egg during fertilization is essential for normal development, since activation before entry of the sperm nucleus can cause parthenogenesis. Conversely, a delay in activation can cause pathological polyspermy. Thus, the sperm must provide a signal for egg activation at the time of membrane binding or fusion between sperm and eggs.

Amphibians contain two groups exhibiting very different blocks to polyspermy (Elinson, 1986; Iwao, 2000). One is a block before sperm-egg fusion, operating in monospermic eggs of anurans and some primitive urodeles, which is a species of the *Hynobius* genus (Iwao, 1989). Another is a block in egg cytoplasm after sperm entry, operating in physiologically polyspermic eggs in other urodeles. In monospermic species, development of an embryo with a diploid genome and a single centrosome (centriole) derived from a sperm is ensured by recruitment of a fast, electrical block to polyspermy on egg membrane, followed by a block at fertilization envelope formed by cortical granule exocytosis. The entry of more than two sperm causes abnormal development in monospermic species. In physiologically polyspermic species, several sperm enter an egg at normal fertilization, but only a single sperm nucleus with a single centrosome ultimately participates in embryonic development, while the other sperm nuclei and centrosomes degenerate before cleavage. Since faster activation seems to be necessary for the polyspermy block in monospermic species compared with physiologically polyspermic species, different mechanisms of egg activation may operate between these species. In this review we will discuss the molecular mechanisms of egg activation and polyspermy

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blocks in amphibians and will make comparisons with other vertebrates, such as fishes and mammals.

Primary role of Ca^{2+} in amphibian egg activation

A fertilizing sperm causes an increase in intracellular free Ca^{2+} ($[Ca^{2+}]_i$) in the eggs of both the anuran *Xenopus laevis* (Fig. 1A) (Busa and Nuccitelli, 1985; Nuccitelli *et al*., 1993; Iwao and Fujimura, 1996; Fontanilla and Nuccitelli, 1998), and the urodeles *Pleurodeles waltl* (Gradin and Charbonneau, 1992) and *Cynops pyrrhogaster* (Fig. 1B) (Yamamoto *et al*., 1999a), as well as in other vertebrates (Stricker, 1999). In *Xenopus* eggs, an initial Ca^{2+} increase occurs near the sperm entry site, followed by a propagative Ca^{2+} wave spreading towards the opposite side of the egg (Nuccitelli *et al*., 1993; Fontanilla and Nuccitelli, 1998). The peak level of $[Ca^{2+}]_i$ is estimated to be about 1.2 μ M in the cortex and 0.7 μ M in the center of the egg (Fontanilla and Nuccitelli, 1998). The Ca^{2+} increase in *Xenopus* eggs is similar to that observed in Ca^{2+} oscillation of mammalian eggs (0.5–2.5 μ M, Miyazaki *et al*., 1993), but lower than that in the eggs of *Oryzias latipes* eggs (30 μ M, Gilkey *et al*., 1978), which is a fish. The Ca^{2+} increase (0.15 μ M) in *Pleurodeles* is less than those in other vertebrates (Gradin and Charbonneau, 1992). Since the velocity of the Ca^{2+} wave in *Xenopus* is somewhat greater in the cortex (8.9 μ m/sec) than in the center of the egg (5.7 μ m/sec)

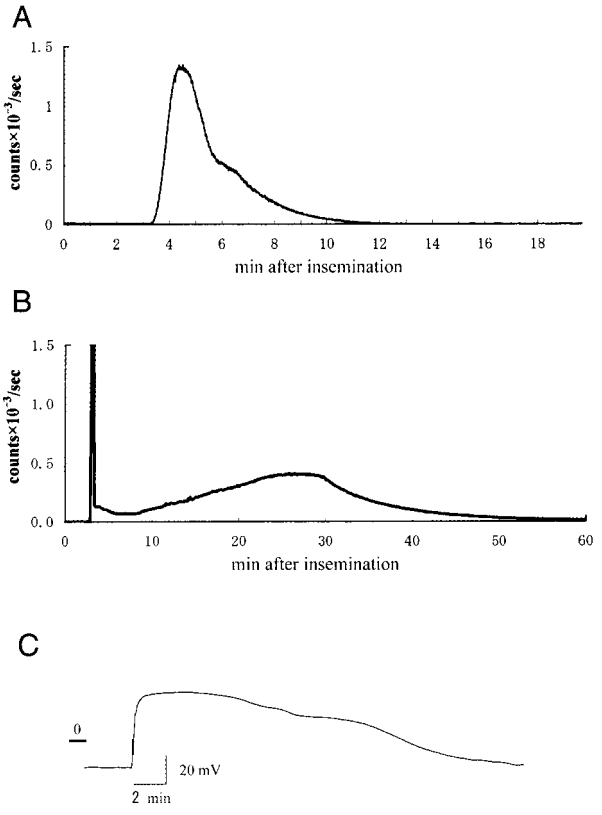

Fig. 1. Ca^{2+} increases at fertilization of *Xenopus* (A) and *Cynops* (B) eggs. The level of $[Ca^{2+}]_i$ was monitored by aequorin injected into the eggs. A positive-going fertilization potential at fertilization of a *Xenopus* egg (C).

(Fontanilla and Nuccitelli, 1998), the Ca^{2+} wave reaches the opposite side of the egg about 10 min after initiation. At *Cynops* fertilization, after an initial spike-like Ca^{2+} increase lasting about 30 sec, a Ca^{2+} wave spreads with a velocity of 5.0–6.0 μ m/sec for about 40 min (Fig. 1B) (Yamamoto *et al*., 1999a,b). The velocity of Ca^{2+} waves in amphibians is somewhat slower than that in fishes (9–12 μ m/sec, Gilkey *et al*., 1978; Lee *et al*., 1999) or mammals (16–28 μ m/sec, Miyazaki *et al*., 1993). Since the single Ca^{2+} increase occurs at amphibian fertilization, a relatively high $[Ca^{2+}]_i$ continues for 10–15 min in *Xenopus* eggs (Fig. 1A) and for 30 - 40 min in *Cynops* eggs (Fig. 1B). *Oryzias* eggs exhibit a single Ca^{2+} wave lasting about 15 min (Gilkey *et al*., 1978), but a repetitive Ca^{2+} increase (Ca^{2+} oscillation) occurs in mammalian eggs, each lasting 0.5–4 min for about 2 hor (Miyazaki *et al*., 1993).

The increase in $[Ca^{2+}]_i$ is both necessary and sufficient for egg activation in amphibians. Prevention of this increase at fertilization by injection of the Ca^{2+} chelator, BAPTA, inhibits all events in egg activation, including elicitation of the fertilization potential, cortical granule exocytosis, cortical contraction in *Xenopus* (Kline, 1988), and resumption of meiosis in both *Xenopus* (Kline, 1988) and *Cynops* (Yamamoto *et al*., 1999a). A $[Ca^{2+}]_i$ increase induced by the Ca^{2+} ionophore A23187 causes egg activation in both anurans (Steinhardt *et al*., 1974; Iwao, 1982) and urodeles (Charbonneau and Picheral, 1983; Iwao and Masui, 1995). Anurans eggs can be activated by introduction of Ca^{2+} into the egg cytoplasm by injection (Cross, 1981) or by pricking with a fine needle (Goldenberg and Elinson, 1980; Iwao *et al*., 1981). Longer treatment with a higher concentration of ionophore A23187 is necessary for activation of *Cynops* eggs in comparison with anuran eggs (Iwao and Masui, 1995). While pricking can cause egg activation in *Pleurodeles* (Aimar and Larousse, 1975) and *Hynobius nebulosus* (Iwao, 1989), the eggs of most urodeles are relatively insensitive to pricking (Fankhauser, 1967; Iwao and Masui, 1995). Injection of Ca^{2+} into *Oryzias* eggs induces egg activation with a Ca^{2+} wave (Iwamatsu *et al*., 1988a,b). The introduction of Ca^{2+} into mammalian eggs does not appear to be sufficient to induce sustained Ca^{2+} oscillation (Swann and Ozil, 1994).

One Ca^{2+} store in amphibian eggs seems to be the endoplasmic reticulum, which is abundant in the egg cortex (Gardiner and Grey, 1983; Campanella *et al*., 1988). Inositol-1,4,5-trisphosphate (IP3)-receptors preferentially localized in the egg cortex (Kume *et al*., 1993) are likely involved in the Ca^{2+} increase at amphibian fertilization. The amount of IP3 in *Xenopus* egg cytoplasm increases 3- to 5-fold at fertilization (Stith *et al*., 1993, 1994; Snow *et al*., 1996). Injection of IP3 into the eggs of both *Xenopus* (Busa *et al*., 1985; Larabell and Nuccitelli, 1992) and *Cynops* (Yamamoto *et al*., 1999b) causes an increase in Ca^{2+}. Injection of heparin, an inhibitor of IP3-receptors, prevents Ca^{2+} waves at fertilization in both *Xenopus* (Nuccitelli *et al*., 1993) and *Cynops* (Yamamoto *et al*., 1999b) eggs. Injection of an antibody against type 1 IP3-receptor into *Xenopus* eggs reduces the Ca^{2+} increase at fertilization (Runft *et al*., 1999). The Ca^{2+} wave in *Xenopus* eggs

seems to be induced by Ca^{2+} that promotes Ca^{2+}-induced Ca^{2+} release (CICR) acting on IP3-receptors directly or through IP3 production (Nuccitelli *et al*., 1993). The faster Ca^{2+} wave in the cortex may be due to abundant endoplasmic reticulum with IP3-receptors in the cortex. However, local and transient Ca^{2+} increases, known as "hot spots", have been observed at fertilization of *Xenopus* eggs injected with heparin (Nuccitelli *et al*., 1993; Fontanilla and Nuccitelli, 1998) or anti-IP3 receptor antibody (Runft *et al*., 1999). Since each hot spot probably represents a Ca^{2+} increase at each sperm entry site, another mechanism that is not mediated by IP3 receptors may operate in the initial phase of the Ca^{2+} increase in *Xenopus* eggs. IP3-injection causes a Ca^{2+} increase in *Oryzias* eggs (Nuccitelli *et al*., 1987; Iwamatsu *et al*., 1988a). Injection of an antibody against IP3-receptors into hamster eggs completely inhibits the Ca^{2+} increase at fertilization (Miyazaki *et al*., 1992). However, IP3 can not fully mimic a Ca^{2+} oscillation in mammalian eggs (Swann and Ozil, 1994). Although ryanodine receptors also seem to function in the Ca^{2+} oscillation in mammalian eggs (Stricker, 1999), they are unlikely to be involved in the Ca^{2+} increase in amphibian eggs, since they have not been observed in *Xenopus* eggs (Parys *et al*., 1992) and injection of cyclic-ADP ribose does not cause a Ca^{2+} increase in *Xenopus* egg homogenate (Whitaker and Swann, 1993) or in *Cynops* eggs (Yamamoto S and Iwao Y, unpublished data).

Signaling pathways in the Ca^{2+} increase at egg activation

IP3 is produced by hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into IP3 and diacylglycerol (DG) with the phospholipase C (PLC) enzymes which include three subgroups: PLC-β, PLC-γ, and PLC-δ, (Fig. 2). Both a functional G-protein/ PLC-β pathway (Kline *et al*., 1988) and a PLC-γ (Yim *et al*., 1994) are present in *Xenopus* eggs, which are responsive to exogenously expressed receptors for serotonin 1C and epidermal growth factor, respectively. However, the Ca^{2+} increase in *Xenopus* eggs is not inhibited by pertussis toxin, which inhibits a Gi family of G-proteins (Kline *et al*., 1991), or by an antibody against a Gq family G-protein (Runft *et al*., 1999). There is no direct evidence in favor of the involvement of a G-protein/ PLC-β pathway in egg activation at amphibian fertilization. The Ca^{2+} increase is not inhibited by injection of the SH2-domain of PLC-γ1 in which tyrosine kinases bind to activate PLC-γ (Runft *et al*., 1999). No PLC-γ 2 is detected in *Xenopus* eggs (Runft *et al*., 1999), although some inhibitors of tyrosine kinases block a Ca^{2+} increase and egg activation in *Xenopus* eggs (Glahn *et al*., 1998; Sato *et al*., 1998; 1999). It has been shown that a Src-related protein tyrosine kinase (Xyk) localized in the egg cortex is activated and translocated to egg cytoplasm (a soluble fraction) at fertilization in *Xenopus* (Sato *et al*., 1996; 1999). Both activation and translocation are induced by a fertilizing sperm, but not by an artificial Ca^{2+} increase induce by an ionophore or electric shock (Sato *et al*., 1999), while injection of a peptide that inhibits Xyk does block egg activation in *Xenopus* (Sato *et al*., 1999). These results suggest that Xyk plays a role in the cascade between sperm-egg binding/fusion and the Ca^{2+} increase. Xyk seems to stimulate PLC-γ through a SH2-domain-independent mechanism, such as a partial proteolysis or noncatalytic interaction with other molecules, since Xyk is associated with PLC-γ and activation of PLC-γ is inhibited by a specific inhibitor of Src-related kinases (Sato K, *et al*., 2000). However, since Ca^{2+} hot spots are seen at fertilization of eggs in which the Ca^{2+} waves were inhibited by a tyrosine kinase inhibitor (Glahn *et al*., 1998), a pathway that is different from the Xyk cascade may be involved in the initial and local Ca^{2+} increase at the sperm entry site. The role of PLC-δ in the Ca^{2+} increase remains to be investigated in amphibian fertilization. Among mammals, injection of GDP-β-S inhibits G-proteins and blocks the Ca^{2+} increase in hamster eggs (Miyazaki *et al*., 1993), while injection of an antibody against the Gq family G-protein does not inhibit activation of mouse eggs (Williams *et al*., 1998). Furthermore, a Ca^{2+} increase in mouse eggs is not blocked by injection of the SH2-domain of PLC-γ (Mehlmann *et al*., 1998).

Mechanisms of sperm-induced initiation of Ca^{2+} increase

While it is unknown just how a fertilizing sperm transmits the initial signal for the Ca^{2+} increase at fertilization, there are at least three different hypotheses for initiation of egg activation in animal eggs: the Ca^{2+}-influx model, the membrane receptor model, and the soluble factor model (Fig. 2). In any case, a fertilizing sperm must stimulate IP3 production in the egg cytoplasm to potentiate the Ca^{2+} wave.

(A) In the Ca^{2+} influx model (Fig. 2A), a fertilizing sperm induces an influx of external Ca^{2+} required for egg activation either through Ca^{2+} channels on the sperm membrane following sperm-egg fusion or on the egg membrane at the sperm-egg binding/fusion. Amphibian eggs can be artificially activated by a Ca^{2+} release from internal stores in the absence of external Ca^{2+} (Steinhardt *et al*., 1974; Yamamoto *et al*., 1999b). The progression of the Ca^{2+} wave is not affected by depletion of external Ca^{2+} (Fontanilla and Nuccitelli, 1998; Yamamoto, 1999b). No cortical flush of the Ca^{2+} increase is seen at the initial phase of the Ca^{2+} increase. The Ca^{2+} increase from internal stores seems to be sufficient for egg activation of amphibian eggs. Since CICR is induced by Ca^{2+} injection (Cross, 1981) and PLCs can be stimulated by 1–10 μ M Ca^{2+} (Hwang *et al*., 1996), it remains to be determined whether the initial Ca^{2+} increase around the sperm entry site is dependent upon external Ca^{2+}. In order to determine a role of the Ca^{2+} influx at fertilization of amphibians, it should be determine whether amphibian eggs can be fertilized and are normally activated in the absence of external Ca^{2+} ions. *Oryzias* eggs can be fertilized, and the Ca^{2+} wave is not affected in the absence of external Ca^{2+} (Gilkey *et al*., 1978). In zebrafish eggs, the external spawning medium triggers an activating Ca^{2+} wave without sperm-egg fusion, but neither sperm nor external Ca^{2+} is required to initiate the Ca^{2+} wave (Lee *et al*., 1999). The exact mechanism of egg activation in fishes remains to be investigated. In mouse eggs, a fertilizing sperm can induce the Ca^{2+} increase even where there is a very low concentration of external Ca^{2+} (13 nM) (Jones *et al*., 1998b).

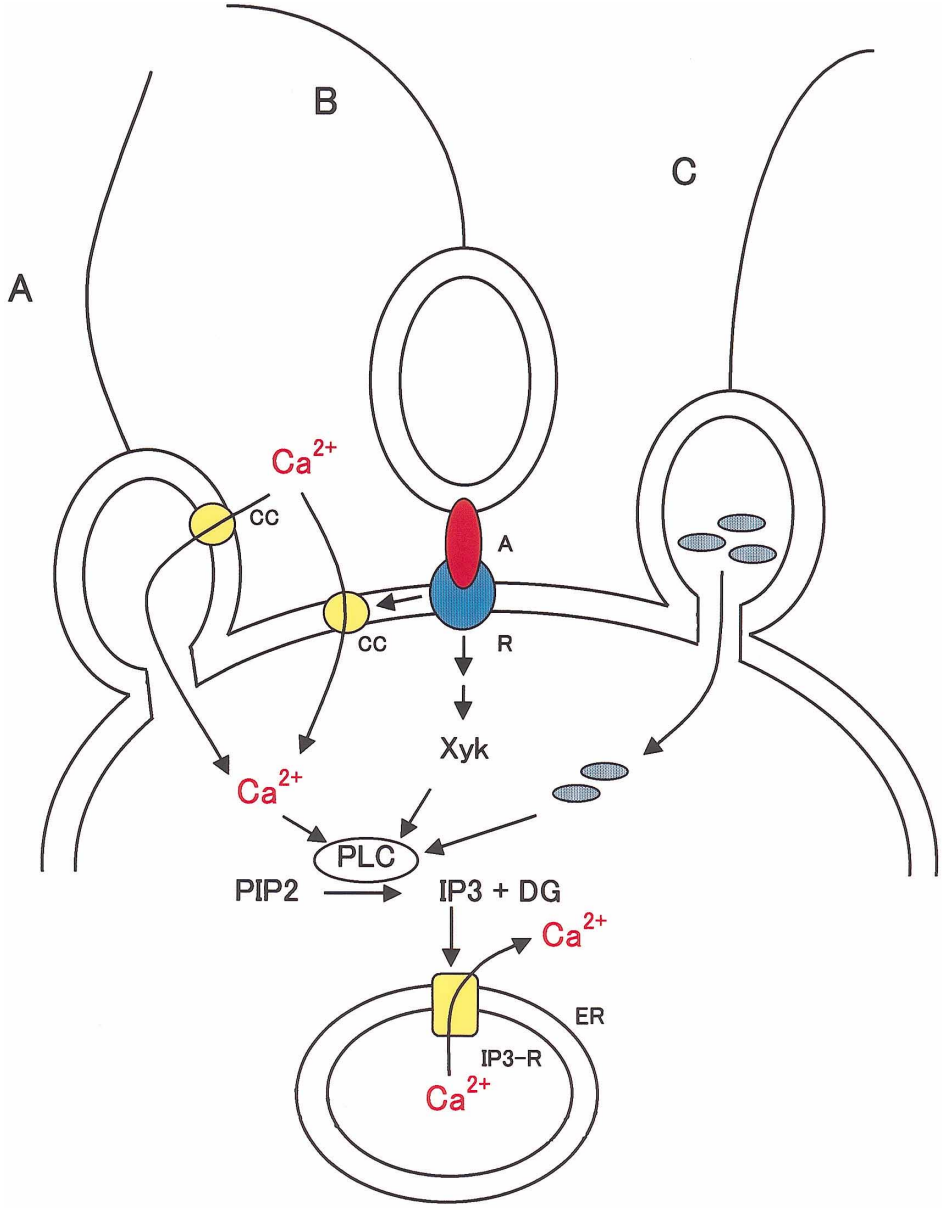

Fig. 2. Models for the mechanism of Ca^{2+} increase at fertilization. Ca^{2+} influx model (A), membrane-receptor model (B), and sperm factor model (C). A, sperm agonist; CC, Ca^{2+} channel; IP3-R, ER, endoplasmic reticulum; IP3-receptor; R, receptor; Xyk, Src-related tyrosine kinase. Blue oval symbols indicating the sperm factor. See text for detail.

(B) The membrane receptor model proposes that an agonist (ligand) on the sperm membrane binds to a receptor on the egg membrane to cause IP3 production in the egg cytoplasm (Fig. 2B). We have suggested that a positively charged molecule(s) on the sperm membrane is involved in sperm-egg binding and fusion, based on the analysis of cross-fertilization between voltage-sensitive and voltage-insensitive species (see below). In *Xenopus* eggs, a Ca^{2+} increase is induced by external treatment with peptides containing an RGD sequence (Iwao and Fujimura, 1996), which is well known as a ligand for integrins. RGD-containing peptides can induce a Ca^{2+} increase in the absence of external Ca^{2+}. The treatment with RGD-containing peptides causes activation in *Hynobius* eggs (Fujumura and Iwao, 1997), but does not in *Cynops* eggs (Iwao Y, unpublished data). *Xenopus* sperm contain a protein of the metalloprotease/disintegrin/cysteine-rich (MDC) family (xMDC16) (Shilling *et al*., 1997). Peptides containing a sequence (KTE) of its disintegrin domain inhibit fertilization (Shilling *et al*., 1997). Treatment with a high concentration of these peptides causes a Ca^{2+} increase and activation in *Xenopus* eggs (Shilling *et al*., 1998). These results indicate that the sperm protein binds to a receptor, probably an integrin(s), on the egg membrane and transmits a signal for the Ca^{2+} increase at fertilization. However, no receptor has been found for either the RGD-containing peptides or the xMDC16. Another potential candidate for the sperm agonist is a sperm acrosomal protease purified from *Cynops* sperm (Fig. 3) (Iwao *et al*., 1994; Mizote *et al*., 1999). *Xenopus* eggs can be fertilized by

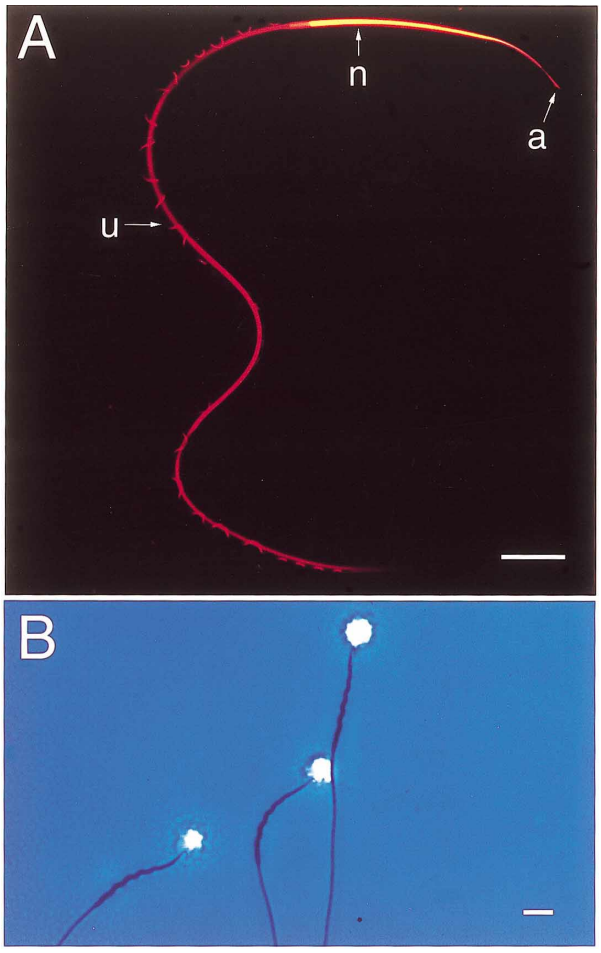

Fig. 3. (A) A conforcal fluorescence image of the newt *Cynops pyrrhogaster* sperm stained by acridine orange and neutral red, showing a nucleus (n) with an arrow head-like acrosome (a) in the head region and a tail with an undulating membrane (u). (B) A protease activity in *Cynops* sperm, showing a halo in each acrosome by digestion of a gelatin film. Bars, 20 μ m.

Cynops sperm and are completely activated by the external application of the protease purified from *Cynops* sperm. The sperm protease causing a Ca^{2+} wave in *Xenopus* eggs (Iwao *et al*., 1995) is a high-molecular weight complex with a unique tryptic protease that efficiently hydrolyzes the C-terminus of double arginine protein residues (Mizote *et al*., 1999). Homologous *Xenopus* fertilization is inhibited by inhibitors for the sperm protease (Mizote *et al*., 1999) and a similar protease is localized on the *Xenopus* sperm membrane (Mizote *et al*., 1999; Iwao Y, unpublished data). The sperm protease might cleave a receptor on the egg membrane, as does a protease-activating receptor for the thrombin system (Vu *et al*., 1991). Thus, there are strong evidences in support of the membrane receptor model for the Ca^{2+} increase in *Xenopus* eggs, although the target of the sperm protease on the egg membrane is still unknown, In mammals, sperm-egg binding is mediated by the interaction between the fertilin α/β MDC proteins on the sperm membrane and the integrin α_6/β_1 on the egg membrane (Almeida *et al*., 1995). Since mouse sperm that lack the fertilin subunit can fertilize the eggs and cause activation (Cho *et al*., 1998), fertilin is probably not involved in egg activation in mammals. Since CD9, which is a member of the transmembrane-4 superfamily, is found on the egg membrane and is required for membrane fusion (Miyado *et al*., 2000; Le Naour *et al*., 2000), its role in Ca^{2+} signaling in mammalian eggs should be investigated further.

(C) The soluble factor model proposes that a soluble component(s) in sperm cytoplasm is transmitted to egg cytoplasm after sperm-egg fusion, which then causes the Ca^{2+} increase. This model, based on the latent period between sperm-egg membrane fusion and the onset of the Ca^{2+} increase in egg cytoplasm (Whitaker and Swann, 1993), may apply to *Cynops* egg activation. Injection of sperm soluble components into *Cynops* eggs causes a Ca^{2+} increase and complete egg activation (Yamamoto *et al*., 1999b). This appears to be consistent with the finding that *Cynops* eggs are resistant to a transient Ca^{2+} increase by pricking and to the treatment with RGD-containing peptides , as discussed above. In support of this, only a small percentage of *Cynops* eggs are activated by external treatment with the sperm protease (Iwao *et al*., 1994). The sperm factor in *Cynops* is known to be a heat-labile and proteinous molecule(s). However, further investigation is necessary to determine whether *Cynops* sperm contains a sufficient amount of sperm factor to activate an egg. Recent studies on mammals strongly support the soluble factor model for Ca^{2+} oscillation (Swann and Parrington, 1999). Injection of a soluble sperm extract into mammalian eggs is known to trigger a Ca^{2+} oscillation (Parrington *et al*., 1996; Swann and Parrington, 1999). While a 33-kDa protein (oscillin) has been proposed as the sperm factor responsible for the Ca^{2+} oscillation (Parrington *et al*., 1996), other recent candidates include a PLC (Dupont *et al*., 1996; Jones *et al*., 1998a), a truncated c-kit (Sette *et al*., 1997), or various perinuclear substances (Perry *et al*., 2000).

Fast polyspermy block at the egg membrane in monospermic amphibians

The unfertilized amphibian eggs are surrounded with several jelly layers and a vitelline envelope which play important roles in polyspermy block (Iwao, 2000). In monospermic amphibian species, the eggs from which external coats have been removed and surrounded with only egg membrane exhibit monospermy, indicating a polyspermy block at the level of the sperm-egg binding or fusion (Elinson, 1973; Katagiri, 1974). The propagative Ca^{2+} wave at egg activation induces a propagative opening of Cl^- channels (halide ion channels) on the egg membrane (Kline and Nuccitelli, 1985), which causes a positive shift in the potential of the egg membrane (fertilization potential) in conditions of low external Cl^- such as fresh water (Fig.1C). While the level of fertilization potential is species specific, the eggs of most species have positive potentials of about +10~+40 mV (Iwao, 2000). The positive fertilization potential prevents entry of a second sperm for 10–15 min after fertilization. When the membrane potential of unfertilized eggs remains higher than 0 mV under voltage-clamp

conditions, both sperm entry and egg activation are blocked (Cross and Elinson, 1980; Charbonneau *et al*., 1983; Jaffe *et al*., 1983a; Iwao 1989; Iwao *et al*., 1994). In contrast, polyspermy occurs when the egg membrane potential remains below 0 mV under voltage-clamp (Cross and Elinson, 1980; Charbonneau *et al*., 1983; Jaffe *et al*., 1983a; Iwao and Jaffe, 1989; Iwao *et al*., 1994) or in the presence of concentrated external halide ions (Grey *et al*., 1982). The voltages that inhibit fertilization correspond well to those of fertilization potentials induced by sperm (Iwao, 2000). Thus, a positive fertilization potential functions as a fast, electrical block to polyspermy in voltage-sensitive species. In physiologically polyspermic urodeles, fertilization is not blocked by any positive potentials (Charbonneau *et al*., 1983; Iwao and Jaffe, 1989). Cross-fertilization between the eggs of voltage-sensitive species and the sperm of voltage-insensitive species is not affected by positive potentials, resulting in polyspermy (Jaffe *et al*., 1983a; Iwao and Jaffe, 1989), while a cross between the eggs of voltage-insensitive species and the sperm of voltage-sensitive species is sensitive to the voltage of the egg membrane (Iwao and Jaffe, 1989). These results indicate that the voltage-sensor for fertilization is localized on the membrane of the sperm, and not on the egg membrane. Potential candidates for the voltage sensor are the sperm protease and the xMDC16 protein, because egg activation by their molecules is voltage-dependent (Iwao *et al*., 1994; Shilling *et al*., 1998). While the fast block is transient, the eggs accomplish a complete polyspermy block by the formation of a fertilization envelope and by hydration of the jelly layers (Iwao, 2000).

*Oryzia*s (bony fish, Osteichthyes) eggs do not elicit a positive fertilization potential and their fertilization is voltage-insensitive (Nuccitelli, 1980), indicating lack of a fast electrical block. Limitation of sperm entry through a narrow micropyle on the egg envelope (chorion) is necessary to ensure monopsermy in bony fishes (Kobayashi and Yamamoto, 1981). In contrast, monospermic fertilization of the lamprey (jawless fishes, Agantha) in fresh water is ensured by a fast electrical block (Kobayashi and Yamamoto, 1994; Kobayashi *et al*., 1994). Lamprey eggs elicit a large positive fertilization potential mediated by the opening of Cl^- channels which mainly localize in the animal pole region (Kobayashi *et al*., 1994). The positive potential blocks sperm-oocyte fusion, but not egg activation. These results suggest that the membrane receptor model can be applied to lamprey fertilization and that molecules with different voltage-sensitivities are involved in sperm-egg membrane fusion and the signaling pathway for egg activation. Fertilized mammalian eggs elicit repetitive hyperpolarizations (negative-going potentials) mediated by the opening of K^+ channels in response to the Ca^{2+} oscillation (Miyazaki and Igusa, 1981; 1982). A fast electrical block does not operate in mammalian eggs (Jaffe *et al*., 1983b), where monospermy is generally accomplished by a zona reaction mediated by cortical granule exocytosis (Wassarmann, 1999).

Behavior of sperm nuclei and centrosomes in physiologically polyspermic urodele eggs

Physiological polyspermy is seen in some invertebrates and in several species of vertebrates, including fishes, urodele amphibians, reptiles, and birds (Austin, 1965). The mechanism of a polyspermy block in physiologically polyspermic eggs is well understood in urodeles (Fankhauser, 1948; Elinson, 1986; Iwao, 2000). Several sperm (2–20 sperm/egg) enter the egg at normal fertilization of *Cynops* (Iwao *et al*., 1985; 1993). While most sperm enter animal hemispheres or equatorial regions, some enter at the vegetal hemispheres (Iwao *et al*., 1993). The number of fertilizing sperm is limited by the hydration of the jelly layers (McLaughlin and Humphries, 1978; Matsuda and Onitake, 1984). All incorporated sperm undergo nuclear decondensation and form sperm pronuclei with functional centrosomes (Fig. 4). Each sperm pronucleus is associated with each sperm aster, but the asters in animal hemispheres are larger than those in vegetal hemispheres (Iwao *et al*., 1997). The size of the asters is dependent upon the state of the egg cytoplasm (Iwao *et al*., 1997), and probably upon the amount of γ-tubulin in the centrosomes which is responsible for microtubule polymerization (Iwao Y, unpublished data). A single sperm pronucleus, the “principal sperm nucleus”, forms a zygote nucleus with an egg pronucleus in the animal hemisphere. Although the exact mechanism for selection of the principal sperm nucleus remains unclear, the sperm nucleus nearest the egg nucleus appears capable of contacting the egg nucleus. All sperm and egg nuclei enter the S phase, but both the onset and the completion of DNA synthesis are earlier in the zygote nucleus (the egg and the principal sperm nuclei) than they are in other accessory sperm nuclei (Iwao *et al*., 1993). When the zygote nucleus enters the pro-metaphase, its single centrosome divides and forms a bipolar spindle with a diploid set of condensed chromosomes (Fig. 4). When the zygote nucleus enters the anaphase, centrosomes in the accessory sperm nuclei do not separate, and the nuclear membranes of the sperm nuclei in the vegetal hemispheres remain distinct and enclose the decondensed chromatins. Accessory sperm nuclei in the equatorial region sometimes form mono-polar spindles with a haploid set of chromosomes. After the first cleavage, all accessory sperm nuclei undergo degeneration. Their chromatins undergo pycnosis and the materials of their centrosomes are dispersed in the egg cytoplasm. Thus, only one sperm nucleus with a centrosome (a centriole) participates in the embryonic development of urodele eggs (Fig. 4).

The degeneration of accessory sperm nuclei is closely related to their failure to enter the M-phase (Iwao, 2000). M-phase promoting factor (MPF), consisting of cdc2 kinase (cdk1) and cyclin B (Lohka *et al*., 1988; Gautier *et al*., 1990), is a key component involved in entering the M-phase in many animal cells (Masui, 1992). Injection of an MPF-rich cytoplasmic fraction prevents some accessory sperm nuclei from degenerating in animal hemispheres or equatorial regions (Iwao and Elinson, 1990). The rescued accessory sperm nuclei form extra bipolar spindles with haploid sets of chromosomes, and then

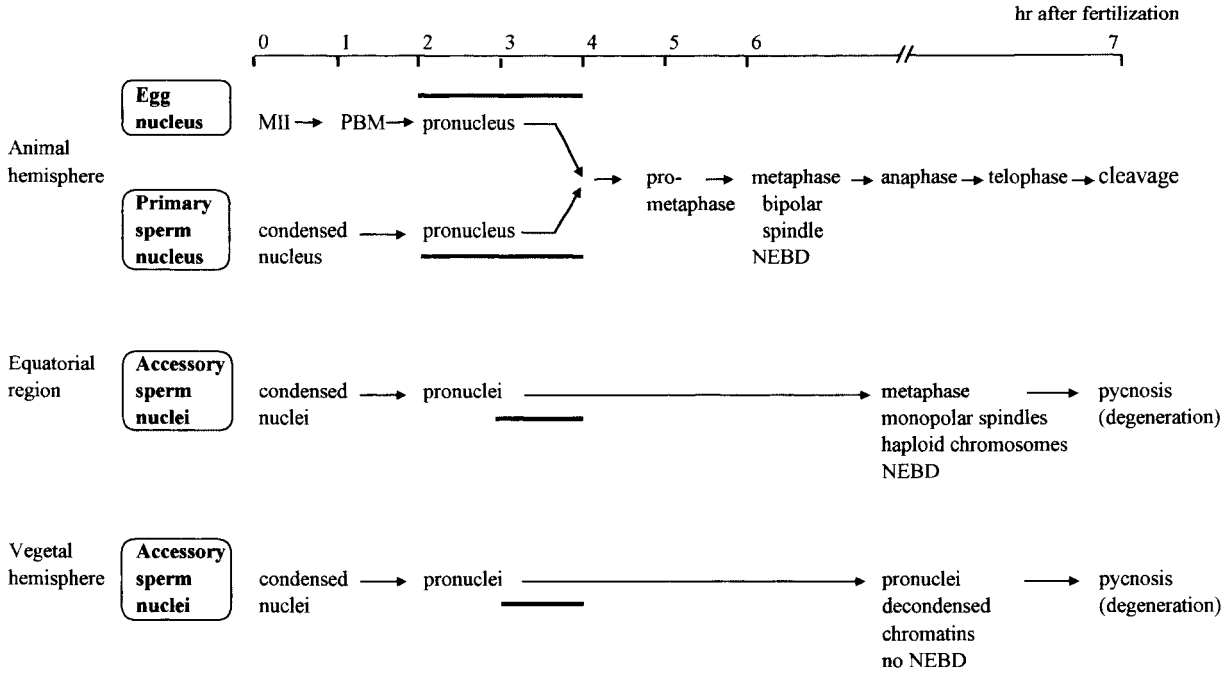

Fig. 4. Fate of sperm nuclei in a physiologically polyspermic *Cynops* egg. MII, the second meiotic metaphase; NEBD, nuclear membrane breakdown; PBM, the second polar body emission; Bars, S phase.

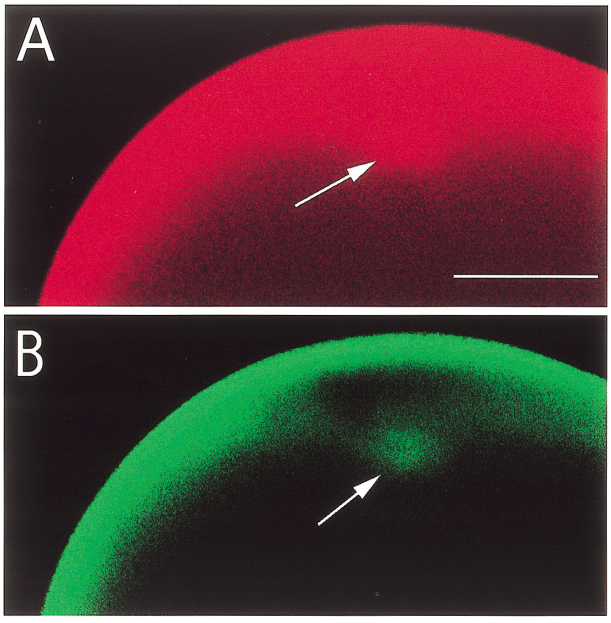

Fig. 5. Localization of cyclin B (A) and α-tubulin (B) in a *Cynops* egg 4 hr after fertilization, showing cyclin B in the cortex and around the zygote nucleus (arrows) in association with microtubules. Bar, 0.2 mm.

induce multipolar cleavage. In addition to the increased MPF activity, there are greater amounts of both cdc2 kinase and cyclin B in animal hemispheres compared with vegetal hemispheres (Sakamoto *et al.*, 1998). Cyclin B is mainly distributed in the egg cortex (Sakamoto *et al.*, 1998), and a large amount of cyclin B is associated with the zygote nucleus (Fig. 5A). Microtubule structures, such as sperm asters and cortical microtubules (Iwao *et al.*, 1997), probably regulate the localization of cyclins in egg cytoplasm (Fig. 5B). Since DNA synthesis and centrosome separation in *Xenopus* eggs is dependent upon cdc2/cdk2 kinases (Chevalier *et al.*, 1995) and cdk2/cyclins (Hinchcliffe *et al.*, 1999), respectively, earlier entry of the zygote nucleus into both the S-phase and the M-phase, as well as centrosome separation, is probably due to the abundant cdc2 or cdk2 around the zygote nucleus. Furthermore, earlier entry into the anaphase in the zygote nucleus is likely stimulated by anaphase-promoting factor (APF) (Peters *et al.*, 1996), which inactivates MPF by destruction of cyclin B through ubiquitin-dependent proteolysis (Aizawa *et al.*, 1996; Tokumoto *et al.*, 1997). Earlier disappearance of cyclin B around the zygote nucleus is seen around the anaphase of the first cleavage in *Cynops* eggs (Iwao Y, unpublished data). These results indicate that the accessory sperm nuclei are exposed to APF without sufficient exposure of MPF, which causes degeneration of accessory sperm nuclei. However, the molecular mechanism of degradation of accessory sperm chromatins and centrosomes remains unknown.

There are few reports of the nuclear behavior in other physiologically polyspermic animals that are comparable with urodele amphibians. In the domestic fowl, some accessory sperm form bipolar spindles at the M-phase for the first cleavage, but they never cause the extra cleavage furrow (Perry, 1987). The accessory sperm nuclei that disperse slightly towards the margin of a germinal disc degenerate after one round of mitosis (Perry, 1987; Waddington *et al.*, 1998). There may be some difference in the ability to induce cleavage in the egg cortex. In the polyspermic invertebrate ctenophore *Bore ovata*,

one sperm nuclei is selected to form the zygote nucleus after an egg nucleus approaches different sperm nuclei along the microtubules in egg cytoplasm (Carré and Sardet, 1984; Rouvière *et al*., 1994). Although each sperm pronucleus is associated with a sperm aster, they do not migrate (Houliston *et al*., 1993). The egg nucleus which enters into the center of the sperm aster forms the zygote nucleus (Carré *et al*., 1991). The mechanisms for suppression of accessory sperm in these species remain to be investigated.

Concluding remarks

Two different models for egg activation may apply in amphibian fertilization: the membrane receptor model for the anuran *Xenopus* and the sperm factor model for the urodele *Cynops*. Although the molecular mechanisms of egg activation are not fully understood in amphibians, the observed voltage-sensitive and voltage-insensitive fertilization seems to correspond well to the membrane receptor model and the sperm factor model, respectively. Since it is estimated that the second sperm probably reaches the egg membrane within several seconds of the arrival of the first sperm (Iwao, 2000), the signal transmission through the membrane receptor seems to be suitable for faster egg activation (faster generation of a positive fertilization potential) to prevent polyspermy in monospermic species. From the phylogenetic perspective, voltage-insensitive fertilization was probably acquired concomitant with the emergence of physiological polyspermy in urodeles. Recent molecular studies indicate that the anuran group may have branched relatively early from the urodele/caecillian (limbless amphibians) group, perhaps during the beginning of the Mesozoic period (240 million years ago), while the urodele and caecillians groups probably branched relatively late, in the late Mesozoic period (160–190 million years ago) (Feller and Hedges, 1998). In this connection, monospermy with the fast electrical block, but without the cortical granule-mediated block, in urodeles belonging to the genus *Hynobius* (Iwao, 1989; 2000), apparently shows an intermediate mode between monospermic anurans, with both fast and cortical granule-mediated blocks, and physiologically polyspermic urodeles, which lack both blocks.

In this context, the mode of fertilization in the ancestor of amphibians may provide an important view of the relationship between the type of polyspermy block and the mode of egg activation. Amphibians are believed to share a common ancestor with the bony fishes of subclass Sarcopterygii (lobe-finned fishes), which contains Crossopterigii (coelacanth, *Latimeria*) and Dipnoi (lung fishes) (Meyer and Dolven, 1992). The molecular analysis in extant animals suggests that lungfishes comprise the closest sister group of tetrapods (amphibians) (Zardoya and Meyer, 1997). *Latimera* is ovoviviparous (Smith *et al*., 1975), but the mode of fertilization in sacropterygian fishes remains unknown. As is the case in monospermic amphibians, lampreys exhibit voltage-sensitive fertilization and their eggs generate a positive fertilization potential mediated by Cl^- channels. A Cl^--dependent fertilization potential may be necessary for monospermic fertilization in vertebrates that live in fresh water. Since fish sperm contain cleavage-initiation activities (Iwamatsu and Ohta, 1974), molecular mechanisms of egg activation in voltage-insensitive monospermic bony fishes and polyspermic cartilaginous fishes merit further investigation. Mammalian eggs exhibit voltage-insensitive fertilization and appear to be activated by the sperm factor. However, the mechanisms of activation of polyspermic yolky eggs of birds and reptiles remain to be investigated.

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REFERENCES

Aimar C, Labrousse J-P (1975) DNA synthesis and evolution, in presence of a somatic nucleus, of the female pronucleus after experimental activation of the egg of *Pleurodeles waltlii*. Dev Growth Differ 17:197–207

Aizawa H, Kawahara H, Tanaka K, Yokosawa H (1996) Activation of the proteasome during *Xenopus* egg activation implies a link between proteasome activation and intracellular calcium. Biochem Biophys Res Commun 218: 224–228

Almeida EA, Huovila A-PJ, Sutherland AE, Stephens LE, Calarco PG, Shaw LM, Mercurio AM, Sonnenberg A, Primakoff P, Myles DG, White JM (1995) Mouse egg integrin $\alpha 6\beta 1$ functions as a sperm receptor. Cell 81: 1095–1104

Austin CR (1965) "Fertilization". Prentice-Hall Inc, New Jersey.

Busa WB, Nuccitelli R (1985) An elevated free cytosolic calcium wave follows fertilization in eggs of the frog, *Xenopus laevis*. J Cell Biol 100: 1325–1329

Busa WB, Ferguson JE, Joseph SK, Williamson JR, Nuccitelli R (1985) Activation of frog (*Xenopus laevis*) eggs by inositol trisphosphate. I. Characterization of calcium release from intracellular stores. J Cell Biol 101: 677–682

Campanella C, Talevi R, Kline D, Nuccitelli R (1988) The cortical reaction in the egg of *Discoglossus pictus*, A study of the changes in the endoplasmic reticulum at activation. Dev Biol 130: 108–119

Carr D, Sardet C (1984) fertilization and early development in *Beroe ovata*. Dev Biol 105:188–195

Carré D, Rouvière C, Sardet C (1991) In vitro fertilization in ctenophores: sperm entry, mitosis, and the establishment of bilateral symmetry in *Beroe ovata*. Dev Biol 147: 381–191

Charbonneau M, Picheral B (1983) Early events in anuran amphibian fertilization: An ultrastructural study of changes occurring in the course of monospermic fertilization and artificial activation. Dev Growth Differ 25: 23–37

Charbonneau M, Moreau M, Picheral B, Vilain JP, Guerrier P (1983) Fertilization of amphibian eggs, A comparison of electrical responses between anurans and urodeles. Dev Biol 98: 304–318

Chevalier S, Tassan JP, Cox R, Philippe M, Ford C (1995) Both cdc2 and cdk2 promote S phase initiation in *Xenopus* egg extracts. J Cell Sci 108: 1831–1841

Cho C, Bunch DO, Faure JE, Goulding EH, Eddy EM, Primakoff P, Myles DG (1998) Fertilization defects in sperm from mice lacking fertilin β. Science 28:1857–1859

Cross NL (1981) Initiation of the activation potential by an increase in intracellular Ca^{2+} in eggs of the frog, *Rana pipiens*. Dev Biol 85: 380–384

Cross NL, Elinson, RP (1980) A fast block to polyspermy in frogs mediated by changes in the membrane potential. Dev Biol 75: 187–198

Dupont G, McGuinness OM, Johnson MH, Berridge MJ, Borgese F (1996) Phospholipase C in mouse oocytes: characterization of β and γ isoforms and their possible involvement in sperm-induced Ca^{2+} spiking. Biochem J 316: 583–591

Elinson RP (1973) Fertilization of frog body cavity eggs enhanced by treatment affecting the vitelline coat. J Exp Zool 183: 291–302

Elinson RP (1986) Fertilization of amphibians, The ancestry of the block to polyspermy. Inter Rev Cytol 101: 59–100

Fankhauser G (1948) The organization of the amphibian egg during fertilization and cleavage. Ann NY Acad Sci 49: 684–708

Fankhauser G (1967) I. System: Procurement, maintenance, and use (Urodeles). In: Wilt FH, Wessells NK (eds) "Method in Developmental Biology". TY Crowell Company, New York, pp 85–99

Feller AE, Hedges SB (1998) Molecular evidence for the early history of living amphibians. Mol Phylogenet Evol 9: 509–516

Fontanilla RA, Nuccitelli R (1998) Characterization of the sperm-induced calcium wave in *Xenopus* eggs using confocal microscopy. Biophys J 75: 2079–2087

Fujimura T, Iwao Y (1997) Species specificity and signal tramsduction in Xenopus egg activation by RGD-peptide. Zool Sci 14 suppl:89

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

Gautier J, Minshull J, Lohka M, Glotzer M, Hunt T, Maller J L (1990) Cyclin is a component of maturation-promoting factor from *Xenopus* Cell 60: 487–494

Gilkey JC, Jaffe LF, Ridgway EB, Reynolds GT (1978) A free calcium wave traverses the activating egg of the medaka, *Oryzias latipes*. J Cell 76: 448–466

Glahn D, Mark SD, Behr RK, Nuccitelli R (1998) Tyrosine kinase inhibitors block sperm-induced egg activation in *Xenopus laevis*. Dev Biol 205: 171–180

Goldenberg M, Elinson RP (1980) Animal/vegetal difference in cortical granules exocytosis during activation of the frog egg. Dev Growth Differ 22: 345–356

Grandin N, Charbonneau M (1992) Intracellular free Ca^{2+} changes during physiological polyspermy in amphibian eggs. Development 114: 617–624

Grey RD, Bastiani, MJ, Webb, DJ, Schertel ER (1982) An electrical block is required to prevent polyspermy in eggs fertilized by natural mating of *Xenopus laevis*. Dev Biol 89: 475–484

Hwang SC, Jhon DY, Bae YS, Kim JH, Rhee SG (1996) Activation of phospholipase C-γ by the concerted action of tau proteins and arachidonic acid. J Biol Chem 271: 18342–18349

Hinchcliffe EH, Li C, Thompson EA, Maller JL, Sluder G (1999) Requirement of Cdk2-cyclin E activity for repeated centrosome reproduction in *Xenopus* egg extracts. Science 283: 851–854

Houliston E, Carré D, Johnston JA, Sardet C (1993) Axis establishment and microtubule-mediated waves prior to first cleavage in *Boroe ovata*. Development 111: 75–87

Iwamatsu T, Ota I (1974) Cleavage initiating activities of sperm fractions injected into the egg of the medaka, *Oryzias latipe*s. J Exp Zool 187: 3–15

Iwamatsu T, Yoshimoto Y, Hiramoto Y (1988a) Mechanism of Ca^{2+} release in medaka eggs microinjected with inositol 1,4,5-trisphosphate and Ca^{2+}. Dev Biol 129: 191–197

Iwamatsu T, Yoshimoto Y, Hiramoto Y (1988b) Cytoplasmic Ca^{2+} release induced by microinjection of Ca^{2+} and effects of microinjected divalent cations on Ca^{2+} sequestration and exocytosis of cortical alveoli in the medaka egg. Dev Biol 125: 451–457

Iwao Y (1982) Differential emergence of cortical granule breakdown and electrophysiological responses during maturation of toad oocytes. Dev Growth Differ 23: 89–100

Iwao Y (1989) An electrically mediated block to polyspermy in the primitive urodele *Hynobius nebulosus* and phylogenetic comparison with other amphibians. Dev Biol 134: 438-445

Iwao Y (2000) Fertilization of amphibians in "Fertilization in protoza and metazoan animals" eds: J.J.Tarin and A. Cano, Springer-Verlag, pp147–191.

Iwao Y, Elinson RP (1990) Control of sperm nuclear behavior in physiologically polyspermic newt eggs, Possible involvement of MPF. Dev Biol 142: 301–312

Iwao Y, Fujimura T (1996) Activation of *Xenopus* eggs by RGD-containing peptides accompanied by intracellular Ca^{2+} release. Dev Biol 177: 55–567

Iwao Y, Jaffe LA (1989) Evidence that the voltage-dependent component in the fertilization process is contributed by the sperm. Dev Biol 134: 446-451

Iwao Y, Masui Y (1995) Activation of newt eggs in the absence of Ca^{2+} activity by treatment with cycloheximide or D_2O. Dev Growth Differ 37: 641–651

Iwao Y, Ito S, Katagiri C (1981) Electrical properties of toad oocytes during maturation and activation. Dev Growth Differ 23: 89–100

Iwao Y, Yamasaki H, Katagiri C (1985) Experiments pertaining to the suppression of accessory sperm in fertilized newt eggs. Dev Growth Differ 27: 323–331

Iwao Y, Sakamoto N, Takahara K, Yamashita, M, Nagahama Y (1993) The egg nucleus regulates the behavior of sperm nuclei as well as cycling of MPF in physiologically polyspermic newt eggs. Dev Biol 160: 15–27

Iwao Y, Miki A, Kobayashi M, Onitake K (1994) Activation of *Xenopus* eggs by an extract of *Cynops* sperm. Dev Growth Differ 36: 469–479

Iwao Y, Kobayashi M, Miki, A, Kubota HY, Yoshimoto Y (1995) Activation of *Xenopus* eggs by *Cynops* sperm extract is dependent upon both extra- and intra-cellular Ca activities. Zool Sci 12: 573–581

Iwao Y, Yasumistu K, Narihira M, Jiang J, NagahamaY (1997) Changes in microtubule structures during the first cell cycle of physiologically polyspermic newt eggs. Mol Reprod Dev 47: 210–221

Jaffe LA, Cross NL, Picheral B (1983a) Studies of the voltage-dependent polyspermy block using cross-species fertilization of amphibians. Dev Biol 98: 319–326

Jaffe LA, Sharp AP, Wolf DP (1983b) Absence of an electrical polyspermy block in the mouse. Dev Biol 96: 317–323

Jones KT, Cruttwell C, Parrington J, Swann K (1998a) A mammalian sperm cytosolic phospholipase-C activity generates inositol trisphosphate and causes Ca^{2+} release in sea urchin egg homogenates. FEBS Lett 437: 297–300

Jones KT, Soeller C, Cannell MB (1998b) The passage of Ca^{2+} and fluorescent markers between the sperm and egg after fusion in the mouse. Development 125: 4627–4635

Katagiri C (1974) A high frequency of fertilization in premature and mature coelomic toad eggs after enzymic removal of vitelline membrane. J Embryol Exp Morphol 31: 573–587

Kline D (1988) Calcium-dependent events at fertilization of the frog egg, Injection of a calcium buffer blocks ion channel opening, exocytosis, and formation of pronuclei. Dev Biol 126: 346–361

Kline D, Nuccitelli R (1985) The wave of activation current in the *Xenopus* egg. Dev Biol 111: 471–487

Kline D, Simocini L, Mandel G, Maue RA, Kado RT, Jaffe LA (1988) Fertilization events induced by neurotransmitters after injection of mRNA in *Xenopus* eggs. Science 241: 464–467.

Kline D, Kopf GS, Muncy LF, Jaffe LA (1991) Evidence for the involvement of a pertussis toxin-sensitive G-protein in egg activation of the frog, *Xenopus laevis*. Dev Biol 143: 218–229

Kobayashi W, Yamamoto TS (1981) Fine structure of the micropylar apparatus of the chum salmon egg, with a discussion of the mechanism for blocking polyspermy. J Exp Zool 217: 265–275

Kobayashi W, Yamamoto TS (1994) Fertilization of the lamprey

(*Lampetra japonica*) eggs: Implication of the presence of fast and permanent blocks against polyspermy. J Exp Zool 269: 166–176
Kobayashi W, Baba Y, Shimozawa T, Yamamoto TS (1994) The fertilization potential provides a fast block to polyspermy in lamprey eggs. Dev Biol 161: 552–562
Kume S, Muto A, Aruga J, Nakagawa T, Michikawa T, Furuichi T, Nakade S, Okano H, Mikoshiba K (1993) The *Xenopus* IP_3 receptor, structure, function, and localization in oocytes and eggs. Cell 73: 555–570
Larabell C, Nuccitelli R (1992) Inositol lipid hydrolysis contributes to the Ca^{2+} wave in the activating egg of *Xenopus laevis*. Dev Biol 153: 347–355
Lee KW, Webb SE, Miller AL (1999) A wave of free cytosolic calcium traverses zebrafish eggs on activation. Dev Biol 214: 168–180
Le Naour F, Rubinstein E, Jasmin C, Prenant M, Boucheix C (2000) Severely reduced female fertility in CD9-deficient mice. Science 2000 287: 319–321
Lohka MJ, Hayes MK, Maller JL (1988) Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. Proc Natl Acad Sci USA 85: 3009–3013
Masui Y (1992) Towards understanding the control of the division cycle in animal cells. Biochem Cell Biol 70: 920–945
Matsuda M, Onitake K (1984) Fertilization of the eggs of *Cynops pyrrhogaster* (Japanese newt) after immersion in water. Roux's Arch Dev Biol 193: 61–63
McLaughlin EW, Humphries AAJr (1978) The jelly envelopes and fertilization of eggs of the newt, *Notophthalmus viridescens*. J Morphol 158: 73–90
Mehlmann LM, Carpenter G, Rhee SG, Jaffe LA (1998) SH2 domain-mediated activation of phospholipase Cγ is not required to initiate Ca^{2+} release at fertilization of mouse eggs. Dev Biol203: 221–232
Meyer A, Dolven S (1992) Molecules, fossils, and the origin of tetrapods. J Mol Evol 35: 102–113
Miyado K, Yamada G, Yamada S, Hasuwa H, Nakamura Y, Ryu F, Suzuki K, Kosai K, Inoue K, Ogura A, Okabe M, Mekada E (2000) Requirement of CD9 on the egg plasma membrane for fertilization. Science 2000 287: 321–324
Miyazaki S, Igusa Y. (1981) Fertilization potential in golden hamster eggs consists of recurring hyperpolarizations. Nature.290: 702–704
Miyazaki S, Igusa Y (1982) Ca-mediated activation of a K current at fertilization of golden hamster eggs. Proc Natl Acad Sci U S A 79: 931–935.
Miyazaki S, Yuzaki M, Nakada K, Shirakawa H, Nakanishi S, Nakade S, 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.
Miyazaki S, Shirakawa H, Nakada K, Honda Y (1993) Essential role of the inositol 1,4,5-trisphosphate receptor/Ca^{2+} release channel in Ca^{2+} waves and Ca^{2+} oscillations at fertilization of mammalian eggs. Dev Biol 158: 62–78
Mizote A, Okamoto S, Iwao Y (1999) Activation of *Xenopus* eggs by proteases, Possible involvement of a sperm protease in fertilization. Dev Biol 208: 79–92
Nuccitelli R (1980) The fertilization potential is not necessary for the block to polyspermy or the activation of development in the medaka egg. Dev Biol 76: 499–504
Nuccitelli R (1987) The wave of activation current in the egg of the medaka fish. Dev Biol 122: 522–534
Nuccitelli R, Kiline D, Busa WB, Talevi R, Campanella C (1988) A highly localized activation current yet widespread intracellular calcium increase in the egg of the frog, *Discoglossus pictus*. Dev Biol 130: 120–132
Nuccitelli R, Yim DL, Smart T (1993) The sperm-induced Ca^{2+} wave following fertilization of *Xenopus* egg requires the production of $Ins(1,4,5)P_3$. Dev Biol 158: 200–212
Parrington J, Swann K, Shevchenko VI, Sesay AK, Lai FA (1996) Calcium oscillations in mammalian eggs triggered by a soluble sperm factor. Nature 379: 364–368
Parys JB, Sernett SW, DeLisle S, Snyder PM, Welsh MJ, Campbell KP (1992) Isolation, characterization, and localization of the inositol 1,4,5-trisphosphate receptor protein in *Xenopus laevis* oocytes. J Biol Chem 267: 18776–18782.
Perry MM (1987) Nuclear events from fertilisation to the early cleavage stages in the domestic fowl. J Anat 150: 99–109
Perry AC, Wakayama T, Cooke IM, Yanagimachi R (2000) Mammalian oocyte activation by the synergistic action of discrete sperm head components: induction of calcium transients and involvement of proteolysis. Dev Biol. 2000 217: 386–393.
Peters JM, King RW, Hoog C, Kirschner MW (1996) Identification of BIME as a subunit of the anaphase-promoting complex Science 274: 1199–11201.
Rouvière C, Houliston E, Carré D, Chang P, Sardet C (1994) Characteristics of pronuclear migration in *Beroe ovata*. Cell Motil Cytoskeleton 29: 301–311
Runft LL, Watras J, Jaffe LA (1999) Calcium release at fertilization of *Xenopus* eggs requires type I IP(3) receptors, but not SH2 domain-mediated activation of PLCγ or G(q)-mediated activation of PLCβ . Dev Biol 214: 399–411
Sakamoto I, Takahara K, Yamashita M, Iwao Y (1998) Changes in cyclin B during oocyte maturation and early embryonic cell cycle in the newt, *Cynops pyrrhogaster*: Requirement of germinal vesicle for MPF. Dev Biol 195: 60–69
Sato K, Aoto M, Mori K, Akasofu S, Tokmakov AA, Sahara S, Fukami Y (1996) Purification and characterization of a Src-related p57 protein-tyrosine kinase from *Xenopus* oocytes. J Biol Chem 271: 13250–13257
Sato K, Iwasaki T, Tamaki I, Aoto M, Tokmakov AA, Fukami Y (1998) Involvement of protein-tyrosine phosphorylation and dephosphorylation in sperm-induced *Xenopus* egg activation. FEBS Lett 424: 113–118
Sato K, Iwao Y, Fujimura T, Tammaki I, Ogawa K, Iwasaki T, Tokmakov AA, Hatano O, Fukami Y (1999) Evidence for the involvement of a Src-related tyrosine kinase in the *Xenopus* egg activation. Dev Biol 209: 308–320
Sato K, Tokmakov AA, Iwasaki T, Fukami Y (2000) Tyrosine Kinase-dependent activation of phospholipase C gamma is required for calcium transient in *Xenopus* egg fertilization. Dev Biol, in press.
Sette C, Bevilacqua A, Bianchini A, Mangia F, Geremia R, Rossi P (1997) Parthenogenetic activation of mouse eggs by microinjection of a truncated c-kit tyrosine kinase present in spermatozoa Development 124: 2267–2274
Shilling FM, Kratzschmar J, Cai H, Weskamp G, Gayko U, Leibow J, Myles, DG, Nuccitelli R, Blobel, CP (1997) Identification of metalloprotease/disintegrins in *Xenopus laevis* testis with potential role in fertilization. Dev Biol 186: 155–164
Shilling FM, Magie CR, Nuccitelli (1998) Voltage–dependent activation of frog eggs by a sperm surface disintegrin peptide. Dev Biol 202: 113–124
Smith CL, Rand CS, Schaeffer B, Atz JW (1975) *Latimeria*, the living coelacanth, is ovoviviparous. Science 190: 1105–1106
Snow P, Yim DL, Leibow JD, Saini S, Nuccitelli R (1996) Fertilization stimulates an increase in inositol trisphosphate and inositol lipid levels in *Xenopus eggs*. Dev Biol 180: 108–118
Steinhardt RA, Epel D, Carroll EJ Jr, Yanagimachi R (1974) Is calcium ionophore a universal activator for unfertilised eggs? Nature 252: 41–43
Stith BJ, Goalstone M, Silva S, Jaynes C (1993) Inositol 1,4,5-trisphosphate mass changes from fertilization through first cleavage in *Xenopus laevis*. Mol Biol Cell 4: 435–443
Stith BJ, Espinoza R, Roberts D, Smart T (1994) Sperm increase inositol 1,4,5-trisphosphate mass in *Xenopus laevis* eggs preinjected with calcium buffers or heparin. Dev Biol 165: 206–

215
Stricker SA (1999) Comparative biology of calcium signaling during fertilization and egg activation in animals. Dev Biol 211: 157–176
Swann K, Ozil JP (1994) Dynamics of the calcium signal that triggers mammalian egg activation. Int Rev Cytol. 152: 183–222
Swann K, Parrington J (1999) Mechanism of Ca^{2+} release at fertilization in mammals. J Exp Zool 285: 267–75
Tokumoto T, Yamashita M, Tokumoto M, Katsu Y, Horiguchi R, Kajiura H, Nagahama Y (1997) Initiation of cyclin B degradation by the 26S proteasome upon egg activation. J Cell Biol 138: 1313–1322
Vu TK, Hung DT, Wheaton VI, Coughlin SR (1991) Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation Cell 64: 1057–1068.
Waddington D, Gribbin C, Sterling RJ, Sang HM, Perry MM (1998) Chronology of events in the first cell cycle of the polyspermic egg of the domestic fowl (*Gallus domesticus*). Int J Dev Biol 42: 625–628
Wassarman PM (1999) Mammalian fertilization: molecular aspects of gamete adhesion, exocytosis, and fusion. Cell 96: 175–183
Whitaker M, Swann K (1993) Lighting the fuse at fertilization. Development 117: 1–12
Williams CJ, Mehlmann LM, Jaffe LA, Kopf GS, Schultz RM (1998) Evidence that Gq family G proteins do not function in mouse egg activation at fertilization Dev Biol 198: 116–127
Yamamoto S, Iwao Y (1998) A sperm factor which induces an increase of intracellular Ca^{2+} level in the newt eggs. Zool Sci 15 Suppl: 64
Yamamoto S, Yamashita M, Iwao Y (1999a) Rise of intracellular Ca^{2+} level causes the decrease of cyclin B1 and Mos in the newt eggs at fertilization. Mol Reprod Dev 53: 341–349
Yamamto S, Iwao Y, Kubota H, Yoshimoto Y (1999b) Rise of intracellular Ca^{2+} level caused by the injection of sperm extract in the newt, *Cynops pyrrhogaster* eggs. Zool Sci 16 suppl: 66
Yim DL, Opresko LK, Wiley HS, Nuccitelli R (1994) Highly polarized EGF receptor tyrosine kinase activity initiates egg activation in *Xenopus*. Dev Biol 162: 41–55
Zardoya R, Meyer A (1997) Molecular phylogenetic information on the identity of the closest living relative(s) of land vertebrates. Naturwissenschaften 84: 389–397

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