Available online at www.sciencedirect.com
SCIENCE DIRECT®
Aquat. Living Resour. 16 (2003) 395–398
Aquatic Living Resources
www.elsevier.com/locate/aquliv

Prospective note

New insights into the mechanism of egg activation in fish

Kevin Coward *, John Parrington

Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT, UK

Received 19 May 2003; accepted 18 June 2003

Abstract

The commercial culture of several important species of fish has long been associated with productivity problems generally attributable to fertilisation rates, hatching rates, and embryonic development. Our present knowledge of the molecular processes accompanying fertilisation in fish is scant at best. Here, we examine how new findings about the molecular mechanisms underlying reproduction in other animal groups may help advance our understanding of how egg activation takes place in fish. A better understanding of egg activation in fish is likely to make a highly valuable contribution to future growth of the aquaculture industry.

Keywords: Egg activation; Fertilisation; Calcium; Fish

Despite continued growth in commercial aquaculture, we still know surprisingly little about the precise physiological mechanisms accompanying egg activation at fertilisation in fish. This is of great concern given the low 'capacity to fertilise' of several commercially important species in captivity. Captive fish often exhibit reductions in fertilisation rate and/or hatching success when compared to their wild counterparts. Certain marine species appear to be particularly prone (see Coward et al., 2002). As a consequence, aquaculture productivity is often constrained due to problems associated with fertilisation, hatching, or early embryonic development. Exogenous and endogenous factors affecting fertilisation, along with detailed discussion of the problems associated with fertilisation in commercial species, are discussed by Coward et al. (2002). Fertility problems of a different sort are found in some wild populations of fish. For instance, Jobling et al. (2002) reported reductions in sperm motility, density and fertilisation success in roach *Rutilus rutilus* (L.) that possessed intersex gonads as a result of exposure to endocrine-disrupting chemicals.

Our knowledge of egg activation in fish has been based almost exclusively on small laboratory species, such as zebrafish *Brachydanio rerio* (Hamilton) and medaka *Oryzias latipes* (Temmink and Schlegel), that, while being important experimental models, have no great commercial importance. Even in these species, the exact molecular mechanisms underlying activation of the egg remain far from clear. However, in other animal groups, such as echinoderms and mammals, there have been significant advancement over the last decade in our scientific understanding of the molecular mechanisms of egg activation. It is from such studies that we ought to be able to gain insights about what is taking place in fish eggs at fertilisation.

Activation of the egg at fertilisation, in practically all species studied thus far, is initiated by a sperm induced increase in free cytosolic calcium (Ca^{2+}) concentration in the egg (Stricker, 1999). This Ca^{2+} rise appears to be essential for successful egg activation, and for early embryonic development (Swann and Parrington, 1999). In sea urchins, frogs, and fish (medaka and zebrafish), sperm–egg fusion leads to a single explosive wave of Ca^{2+} traversing the egg from one pole to the other (Jaffe, 1983; Whitaker and Swann, 1993; Stricker, 1999). By contrast, in mammals, nemertean worms, and ascidians, a series of periodic increases in Ca^{2+} ('oscillations') are evident (Miyazaki et al., 1993; Swann and Ozil, 1994; Stricker, 1999). A primary mechanism of Ca^{2+} release in the egg appears to involve increased levels of inositol 1,4,5–triphosphate (IP_3) subsequently causing release of Ca^{2+} from intracellular egg stores via an IP_3 receptor-mediated process (Miyazaki et al., 1993; Jellerette et al., 2000). There are numerous studies providing evidence for the involvement of IP_3 in this process. For example, if binding of IP_3 to the IP_3–receptor is blocked by injecting the IP_3

* Corresponding author.
E-mail address: kevin.coward@pharm.ox.ac.uk (K. Coward).

doi:10.1016/S0990-7440(03)00068-8

antagonist heparin, or a functionally inhibitory IP_3–receptor antibody, Ca^{2+} release at fertilisation and egg activation is also blocked (Miyazaki et al., 1993). Furthermore, there appears to be an increased turnover of phosphoinositide lipids and increase in IP_3 levels in sea urchin and frog eggs at fertilisation (Nuccitelli, 1991; Ciapa et al., 1992; Snow et al., 1996; Lee and Shen, 1998) and Ca^{2+} release at egg activation can be inhibited in both species with the PLC inhibitor U73122 (Dupont et al., 1996; Lee and Shen, 1998). However, we must not dismiss nor neglect the possible involvement of other naturally-occurring calcium releasing agents and their respective signalling pathways; cyclic ADP-ribose (cADPR), nicotinic acid adenine dinucleotide phosphate (NAADP), nitric oxide (NO), and cyclic GMP (cGMP) pathways all play crucial roles in Ca^{2+} release in various physiological signalling mechanisms (Lee, 1977; Galione et al., 2000; Bootman et al., 2001; Leckie et al., 2003). Particularly noteworthy is the recent finding that sea urchin sperm appear to deliver the Ca^{2+} mobilising molecule NAADP to the egg (Churchill et al., 2003). Furthermore, studies appear to suggest that the Ca^{2+} wave seen at fertilisation in the sea urchin is due to more than one mechanism. For instance, Lee et al. (1993) revealed that both cADPR and IP_3 receptors were activated during fertilisation in the sea urchin; blockage of either of the two systems was not sufficient to prevent the sperm-induced Ca^{2+} transient. Galione et al. (1993) further reported that both IP_3 and ryanodine receptor channels were implicated in the Ca^{2+} wave seen in the sea urchin egg at fertilisation; authors hypothesised that these alternative mechanisms of Ca^{2+} release implied redundancy but probably also allowed for modulation and diversity in the generation of Ca^{2+} waves.

Existing data derived from fish studies, do demonstrate clear changes in Ca^{2+} at fertilisation; indeed some of the pioneering studies concerning the general role of Ca^{2+} of fertilisation were undertaken in fish. An explosive Ca^{2+} wave was seen traversing the activating medaka egg during fertilisation, followed by a slow return to resting levels (Ridgway et al., 1977; Gilkey et al., 1978). Similar results were subsequently observed in zebrafish eggs (Lee et al., 1999). Injecting IP_3 or Ca^{2+} into medaka eggs also caused Ca^{2+} release (Iwamatsu et al., 1998). Lee et al. (1996) further reported a single wave of Ca^{2+} traversing the zebrafish egg when activated by ionomycin, a chemical that selectively allows Ca^{2+} across cell membranes. Subsequent studies highlight the importance of another Ca^{2+}–mobilising agent, cyclic-ADPR (cADPR). For example, microinjection of cADPR triggered a regenerative wave of Ca^{2+} release in medaka eggs (Fluck et al., 1999). Polzonetti et al. (2002) further showed that cADPR can induce Ca^{2+} release in an homogenate made from Gilthead sea bream *Sparus aurata* (L.) eggs, indicating a potential role at fertilisation; data further revealed that the level of cADPR in the sea bream egg increased after sperm entrance. Since sea bream eggs are pelagic, floating eggs are considered viable whilst those that sink are non-viable. Polzonetti et al. (2002) compared viable (floating) eggs with non-viable (sinking) eggs in terms of nucleoside/nucleotide content, specific enzymic activity of those enzymes involved in NAD^+ metabolism (cADPR is a product of NAD^+ metabolism), and cADPR content. Results showed that although enzymic activity was similar in the two types of eggs, sinking non-viable eggs exhibited a significantly lower nucleoside content and that in these eggs, cADPR was undetectable.

The precise mechanism by which sperm initiate the signalling cascade that results in Ca^{2+} release at fertilisation remains a hotly debated issue (see Parrington, 2001). Two main theories have been put forward. The first suggests that Ca^{2+} is released from internal egg stores at egg activation via a surface mediated interaction between a ligand on the sperm and an egg receptor. An alternative hypothesis suggests that egg activation is triggered by a soluble 'sperm factor' that is introduced into the egg at sperm–egg fusion (for discussion see Parrington, 2001). In mammals, there is now convincing evidence that a soluble 'sperm factor' is introduced into the egg at sperm–egg fusion. Numerous studies have now established that the injection of soluble sperm protein extracts from various mammalian species can trigger Ca^{2+} oscillations (similar to those seen at fertilisation) in mouse, hamster, human and cow eggs (Swann, 1990; Swann, 1992; Homa and Swann, 1994; Palermo et al., 1997). The sperm factor appears to be a sperm phospholipase C (PLC) with distinctive properties (Jones et al., 1998; Parrington et al., 1999; Rice et al., 2000; Jones et al., 2000; Parrington et al., 2000; Parrington et al., 2002). The PLC responsible was recently identified at the molecular level in the mouse, human and cynomolgus monkey as a sperm-specific novel isoform, which has been named PLCζ (Saunders et al., 2002; Cox et al., 2002).

Soluble sperm extracts also induce Ca^{2+} oscillations in some non-mammals such as marine worms (Stricker, 1997), ascidians (Kyozuka et al., 1998) and sea urchins (Jones et al., 1998, 2000). Sperm extracts from chicken and *Xenopus* (Dong et al., 2000), and even a flowering plant (Li et al., 2001), have also been shown to contain a cytosolic soluble sperm factor that can trigger calcium oscillations when injected into mouse eggs. Dong et al. (2000) suggested that the discovery of sperm factor activity in non-mammals implied that the ability of the sperm factor to induce calcium oscillations in mouse eggs may not be species-specific, at least in vertebrates. The discovery of a cytosolic sperm factor in a species of plant is particularly interesting since it shows that although plants and mammals are evolutionary divergent, the activity of the putative sperm factor in triggering calcium release in mammalian eggs may not be specific to the animal kingdom (Li et al., 2001). However, it has yet to be firmly established whether a sperm factor does play a universal role during fertilisation in animals and plants.

Further evidence for the existence of a sperm-factor mechanism in non-mammalians came with our own recent discovery that soluble protein extracts prepared from fish spermatozoa (*Oreochromis niloticus* L.) induced calcium release in an established calcium bioassay, and also triggered

calcium oscillations when injected into mouse oocytes. Furthermore, we detected a PLC activity with similar properties to that in mammalian sperm in tilapia sperm extracts (Coward et al., 2003). These novel findings strongly suggest that the sperm of this commercially-important fish may contain a sperm factor similar to that found in mammalian species. These findings are likely to make an important preliminary contribution to a better understanding of egg activation in commercially-important fish, which is of obvious potential interest to aquaculture given the current concern over the fertilisation rates of some farmed teleost species

Although these findings suggest the existence of a sperm factor similar to that in mammals in tilapia sperm, a non-mammalian homologue of PLCζ has yet to be identified. It also remains the case that quite different mechanisms of egg activation may be operating in fish compared to those in mammals. In sea urchins for example, the commonly-favoured theory involves an egg receptor/sperm ligand mechanism possibly involving an egg PLCγ (Jaffe et al., 2001). Moreover, even though we have strong evidence for a sperm factor molecule in tilapia, this might not be the case in other fish species. For instance, one study has suggested that zebrafish eggs may be activated merely by contact with the spawning medium (Lee et al., 1999) whilst medaka eggs appear to specifically require contact with sperm for activation to occur (Gilkey et al., 1978; Yoshimoto et al., 1986), possibly indicating the existence of two conflicting mechanisms.

In addition to egg activation mechanisms involving the phosphoinositide pathway, it is important not to overlook Ca^{2+} signalling agents such as cADPR and NAADP (Lee, 1977; Galione et al., 2000; Bootman et al., 2001). cADPR, NO and cGMP pathways are also thought to be involved at fertilisation in sea urchin eggs (Leckie et al., 2003). Future research on fish eggs must, therefore, address the possible functional role of such molecules at fertilisation. There are many unanswered questions. We especially need to identify which molecules are specifically involved in fish egg activation and notably whether the mechanism involves novel or known molecules. The potential role of all existing Ca^{2+} releasing molecules should be addressed using inhibitors and blocking molecules. Selected blocking molecules might include heparin (IP_3 receptor), U73122 (PLC), or 8-amino-cADP-ribose (cADPR). Research should address the potential involvement of each of the known PLC isoforms (β, γ, δ, ε, and ζ). Novel genes will need to be cloned and characterised from selected fish species, and genome mapping projects are likely to assist greatly in this area. Bioassays of Ca^{2+} release such as the sea urchin egg homogenate, and others developed specifically for fish, are likely to be important in initial experiments investigating the Ca^{2+} releasing abilities of any novel genes cloned from fish. Functionality in vivo can be investigated by injecting appropriate eggs with mRNA or recombinant protein produced from selected signalling molecules, and imaging changes in intracellular calcium by use of Ca^{2+} sensitive reporter dyes and confocal microscopy. Further research should address the specific role these molecules might play along with their location, expression and regulation. The probability of interactions with other molecules in fish eggs and sperm will need to be explored.

Clearly, there is much to be done at the biochemical, molecular and genomic level. Future research needs to make full use of genomic/proteomic databases, recombinant technology, molecular biology, bioassays, and incorporate recent advances in single cell fluorescent imaging systems. It is evident that these are exciting times for those involved with fish reproduction. However, we conclude on a note of caution; teleost reproductive strategies, and more importantly, teleost oocytes and spermatozoa, exhibit a remarkable variety of biological adaptations. As a consequence, the elucidation of the precise mechanisms acting at fertilisation in fish is likely to be a tedious and time-consuming process that will most probably have to be undertaken in a species-specific fashion. Nevertheless, this research area remains an important one and deserves much attention.

Acknowledgements

Some of the work described herein was funded by a grant from the Fisheries Society of the British Isles to K. Coward, and an MRC Senior Non-Clinical Fellowship to J. Parrington. The authors also wish to acknowledge Antonio-Campos Mendoza, Niall Bromage and Brendan McAndrew (Institute of Aquaculture, University of Scotland), for their contributions to this project.

References

Bootman, M., Collins, T.J., Peppiatt, L.S., Prothero, L.S., Mackenzie, L., De Smet, P., Travers, M., Tovey, S.C., Seo, J.T., Berridge, M.J., Ciccolini, F., Lipp, P., 2001. Calcium signalling–an overview. Semin. Cell Dev. Biol. 12, 3–10.

Churchill, G.C., O'Neill, J.S., Masgrau, R., Patel, S., Thomas, J.M., Genazzani, A.A., Galione, A., 2003. Sperm deliver a new second messenger. NAADP. Curr. Biol. 13, 125–128.

Ciapa, B., Borg, B., Whitaker, M.J., 1992. Polyphosphinositide metabolism during the fertilization wave in sea urchin eggs. Development 115, 187–195.

Coward, K., Bromage, N.R., Hibbitt, O., Parrington, J., 2002. Gametogenesis, fertilization and egg activation in teleost fish. Rev. Fish Biol. Fish. 12, 33–58.

Coward, K., Campos-Mendoza, A., Larman, M., Hibbitt, O., McAndrew, B., Bromage, N., Parrington, J., 2003. Teleost fish spermatozoa contain a cytosolic protein factor that induces calcium release in sea urchin egg homogenates and triggers calcium oscillations when injected into mouse oocytes. Biochem. Biophys. Res. Commun. 305, 299–304.

Cox, L.J., Larman, M.G., Saunders, C.M., Hashimoto, K., Swann, K., Lai, F.A., 2002. Sperm phospholipase Cζ from humans and cynomolgus monkeys triggers Ca^{2+} oscillations, activation and development of mouse oocytes. Reproduction 124, 611–623.

Dong, J.-B., Tang, T.-S., Sun, F.-Z., 2000. *Xenopus* and chicken sperm contain a cytosolic soluble protein factor, which can trigger calcium oscillations in mouse eggs. Biochem. Biophys. Res. Commun. 268, 947–951.

Dupont, G., McGuinness, O.M., Johnson, M.H., Berridge, M.J., Borgese, F., 1996. Phospholipase C in mouse oocytes: characterisation of isoforms and their possible involvement in sperm-induced Ca^{2+} spiking. Biochem. J. 316, 583–591.

Fluck, R., Abraham, V., Miller, A., Galione, A., 1999. Microinjection of cyclic ADP-ribose triggers a regenerative wave of Ca^{2+} release and exocytosis of cortical alveoli in medaka eggs. Zygote 7, 285–292.

Galione, A., McDougall, A., Busa, W.B., Willmott, N., Gillot, I., Whitaker, M., 1993. Redundant mechanisms of calcium-induced calcium release underlying calcium waves during fertilization of sea urchin eggs. Science 261, 348–352.

Galione, A., Patel, S., Churchill, G.C., 2000. NAADP-induced calcium release in sea urchin eggs. Biol. Cell 92, 197–204.

Gilkey, J.C., Jaffe, L.F., Ridgway, E.B., Reynolds, G.T., 1978. A free calcium wave traverses the activating egg of the medaka, *Oryzias latipes*. J. Cell Biol. 76, 448–466.

Homa, S.T., Swann, K., 1994. A cytosolic sperm factor triggers calcium oscillations and membrane hyperpolarizations in human oocytes. Hum. Reprod. 9, 2356–2361.

Iwamatsu, T., Yoshimoto, Y., Hiramoto, Y., 1998. Mechanism of Ca^{2+} release in medaka eggs microinjected with inositol 1,4,5–triphosphate and Ca^{2+}. Dev. Biol. 129, 191–197.

Jaffe, L.F., 1983. Sources of calcium in egg activation: a review and hypothesis. Dev. Biol. 99, 265–276.

Jaffe, L.A., Giuisti, A.F., Carroll, D.J., Foltz, K.R., 2001. Ca^{2+} signalling during fertilization of echinoderm eggs. Sem. Cell. Dev. Biol. 12, 45–51.

Jellerette, T., He, C.L., Wu, H., Parys, J.B., Fissore, R.A., 2000. Down-regulation of the inositol 1,4,5–triphosphate receptor in mouse eggs following fertilization or parthenogenetic activation. Dev. Biol. 223, 238–250.

Jobling, S., Coey, S., Whitmore, J.G., Kime, D.E., Van Look, K.J.W., McAllister, B.G., Beresford, N., Hensaw, A.C., Brighty, G., Tyler, C.R., Sumpter, J., 2002. Wild intersex roach (*Rutilus rutilus*) have reduced fertility. Biol. Reprod. 67, 515–524.

Jones, K.T., Crutwell, C., Parrington, J., Swann, K., 1998. A mammalian sperm cytosolic phospholipase C activity generates inositol triphosphate and causes Ca^{2+} release in sea urchin egg homogenates. FEBS Lett. 437, 297–300.

Jones, K.T., Matsumo, M., Parrington, J., Katan, M., Swann, K., 2000. Different Ca^{2+} releasing abilities of sperm extracts compared with tissue extracts and phospholipase C isoforms in sea urchin egg homogenates and mouse eggs. Biochem. J. 346, 743–749.

Kyozuka, K., Deguchi, R., Mohri, T., Miyazaki, S., 1998. Injection of sperm extract mimics spatiotemporal dynamics of Ca^{2+} responses and progression of meiosis at fertilization of ascidian oocytes. Development 125, 4099–4105.

Leckie, C., Empsom, R., Becchetti, A., Thomas, J., Galione, A., Whitaker, M., 2003. The NO pathway acts late during the fertilization response in sea urchins. J. Biol. Chem. 278, 12247–12254.

Lee, H.C., 1977. Mechanisms of calcium signalling by cyclic ADP-ribose and NAADP. Physiol. Rev. 77, 1133–1164.

Lee, S.J., Shen, S.S., 1998. The calcium transient in sea urchin eggs during fertilization requires the production of inositol 1,4,5–triphosphate. Dev. Biol. 193, 195–208.

Lee, H.C., Aarhus, R., Walseth, T.F., 1993. Calcium mobilization by dual receptors during fertilization of sea urchin eggs. Science 261, 352–355.

Lee, K.W., Baker, R., Galione, A., Gilland, E.H., Hanlon, R.T., Miller, A.L., 1996. Ionophore-induced calcium waves activate unfertilized zebrafish (*Danio rerio*) eggs. Biol. Bull. 191, 265–267.

Lee, K.W., Webb, S.E., Miller, A.L., 1999. A wave of free cytosolic calcium traverses zebrafish eggs on activation. Dev. Biol. 214, 168–180.

Li, S.-T., Huang, X.-Y., Sun, F.-Z., 2001. Flowering plant sperm contains a cytosolic soluble protein factor, which can trigger calcium oscillations in mouse eggs. Biochem. Biophys. Res. Commun. 287, 56–59.

Miyazaki, S., Shirakawa, H., Nakada, K., Honda, Y., 1993. Essential role of the inositol 1,4,5–triphosphate/Ca^{2+} channel in Ca^{2+} waves and Ca^{2+} oscillations at fertilization of mammalian eggs. Dev. Biol. 158, 62–78.

Nuccitelli, R., 1991. How do sperm activate eggs? Curr. Top. Dev. Biol. 25, 1–16.

Palermo, G.D., Avredch, O.M., Columbero, L.T., Wu, H., Wolny, Y.M., Fissore, R.A., Rosenwaks, Z., 1997. Human sperm cytosolic factor triggers Ca^{2+} oscillations and overcomes activation failure of mammalian oocytes. Mol. Hum. Reprod. 3, 367–374.

Parrington, J., 2001. Does a soluble sperm factor trigger calcium release in the egg at fertilization? J. Androl. 22, 1–11.

Parrington, J., Jones, K.T., Lai, A., Swann, K., 1999. The soluble sperm factor that causes Ca^{2+} release from sea urchin (Lytechinus pictus) egg homogenates also triggers Ca^{2+} oscillations after injection into mouse eggs. Biochem. J. 341, 1–4.

Parrington, J., Lai, F.A., Swann, K., 2000. The soluble sperm factor protein that triggers Ca^{2+} oscillations in eggs: evidence for expression of mRNA(s) coding for sperm factor protein(s) in spermatogenic cells. Biol. Cell. 92, 267–275.

Parrington, J., Jones, M.L., Tunwell, R., Devader, C., Katan, M., Swann, K., 2002. Phospholipase C isoforms in mammalian spermatozoa: Potential components of the sperm factor that causes Ca^{2+} release in eggs. Reproduction 123, 31–39.

Polzonetti, V., Cardinali, M., Mosconi, G., Natalini, P., Meiri, I., Carnevali, O., 2002. Cyclic ADPR and calcium signalling in sea bream (*Sparus aurata*) egg fertilization. Mol. Reprod. Dev. 6, 213–217.

Rice, A., Parrington, J., Jones, K.T., Swann, K., 2000. Mammalian sperm contain a Ca^{2+}-sensitive phospholipase C activity that can generate InsP3 from PIP2 associated with intracellular organelles. Dev. Biol. 228, 125–135.

Ridgway, E.B., Gilkey, J.C., Jaffe, L.F., 1977. Free calcium increases explosively in activating medaka eggs. Proc. Natl. Acad. Sci. USA 74, 623–627.

Saunders, C.M., Larman, M.G., Parrington, J., Cox, L.J., Royse, J., Blayney, L.M., Swann, K., Lai, F.A., 2002. PLCζ: a sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. Development 129, 3533–3544.

Snow, P., Yim, D.L., Leibow, J.D., Saini, S., Nuccitelli, R., 1996. Fertilization stimulates an increase in inositol triphosphate and inositol lipid levels in *Xenopus* eggs. Dev. Biol., 108–118.

Stricker, S.A., 1997. Intracellular injections of a soluble sperm factor trigger calcium oscillations and meiotic maturation in unfertilized oocytes of a marine worm. Dev. Biol. 186, 185–201.

Stricker, S.A., 1999. Comparative biology of calcium signalling during fertilization and egg activation in animals. Dev. Biol. 211, 57–76.

Swann, K., 1990. A cytosolic sperm factor stimulates repetitive calcium increases and mimics fertilization in hamster eggs. Development 110, 1295–1302.

Swann, K., 1992. Different triggers for calcium oscillations in mouse eggs involve a ryanodine-sensitive calcium store. Biochem. J. 287, 79–84.

Swann, K., Ozil, J.P., 1994. Dynamics of the calcium signal that triggers mammalian oocyte activation. Int. Rev. Cytol. 152, 183–222.

Swann, K., Parrington, J., 1999. Mechanism of Ca^{2+} release at fertilization in mammals. J. Exp. Zool. (Mol. Dev. Evol.) 282, 267–275.

Whitaker, M., Swann, K., 1993. Lighting the fuse at fertilization. Development 117, 1–12.

Yoshimoto, Y., Iwamatsu, T., Hirano, K., Hiramoto, Y., 1986. The wave pattern of free calcium release upon fertilization in medaka and sand dollar eggs. Dev. Growth Diff. 28, 583–596.