Fertilization and egg activation in fish – a new perspective

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In all species studied so far, sperm activate an egg to begin developing into an embryo by triggering an explosive wave of intracellular calcium in the egg.

The identity of the signalling molecules that mediate this fundamental event has remained a mystery until recently. Now, new information emerging from the genome projects has made it possible to isolate and characterise the protein responsible. This discovery is not only of huge importance to human fertility; findings might also have great relevance to commercial aquaculture.

Fertilization, egg activation and aquaculture

Despite the continued growth and importance of aquaculture to worldwide human consumption, we still know very little about the precise mechanisms associated with fertilization and egg activation in fish. Indeed, many species of commercial importance suffer from problems associated with fertilization, hatching and early embryonic development (Coward et al., 2002). Over the last ten years, our knowledge of fertilization and activation in mammalian eggs has grown enormously. It is vital that lessons learnt from this field are swiftly applied to the problems facing fish fertility. Collaboration between the Department of Pharmacology (University of Oxford) and the Institute of Aquaculture (University of Stirling) is already attempting to address these issues. Here, we aim to introduce some of our research.

Egg activation – the role of calcium

Changes in the concentration of calcium within a cell constitutes one of the most important signalling mechanisms in the body. One of the most fundamental biological processes in which calcium signalling plays a central role is the activation of the egg by the sperm. It is commonly known that sperm supply the paternal set of genes for a new organism. It is less well known that sperm must also activate the egg to begin developing into an embryo. The egg needs to be activated because it is frozen in its cell cycle until fusion with a sperm. The process of activation enables the fertilized egg to start producing the proteins that will perform vital functions in the embryo and to break from its state of arrest in the cell cycle. The mechanism by which the sperm activates the egg has been studied for over a century but it is only relatively recently that we have known that sperm activate eggs by triggering a release of calcium from internal stores within the egg.

The 'sperm factor' theory

Until recently, the most popular theory was that sperm activate eggs through an interaction between proteins on their outer surface. Recent progress, however, has provided strong evidence of the existence of a 'sperm factor'. This theory suggests calcium release is caused by a soluble protein that enters the egg when it fuses with the sperm (Figure 1). For an in-depth discussion of the theories put forward to explain egg activation, see Parrington (2001). In brief, the soluble protein ('sperm factor') introduced into the egg at fertilization triggers a signalling cascade. The sperm factor (now known to be a novel member of the phospholipase C [PLC] family) causes release of inositol triphosphate (IP3) by hydrolysing phosphotidyl 4, 5-bisphosphate (PIP2) within the egg. IP3 then causes release of calcium from internal egg stores such as the endoplasmic reticulum (see Figure 1). Remarkably, in all species studied so far, in organisms as diverse as human beings and plants, a rise in intracellular calcium levels was the universal trigger for egg activation. However, despite its apparent universal nature, the form that the calcium increase takes varies significantly between species. In sea urchins, frogs and fish (medaka), a single, explosive wave of calcium is observed crossing the egg at fertilization. In contrast, mammals, some marine worms, and sea slugs, exhibit a series of calcium increases called "calcium oscillations".

Identity of the mammalian "sperm factor"

The cytosolic protein factor carried by sperm and responsible for initiating calcium release at egg activation has

From left; Kevin Coward, John Parrington, and Olivia Hibbitt

recently been identified at the molecular level in mouse, human and cynomolgus monkeys as a sperm-specific novel isoform of PLC which has been named PLCæ (Saunders et al., 2002). Injection of cRNA encoding for PLCæ triggered Ca^{2+} oscillations in mouse eggs that were indistinguishable from those seen at fertilization. Moreover, removal of endogenous PLCæ from sperm extracts totally abolished Ca^{2+} release in eggs. A non-mammalian version has yet to be isolated.

Future perspectives – potential for aquaculture

Currently, studies of egg activation in fish are confined to small laboratory species that are of little or no commercial importance, eg., zebrafish, *Zebradanio rerio* and medaka, *Oryzias latipes*. This very limited data suggests that egg activation in both these species is accompanied by an increase in egg intracellular calcium, though curiously the mechanism of calcium release appears to be quite different. Egg activation in medaka is initiated only through direct contact with sperm suggesting the involvement of a sperm-specific factor, while zebrafish eggs appear to only require contact with the spawning medium. In view of the highly variable fertility rates evident in many commercially important teleosts at the present time, it is important that some of the lessons learnt from our mammalian work are swiftly applied to representative groups of fish using a combination of physiological, molecular, recombinant and genomic/proteomic technology. We now know that sperm trigger an explosive rise in egg calcium at fertilization in many different animal species (and plants), and that this characteristic rise is vital to the success of subsequent embryonic development. The Molecular Mechanisms of Reproduction Group at Oxford, together with Professor Bromage and Professor McAndrew and Antonio Campos-Mendoza of the Institute of Aquaculture, have already started to address this shortfall via a long-term and on-going collaborative project. Findings are likely to be of great significance to commercial aquaculture whilst also providing vital comparison with other animal groups. We already have strong evidence to suggest the existence of a 'sperm factor' in tilapia sperm (Coward et al., 2003); tilapia sperm extracts induced calcium release in an established calcium bioassay, and triggered calcium oscillations when injected into mouse oocytes. Detailed knowledge of the precise mechanisms and molecules involved in the activation of fish eggs may pave the way to improved fertilization and hatching rates and thus help optimise the management of culture species.

References

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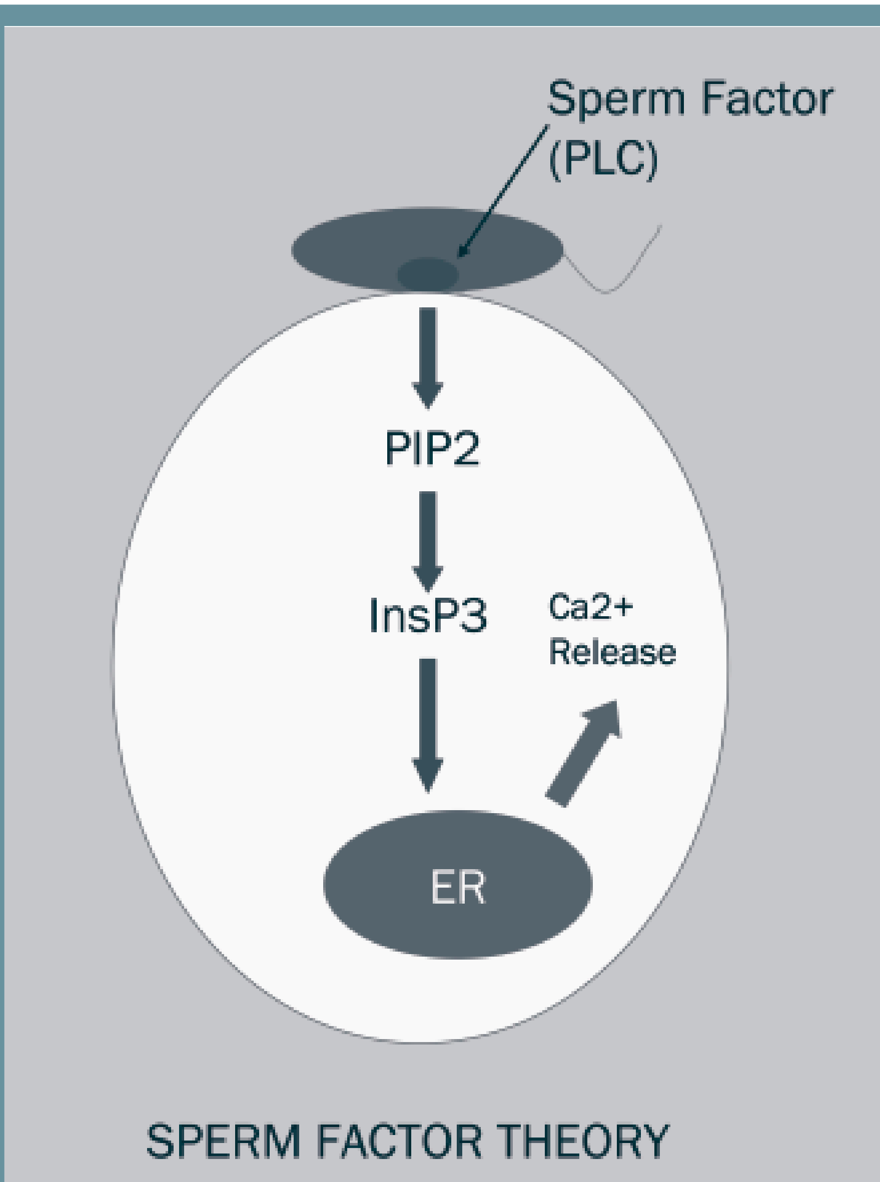

Figure 1: The sperm factor theory (PIP2 = phosphotidyl 4,5 – bisphosphate; IP3 = inositol triphosphate, ER = endoplasmic Reticulum)

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