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Molecular Signalling Mechanisms Involved in the Development of Fertilizing Capacity by Mammalian Spermatozoa

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Ⅰ. INTRODUCTION

Four important attributes for successful fertilization by a spermatozoon are (i) correct morphology, (ii) correct presentation of egg recognition and fusion molecules, (iii) progressive motility and (iv), correct transfer of signalling molecules from sperm to egg for activation of development. In this presentation, these topics will be described and illustrated with emphasis on the endogenous control mechanisms that enable spermatozoa to respond to external signals.

Ⅱ. MORPHOLOGY OF SPERMATOZOA

The morphology of spermatozoa is highly species specific and it is well known that aberrant shape is closely associated with infertility in man and animals (Yanagimachi, 1994). In *azh* mutant mice for example, which produce large numbers of spermatozoa with misshapen heads, fertility is reduced to ~10% of wild-types (Meistrich et al., 1994). Sperm morphology is tightly regulated at the genetic level to such an extent that it is possible to transfect rat spermatogonia into mouse testis *in vivo* and not only are the rat germ cells not rejected, but morphologically normal rat spermatozoa are produced in combination with mouse spermatozoa (Brinster & Zimmermann, 1994). Correct morphology is established during spermiogenesis in the testis when round spermatids differentiate into elongated spermatozoa and seems to be controlled by the cytoskeleton, which in turn is regulated by the activity of a group of small actin regulatory proteins such as β-thymosins, destrin/cofilin and profilin (Sun et al., 1994). Individual actin binding proteins have specific effects on the cytoskeleton and *in vivo* their combined action is complex, since they interact simultaneously with each other and with various signalling processes in the cytoplasm. Testis-specific transcripts of these proteins have been identified (Hurst et al., 1998) and their activity is very sensitive to intracellular to pH, ATP, Ca^{2+} etc., thereby enabling wide-ranging changes in the cytoskeleton to take place from relatively simple stimuli. External influences such as heat, selenium deficiency and environmental pollutants also cause abnormal sperm morphology by interfering with some or many of the intracellular remodelling processes.

Ⅲ. CORRECT PRESENTATION OF EGG RECOGNITION MOLECULES ON SPERMATOZOA

Although much is known about the nature of the receptors molecules for spermatozoa on the zona pellucida (Wassarman, 1990), there is considerable debate over the identity of the complementary binding proteins on spermatozoa

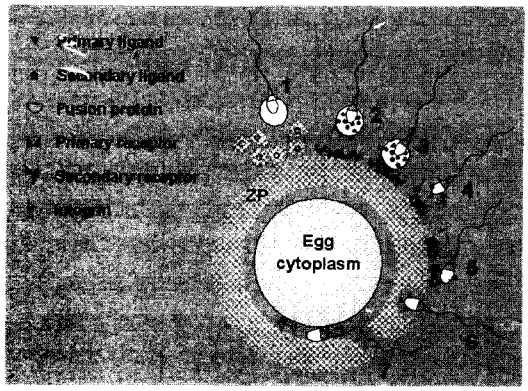

Fig. 1. Proposed sequence of events during binding of the fertilizing spermatozoon to the zona pellucida and oolemma of the egg. Steps 2 to 4 represent primary binding and steps 5 to 6 secondary binding. For further details see Jones et al. (1996).

that allow them to recognise and interact very specifically with homologous eggs. These zona-binding proteins can be classified as primary or secondary depending on whether they participate in the initial attachment of acrosome-intact sperm to the zona or whether they function to retain sperm on the zona surface after the acrosome reaction. (Fig. 1). Examples of the former include galactosyltransferase (Miller & Shur, 1994), sp56 (Bookbinder et al., 1995), M42 (Saling & Lakoski, 1985), FA-1 (Naz, 1987) and SP17 (O'Rand et al., 1988). Fewer secondary binding molecules have been identified, the two most outstanding being proacrosin/acrosin (Jones et al., 1996) and PH20 (Phelps & Myles, 1987) which are located within the acrosomal vesicle and inner acrosomal membrane. However, since spermatozoa are transcriptionally inactive and cannot, therefore, respond to agonists by making new proteins, they have devised several strategies for regulating their fertilizing capacity. One of the most important strategies is processing and re-positioning of molecules from regions on the sperm where they are inactive to regions where they become active. Examples of this are guinea pig sperm fertilin and rat 2B1 glycoprotein which are endoproteolytically cleaved during epididymal maturation and then migrate to new regions within in the plasma membrane. In the case of 2B1 glycoprotein, (the orthologue of guinea pig PH20, a membrane-bound hyaluronidase) which contains a GPI anchor, cleavage takes place at a specific internal arginine residue creating a two-chain molecule cross-linked by disulphide bridges (Jones et al., 1996). This affects its pH optimum for degradation of hyaluronic acid. Cleavage also appears to be a prerequisite for the ability of 2B1 to migrate from the sperm tail to the acrosmal domain during capacitation within the plane of the plasma membrane (Jones et al., 1990). The mechanisms controlling migration of 2B1 over this large distance (the majority of plasma membrane proteins diffuse over relatively short distances and within defined surface domains) are not known but they involve Ca^{2+} and are temperature sensitive. The most immediate possibilities are directional transport in combination with the effects of an intramembranous barrier in the posterior ring. Recent analysis using photobleaching (FRAP) techniques indicates that lateral diffusion of lipids within the plasma membrane varies between different regions suggesting that they have a different lipid composition and/or organisation due to lateral asymmetry within the plasma membrane (Wolfe et al., 1998; James et al., 1999).

A second strategy employed by spermatozoa for correct presentation of zona binding molecules is to enclose them within the acrosomal vesicle so that they are targeted passively onto the zona surface at the time of the acrosome reaction. Consequently, a lower specificity of recognition by such molecules could be tolerated.

Much evidence (mostly from work on the pig but also in the rabbit) indicates that proacrosin/acrosin plays such a role and that it should be regarded as a multifunctional protein (Jones & Brown, 1987; Jones, 1991; Jansen et al., 1995, 1998; Richardson & O'Rand, 1996). Proacrosin/acrosin binds in a stereospecific manner to polysulphate groups on zona glycoproteins and can be inhibited by sulphated drugs such as suramin and its analogues (Jones et al., 1996). Suramin is also active in preventing fertilization *in vitro* (mouse model) and has been shown to bind directly to proacrosin/acrosin on western blots (Jones et al., 1996). Thus, proacrosin/acrosin is released at the appropriate time and place to mediate secondary binding.

Ⅳ. REGULATION OF SPERM MOTILITY

Progressive motility by spermatozoa is well known to be a prerequisite for high fertility and is regulated by interplay between several intracellular systems of which intracellular pH, cAMP and Ca^{2+} levels are arguably the most important (Vijayarahavan et al., 1985; 1996). cAMP activates protein kinases that phosphorylate axonemal proteins to stimulate flagellar activity. Phosphorylation is opposed by protein phosphates (specifically PP1 and PP2A) that are regulated by the activity of inhibitors (e.g. I2) which in turn are controlled by glycogen synthetase kinase 3 (GSK3) activity in combination with Ca^{2+}-calmodulin (Fig. 2). The result is several net-worked feedback loops that can be influenced by other agonists. The central role of protein phosphatases in suppressing the motility of immature spermatozoa in the epididymis has been shown most recently in experiments using specific inhibitors such as okadaic acid and calyculin (Vijayaraghavan et al., 1996). Not only did these inhibitors induce motility in immature spermatozoa from the caput epididymidis of the bull, but the forward velocity of the activated

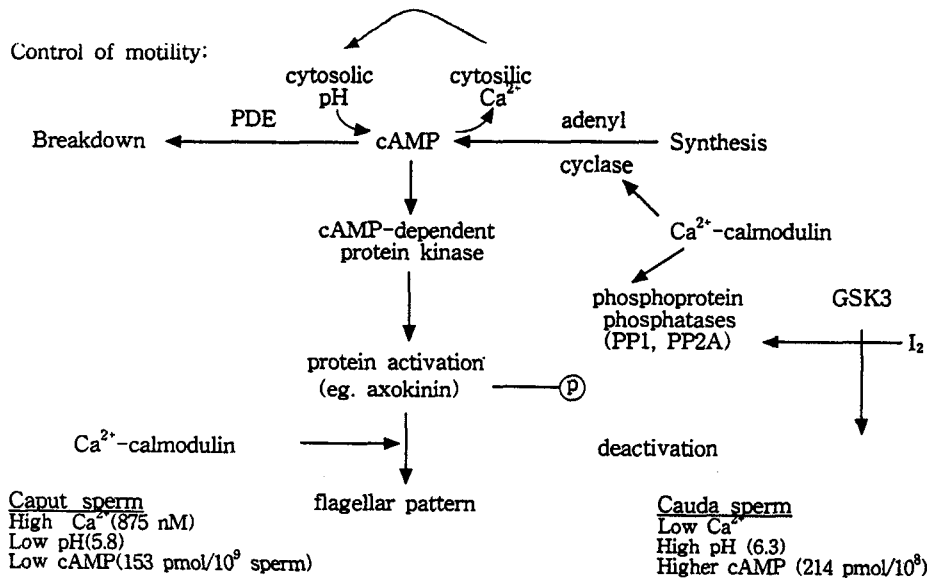

Fig. 2. Proposed sequence of signalling molecules and principle pathways that regulate sperm motility.

sperm was comparable to that of mature spermatozoa from the cauda epididymidis. Whether this 'unblocking' of motility-regulating mechanisms is something that is intrinsic to spermatozoa or whether it is due a subtle effect of epididymal secretions is not known, but intuitively the latter seems the more likely given the many changes in the composition of epididymal luminal fluid at different levels of the duct that could influence intracellular pH, Ca^{2+}.

V. ACTIVATION OF DEVELOPMENT BY SPERM FACTORS

It is now apparent that following fusion of the sperm plasma membrane with the oolemma, specific sperm factors (in addition to nucleoprotein) are transferred into the egg cytoplasm that are responsible for activating further development. Foremost among these soluble factors is one termed oscillin which triggers release of C^{2+} from intracellular stores leading to Ca^{2+} oscillations that have the same periodicity and amplitude as those induced by a fertilizing spermatozoon (Swann, 1996). The initial release of Ca^{2+} begins at the site of sperm entry (or injection of oscillin) and then spreads in a wave-like fashion across the remainder of the egg. Thereafter, Ca^{2+} oscillations begin in a non-polarised position within the cytoplasm and continue for several hours. It seems likely that IP_3 is involved at some point as microinjection of this ubiquitous signalling molecule into mouse eggs will elicit Ca^{2+} oscillations. Contrary to earlier claims that oscillin was a hexose phosphate isomerase (Parrington et al., 1996), recent observations indicate that it has characteristics of a phospholipase C (Swann, 1999). However, it is not clear how a small amount of phospholipase C carried by a single spermatozoon is sufficient to stimulate Ca^{2+} oscillations in a cell as large as a mammalian egg.

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