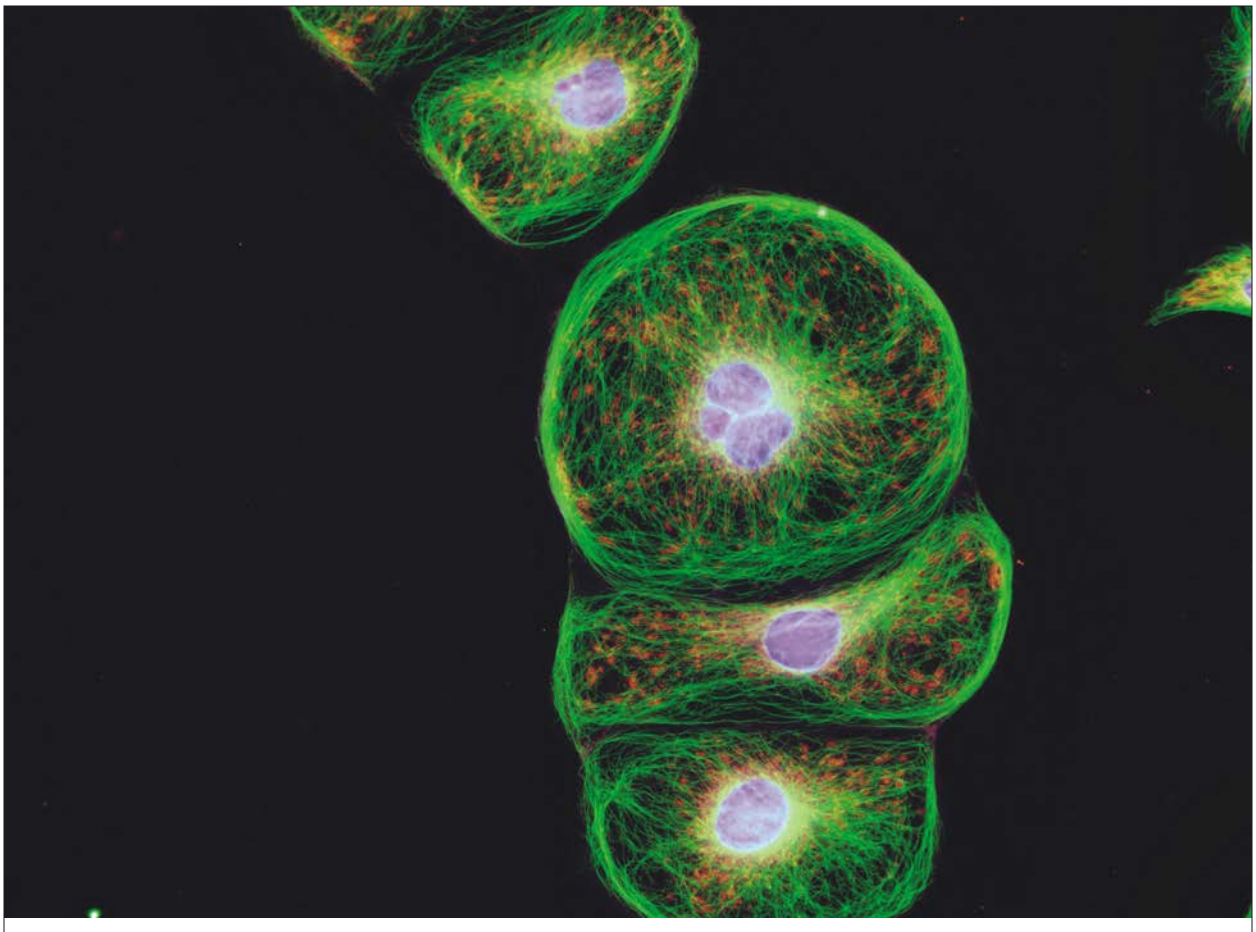

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Acrosome Reaction in Marine Animals: Gateway to Sperm Fusion with the Egg

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Abstract: Acrosome Reaction (AR) is of fundamental importance in the fertilization of egg by spermatozoon. In external fertilizers like starfish, sea urchin and ascidian egg and sperm are spawned simultaneously into the surrounding water. Upon swimming, sperm must obtain motility and then they must swim towards or respond to the egg in some way. Components from the jelly layer of the egg influence ion permeability changes in sperm that regulate chemotaxis and the AR prior to fertilization. The acrosomal process is surrounded by a new membrane that allows sperm to interact with the egg's vitteline layer and, subsequently, to fuse with the egg plasma membrane. This review summarizes the mechanisms and signal transduction pathways involved in AR.

Key words: Acrosome reaction, fertilization, spermatozoon, starfish, sea urchin, sperm activation, channel regulation, plasma membrane

Introduction

The Sperm Acrosome Reaction (AR) is an essential step for fertilization in many species (Wassarman, 1999). Fertilization, the bridge between generations, is defined as the fusion of two haploid cells (sperm and egg) forming a diploid zygote (the fertilized egg) with genetic potentials derived from both parents. Components from the external layers of the egg profoundly influence sperm physiology, priming it for fertilization. Sperm are tiny differentiated terminal cell that serve three major functions. In order to fertilize an egg, a spermatozoon must undergo three important steps. First, the spermatozoon must attain activation of motility as a result it can travel a distance to the egg. Secondly, it must be stimulated by or attracted to the egg. Finally, the spermatozoon needs to undergo changes that allow it to bind to and fuse with the egg plasma membrane. In external fertilizers i.e., sea urchins, starfish and teleosts fishes, sperm develop the potential for motility only after leaving the testis. For example, a sea urchin can spawns as many as 40 billion sperm into the sea (Darszon *et al*., 2001). Upon release, these cells start swimming, powered by chemical signals from the environment and the egg. From the millions of sperm released by a male, only a few will find the egg to initiate the crucial event of fertilization (Garbers, 1989; Trimmer and Vacquier, 1986).

The sperm is not a deterministic device oblivious to the external medium, turned only to the chemical signals from the egg outer layer. It must avoid fusing with any other cells but the egg. The concentration of ions, pollutants, pH, temperature and other physico-chemical variables influence sperm behavior and metabolism. Importantly, signals from the egg modulate sperm physiology, inducing sperm to undergo a series of ordered changes in configuration that enable it to complete fertilization.

The AR process was first discovered by J. C. Dan in 1950, when she was working at Misaki Marine Biological station, Japan (Dan, 1952). The acrosome is a single, large, Golgi-derived, secretory vesicle found in the anterior head of the sperm from many animal species. It filled with a host of enzymes such as acid glycohydrolases, proteases, esterases, acid phosphatases, aryl sulfatases, etc. AR occurs in all metazoans and has been recognized as a coupled reaction involving exocytotic step, which is accomplished by physiological, biochemical and morphological changes including dramatic changes in cell shape (Garbers, 1989; Trimmer and Vacquier, 1986). Furthermore, AR is known to be a signal transduction event linked to ion fluxes, membrane depolarization and changes in the intracellular pH (pHi) and Ca^{2+} concentration (Trimmer and Vacquier, 1986; Darszon *et al*., 1996). It generally occurs on etracellular matrix called egg coat, which covers the eggs of virtually all organisms, prior to actual fusion of plasma membranes of two gametes. Though egg coats vary from animals to animals, the glycans in egg coats play a central role in sperm-egg interactions for efficient fertilization, particularly in triggering AR.

In this review the main theme is the mechanism of AR and its role in fertilization are described. The detailed structure and mechanisms of AR have been described in previous research (Darszon *et al*., 1999; Darszon *et al*., 2001; Neil and Vacquier, 2004; Inaba, 2003).

Morphology and Characteristics of Sperm

Sperm are quite small cells and display similar general design in almost all species (Fig. 1). It consist of (1) a head (2-5 μm in diameter), containing condensed packages of chromosomes in the nucleus (which occupies a significant proportion of the head), two centrioles and in many species the acrosome, a membranous structure overlying the nucleus in the anterior part of the sperm head; (2) the flagellum or tail, which varies in length, depending on the species (10-100 μm) and contains the axoneme. The 9 + 2 structure and molecular composition of the axoneme are well conserved among eukaryotic cilia and flagella from protozoan to human. The doublet microtubules are sliding units containing radial spokes, dynein arms and dynein docking and regulatory complexes. The nine doublets are interconnected and the central pair bridge joins the inner microtubules. The total number of proteins present in the axoneme is about 250, but the exact composition varies according to species (Inaba, 2003). Dyneins are ATPases producing the motility force and phosphorylation is important in regulation of axonemal movement; and (3) mitochondria at the base of the tail, contributing to power its movement. They can be inside the sperm head as in sea urchins, or spirally arranged in the flagellar midpiece, as in mammals. The cytoplasmic volume of sperm is very small; the internal volume per sea urchin and human sperm has been estimated to be ~35 and 15 fl, respectively (Kleinhans *et al*., 1992; Schackmann *et al*., 1984). Spermatozoa are unable to synthesize proteins or nucleic acids. They are specialized cells committed to find, fuse and deliver their genetic information to the egg.

Sperm Activation

Sea urchin sperm can not swim in the male gonads. When they spawn into the seawater, begin to swim vigorously. When sperm contact to seawater an ionic changes occur, responsible for inducing the physiological changes required for the activation of motility. Within the gonad, high CO_2 tension in semen maintains intracellular pH (pHi) at ~7.2 with respect to seawater (Johnson *et al*., 1983). Dynein, the ATPase that drives the flagella, is inactive below pH 7.3, repressing motility and respiration (Christen *et al*., 1982; Lee *et al*., 1983). When sperm are spawned into seawater, the CO_2 concentration decreases, H^+ release and pHi increases to 7.5-7.6. Production of ADP activates mitochondrial respiration 50-fold and initiates motility (Johnson *et al*., 1983; Christen *et al*., 1982).

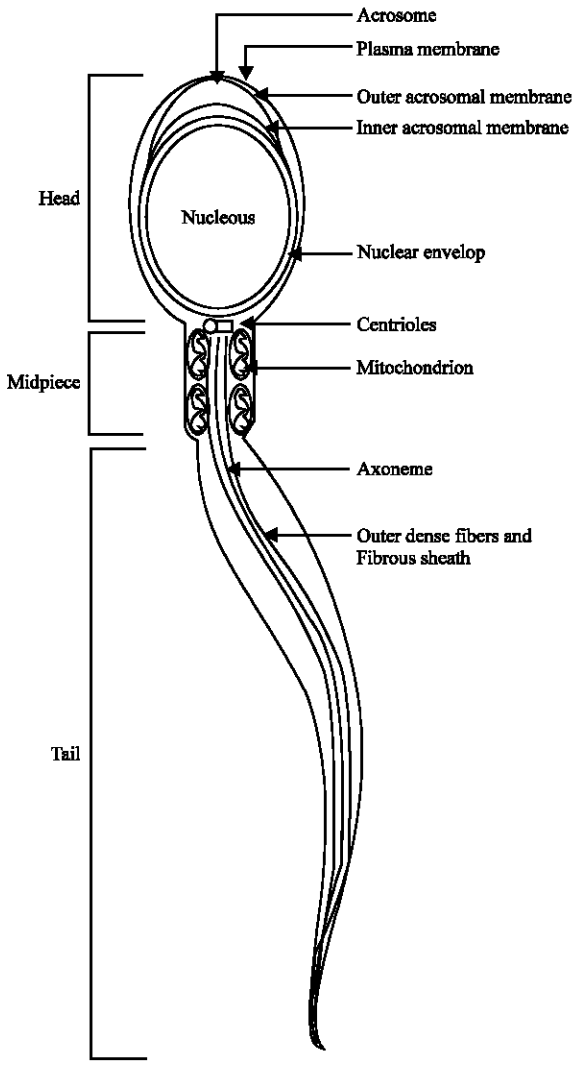

Fig. 1: Structure of the sperm head, midpiece and tail region

The activation of motility depends on the concentration of external Na^+ ($[Na^+]_0$), external K^+ ($[K^+]_0$) and pHi (Johnson *et al*., 1983; Christen *et al*., 1982; Lee *et al*., 1983; Bibring, *et al*., 1984; Christen *et al*., 1983). $[K^+]_0$ is higher in semen than it is in seawater (Christen *et al*., 1986) and the transition from high $[K^+]_0$ in the testis to lower $[K^+]_0$ in seawater may result in plasma membrane hyperpolarization. The hyperpolarization could stimulate the voltage-dependent Na^+/H^+ exchange and contribute to the pHi rise that accompanies sperm activation (Lee, 1984; Lee, 1984). Hyperpolarization could contribute to activation both by participating in the pHi rise and by activating adenylyl cyclase (AC). A cyclic AMP (cAMP) increase may activate a cAMP-dependent protein kinase (PKA), which phosphorylates axonemal proteins contributing to sperm motility (Garbers, 1989; Morisawa, 1994).

Receptors for Diffusible Egg Components

Sea urchin

Diffusible components from the outer layer of eggs influence sperm swimming trials in many marine invertebrates (Morisawa, 1994; Miller, 1985). The egg jelly-associated peptide in the sea urchins *Hemicentrotus pulcherrimus* and *Strongylocentrotus purpuratus* called speract (sperm activating peptides, SAPs), is a peptide consisting of ten amino acids. When they bind to sperm, SAPs causes a cellular activation, resulting in either a chemotactic or chemokinetic response and they often act in a species-specific manner (Suzuki *et al*., 1981; Hansbrough and Garbers, 1981). They may also play a role in induction of the AR. About 100 SAPs have been identified from sea urchin (Suzuki, 1995) and starfish (Nishigaki *et al*., 1996) egg jelly (EJ).

Speract, a decapeptide (Gly-Phe-Asp-Leu-Asn-Gly-Gly-Gly-Val-Gly) isolated from *S. purpuratus* and *H. pulcherrimus* EJ, induce sperm phospholipids metabolism, respiration and motility at Pico molar concentrations at an extracellular pH, (pH)0 of 6.6 (Hansbrough *et al*., 1980; Suzuki and Yoshino, 1992). In normal seawater (pH 8.0), speract induces a number of changes in sperm, including Na^+ and Ca^{2+} influx, K^+ and H^+ efflux and increases in the concentrations of cAMP and cGMP (Darszon *et al*., 1999; Darszon *et al*., 2001). Receptors for the peptide were identified on the sperm membrane in *H. pulcherrimus)* (Shimizu *et al*., 1994) and in *S. purpuratus* (Dangott and Garbers, 1984) with molecular masses of 71 and 77 kDa, respectively. The binding of speract to the receptor activates a GC on the plasma membrane (Suzuki *et al*., 1984). A 14-amino acid peptide, resact (SAPIIA), from another species of sea urchin, *Arbacia punctulata*, binds directly to GC on the sperm plasma membrane (Suzuki, *et al*., 1984; Shingh *et al*., 1988).

Starfish

In starfish, *Asterias amurensis* the outermost egg coat is a relatively thick gelatinous layer called the jelly coat. This jelly layer consists of three components, namely ARIS (acrosome reaction-inducing substance), Co-ARIS and asterosap (asteroidal sperm activating peptide), cooperatively trigger the AR of sperm (Hoshi *et al*., 1994). ARIS is a sulfated proteoglycan-like molecule of an extremely large molecular size (Ikadai and Hoshi, 1981; Koyota *et al*., 1997), Co-ARIS is a group of sulfated steroidal saponins (Nishiyama *et al*., 1987) and asterosap is a group of equally active isoforms of sperm-activating peptide (Nishigaki *et al*., 1996). Asterosap binds to 130 kDa membrane protein that is likely to be a GC (Nishigaki *et al*., 2000). On the other hand, receptor for ARIS on sperm remained to be identified.

Ascidian

In the ascidians *Ciona intestinalis* and *C. savignyi*, a novel sulfated steroid, SAAF (sperm-activating and attracting factor), induces sperm activation and chemotaxis, but the receptor on sperm remains to be identified (Yoshida *et al*., 2002).

Ion Channel Regulation by the Components of EJ

Sea Urchin

Signaling by speract involves ion channel and transporters (Darszon *et al*., 1999). Speract binding to its receptor(s) (Dangott and Garbers, 1984; Yoshino and Suzuki, 1992) activates GC (Bentley *et al*., 1988). In *A. punctulata*, the peptide (resact, Cys-Val-Thr-Gly-Ala-Pro-Gly-Cys-Val-Gly-Gly-Gly-Arg-Leu) binds directly to GC (Shingh *et al*., 1988). Activation of GC results in an increase of cGMP, which in turn opens a cGMP-dependent K^+ channel, leading to the

hyperpolarization of the plasma membrane (Babcock *et al*., 1992; Galindo *et al*., 2000). This hyperpolarization may enhance Na^+/Ca^{2+} exchange to maintain low intracellular Ca^{2+} ($[Ca^{2+}]_I$) (Bridge *et al*., 2000). Blocking of Na^+/Ca^{2+} and K^+-dependent Na^+/Ca^{2+} exchangers does not alter the kinetics of $[Na^+]_I$ fluxes, indicating that these types of channels are not directly involved in the speract response (Rodriguez and Darszon, 2003). However, activity of a flagellar K^+-dependent Na^+/Ca^{2+} exchanger is required for sperm motility, presumeably to maintain low $[Ca^{2+}]_I$ (Su and Vacquier, 2002). Some phosphatases and phosphodiesterases may be pHi-sensitive and rapidly inactivate GC, decreasing $[cGMP]_i$ (Garbers, 1989). High K^+ seawater blocks all sperm responses to speract except for large $[cGMP]_i$ increase (Harumi *et al*., 1992).

The speract-induced hyperpolarization also stimulates Na^+/H^+ exchange (Lee and Garbers, 1986), AC (Beltran *et al*., 1996) and possibly a cation channel named SPIH (Gauss *et al*., 1998). These changes lead to increases in pHi, $[cAMP]_i$ and Na^+ influx. SPIH has been cloned and belongs to the hyperpolarization-activated and cyclic nucleotide-gated K^+ channel (HCN) family (Gauss *et al*., 1998). SPIH is activated by hyperpolarizing potentials and potently up-regulated by cAMP. SPIH is found mainly in the flagellum and HCN channels are involved in periodicity, this channel could modulate flagellar beating and participate in sea urchin sperm chemotaxis (Kaupp and Seifert, 2001). Indeed, a rhythmic pattern of Ca^{2+} increases has been observed in sperm flagella in response to speract (Wood *et al*., 2003).

Resact, the only sea urchin SAP with demonstrated chemotactic capacity, requires external Ca^{2+} to alter sperm motility (Ward *et al*., 1985). It is worth nothing that *S. purpuratus* and *A. punctulata* are separated by ~200 million years in evolution (Smith, 1988); therefore, there could be differences in the way SAPs modulate sperm motility. High Ca^{2+} concentrations trigger asymmetric flagellar beating in demembranated sperm (Brokaw, 1979) and intact sperm (Cook *et al*., 1994). Though $[Ca^{2+}]_I$ increase during this process (Schackmann and Chock, 1986). However, consistent with the Ca^{2+}-dependent depolarization caused by speract (Reynaud *et al*., 1993), a cAMP-regulated Ca^{2+} channel may contribute to this uptake (Cook and Babcock, 1993).

Starfish

In starfish, asterosap transiently increases the $[cGMP]_i$, pHi and $[Ca^{2+}]_I$ via the activation of asterosap receptor, GC (Nishigaki *et al*., 2000; Matsumoto *et al*., 2003), while ARIS slightly elevated the basal concentration of $[Ca^{2+}]_I$ (Kawase *et al*., 2005). However, when sperm were simultaneously treated *in vitro* with ARIS and asterosap, a sustained elevation in $[Ca^{2+}]$I occurred. The loaded with the caged form of cGMP evoked a transient increase in $[Ca^{2+}]_I$ level in starfish (Matsumoto *et al*., 2003). But the amplitude of the $[Ca^{2+}]_I$ signal induced by caged cAMP was significantly less than cGMP. A K^+-dependent Na^+/Ca^{2+} exchanger from starfish testis has been cloned by this author (unpublished data). This author found that asterosap causes a transient Ca^{2+} elevation by the Na^+/Ca^{2+} exchanger and a significant inhibition of the $[Ca^{2+}]_I$ concentration occurred when added KB-R7943 mesylate (a potent, selective inhibitor for Na^+/Ca^{2+} exchanger) (Watano *et al*., 1999; Watano *et al*., 1996). Nifedepine, nitrendipine, verapamil are voltage-dependent Ca^{2+} antagonist and Ni^{2+}, a non-specific Ca^{2+} channel antagonist has no effect on asterosap-induced Ca^{2+} elevation. Figure 2 provides a general scheme of the signaling pathway in starfish sperm by the EJ.

Acrosome Reaction

All sperm species possessing an acrosome must undergo the AR to fertilize the egg. This exocytotic reaction enables sperm to penetrate the outer envelope of the egg and to recognize and fuse

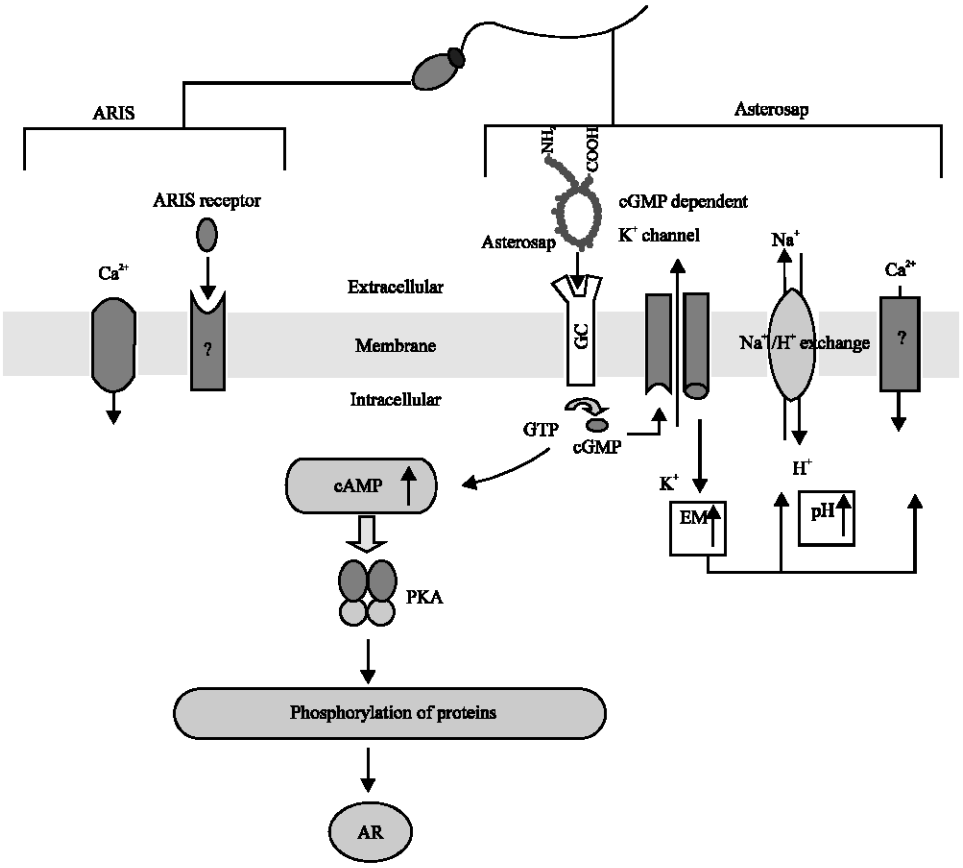

Fig. 2: Signaling cascade in *A. amurensis* sperm by the EJ. Binding of asterosap to its receptor activates a GC, enhancing K^+ efflux through a cGMP-dependent channel causing a decrease in sperm membrane potential (Em). This Em hyperpolarization activates Na^+/H^+ exchange leading to intracellular alkalinization. Em and increased pHi may enhance to open a Ca^{2+} channel. Asterosap-induced Ca^{2+} could be entered by Na^+/Ca^{2+} exchanger (denoted as ? mark, as it is an unpublished data of this author). On the other hand, binding of ARIS to the EJ promote a small increase of Ca^{2+}. The receptor for ARIS remains unknown (denoted as ?). Moreover, during EJ induced signaling a small amount of cAMP produce that regulate the PKA activity and other cellular responses. Upward arrows in the boxes are denoting an elevation

with the egg plasma membrane (Yanagimachi, 1994). Thus, the egg is not simply a protective coat but it plays a crucial role as the physiological signal molecule(s) for triggering the AR. The AR is defined as exocytosis of the acrosomal vesicle, in which the contents of the acrosomal vesicle including hydrolytic enzymes are released to the exterior surrounding. As a result of exocytosis, the acrosomal inner membrane is exposed as a new part of sperm plasma membrane, which has specific device for binding to and fusion with the plasma membrane of eggs. Therefore, the AR is an essential process for fertilization in various animals. In many marine invertebrates, the exocytosis of acrosomal vesicle is accompanied by the formation of an acrosomal process (Fig. 3), which projects from the anterior end

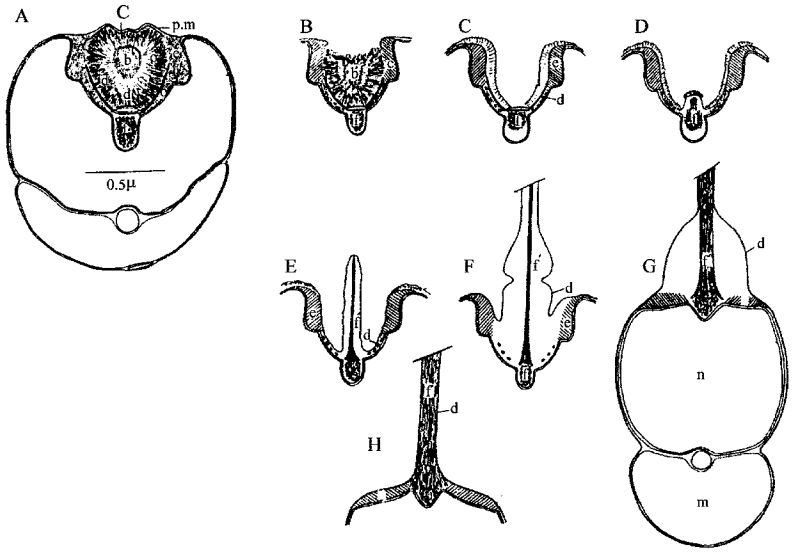

Fig. 3: Acrosome reaction of *A. amurensis* spermatozoon. (A) The intact acrosome. Stages (B-E) were found in spermatozoa fixed 1 second after treatment with jelly solution; (F) 4 seconds; (G) 8 seconds; (H) 60 seconds. (a-e) components of acrosomal vesicles, (f) the fiber-precursor, (f') fibrous shaft, (m) mitochondrion, (n) nucleus, (p. m) plasma membrane (Dan, 1967 with modifications) (Dan, 1967)

of the sperm. Although the actual details of fertilization vary enormously from species to species, the central version of sperm entry into the egg includes a vulgar pathway as depicted in Fig. 4A. This common version is clearly reflected in Fig. 4B that represents a comparison of AR of starfish and sea urchin.

Both extracellular Ca^{2+} and Na^{+} are required for the AR (Dan, 1954; Schackmann and Shapiro, 1981) which is characterized by two major physiological events: the exocytosis of the acrosomal vesicle and the extension of the acrosomal process. Acrosomal exocytosis releases the protein binding (Vacquier and Moy, 1977; Vacquier *et al*., 1995; Zigler and Lessios, 2003), which mediates the species-specific adhesion of sperm to egg (Glabe and Lennarz, 1979). The acrosomal process is formed by the pHi-dependent polymerization of actin (Tilney *et al*., 1978). The process extends ~1 μm from the tip of the sperm head and is covered by the bindin-coated membrane that will fuse with the egg plasma membrane (Barre *et al*., 2003). The interaction between the plasma membranes of sperm and egg is a receptor-mediated event, with the egg receptor for bindin recognizing and binding species especially to sperm bindin (Kamei and Glabe, 2003).

The AR-inducing component in EJ of *S. purpuratus* is a fucose sulfate polymer (FSP) (Alves *et al*., 1998), while in *Echinometra lucunter* (Alves *et al*., 1997) it is a galactose sulfate polymer (sulfated glycan, SG). In the starfish *A. amurensis*, a pentasaccharide repeat containing xylose, sulfated fucose and galactose is the AR inducer (Koyota *et al*., 1997). Thus, differences in the fine structure of sulfated polysaccharides in EJ contribute to species specificity of fertilization in marine animals.

Within seconds, binding of FSP to the sperm receptor for EJ (REJ, now suREJ1, a 210 kDa membrane glycoprotein) induces ion fluxes; Na^{+} and Ca^{2+} influx, while K^{+} and H^{+} efflux (Darszon *et al*., 1999; Darszon *et al*., 2001). These ion fluxes result in changes in membrane potential

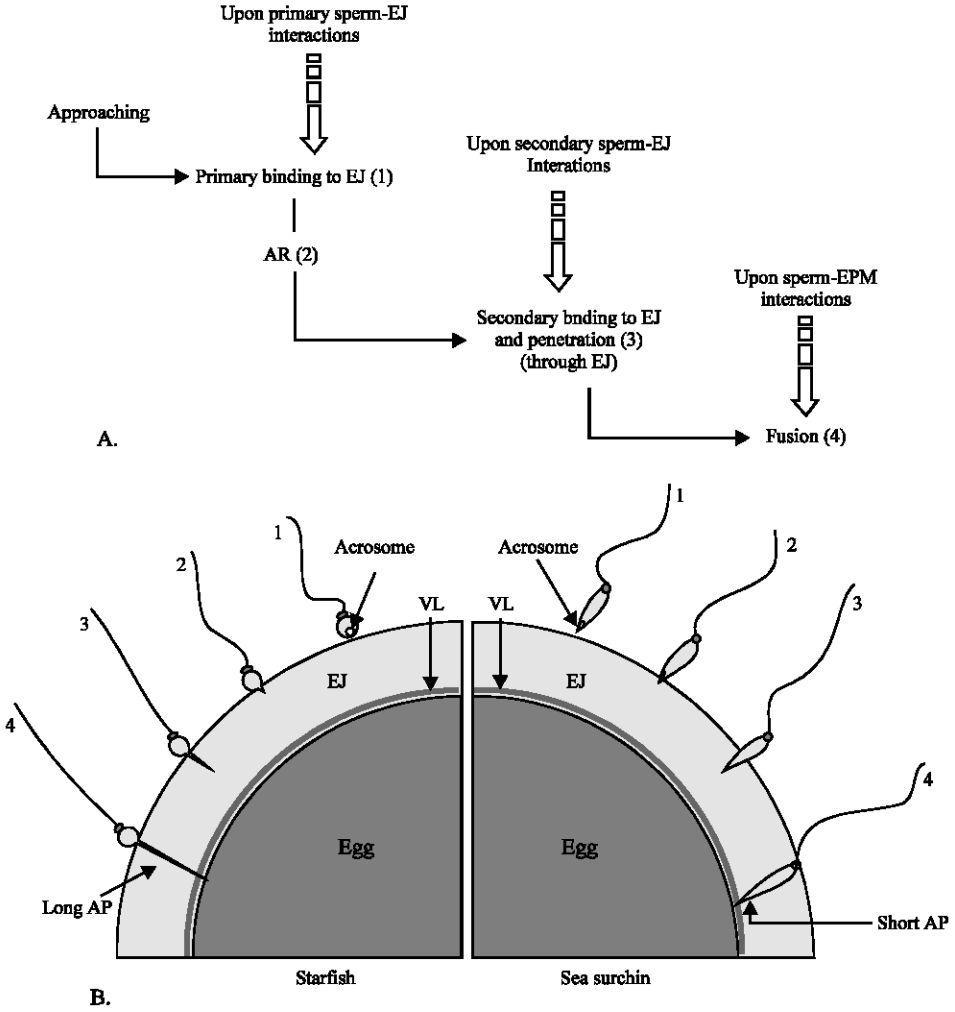

Fig. 4 : A. A general pathway of sperm entry into the egg during fertilization. Numbers indicate the major steps, which are schematically elaborated in B for starfish and sea urchin. B. A comparison of the process of fertilization between starfish and sea urchin. Starfish spermatozoa undergo the AR upon encountering the EJ. They extrude a long acrosomal process (AP) (up to 25 μm) and immediately stop swimming. On the other hand, sea urchin spermatozoa have a short acrosomal process (0.5 μm) and keep swimming until they reach the vitelline layer (VL)

(Schackmann *et al*., 1981; Gonzalez-Martinez and Darszon, 1987), an increase in $[Ca^{2+}]_i$ (Guerrero and Darszon, 1989b) and a Na^+-dependent increase in pHi of ~0.25 units (Lee *et al*., 1983; Guerrero and Darszon, 1989b). Binding of FSP also leads to a number of other physiological changes: a ten fold increase in inositol 1,4,5-triphosphate (IP_3) (Domino and Garbers, 1988), a Ca^{2+}-dependent activation AC (Watkins *et al*., 1978) that leads to an increase in cAMP (Garbers and Kopf, 1980) and increases

in the activities of protein kinase A (Garbers *et al*., 1980; Porter and Vacquier, 1986; Garcia-Soto *et al*., 1991), phospholipase D (Domino *et al*., 1989) and NO synthase (Kuo *et al*., 2000).

Upon binding of FSP to *Lytechinus pictus* sperm, it induces a transient hyperpolarization followed by a membrane depolarization (Watkins *et al*., 1978). These membrane potential changes are most likely occurring in *S. purpuratus* sperm as well; when $[K^+]_0$ is raised from 10 to 40 mM, the Ca^{2+} increase and AR are inhibited (Schackmann *et al*., 1978), as is the increase in pHi (Guerrero and Darszon, 1989b). The Na^+ dependence of the increase in pHi suggests a role for hyperpolarization-activated Na^+/H^+ exchange (Gonzalez-Martinez *et al*., 1992). However, this Na^+/H^+ exchange is probably not mediated by the same pathway as the speract-induced Na^+/H^+ exchange, because in the AR this exchange is Ca^{2+} dependent (Guerrero *et al*., 1998), while in the speract response it is not (Schackmann and Chock, 1986), Even if Na^+/H^+ exchange is involved in Na^+ influx, $[Na^+]_i$ saturates well after pHi saturates, implying that another channel is involved in the $[Na^+]_i$ increase (Rodriguez and Darszon, 2003).

Elevation of Ca^{2+} that occurs in response to FSP has two distinct phases; the influxes associated with these phases occur through separate channels (Guerrero and Darszon, 1989b). Binding of FSP triggers the opening of the first channel, which is Ca^{2+} selective, blocked by verapamil and dihydropyridines and inactivates after opening. The second channel opens 4s later, is sensitive to Ni^{2+}, insensitive to verapamil and dihydropyridines, is permeable to Mn^{2+} and does not inactivates, but produces a sustained Ca^{2+} influx. The first channel will open even if the pHi increase is blocked, but the second channel will not. If the opening of the first channel is blocked, the second channel will not open (Guerrero and Darszon, 1989b; Guerrero and Darszon, 1989). Thus, the opening of these two channels is physiologically linked even though they represent distinct modes of Ca^{2+} entry.

For a successful AR it is necessary to open both channels (Darszon *et al*., 1999; Hirohashi and Vacquier, 2002). The second channel alone can be opened by a lower molecular weight hydrolyzed form of FSP (hFSP), but the AR does not take place (Hirohashi and Vacquier, 2002). hFSP does cause an increase in pHi, further indicating that a rise in pHi is an important signal for the second channel to open.

Second Ca^{2+} channel is a store-operated Ca^{2+} channel (SOC) (Gonzalez-Martinez *et al*., 2001; Hirohashi and Vacquier, 2003a). The increase in IP_3 (Domino and Garbers, 1988) that occurs in response to FSP, coupled with the fact that IP3 receptors have been detected in sea urchin sperm (Zapata *et al*., 1997), suggest that this signaling system may function during the AR. IP_3-mediated release of Ca^{2+} from intracellular stores is a crucial step in store-operated Ca^{2+} entry (Putney *et al*., 2001). Although sperm lack an endoplasmic reticulum, it has been suggested that the acrosomal vesicle may be acting as the $[Ca^{2+}]_i$ store (Gonzalez-Martinez *et al*., 2001). In sea urchins, opening of the SOC alone is sufficient to trigger acrosomal exocytosis, but not for a complete AR (Hirohashi and Vacquier, 2003a).

SG, another jelly component can serve to potentiate the FSP induction. It causes an increase in pHi, but SG alone can not induce the AR. The FSP-induced rise in pHi can be blocked by either nifedepine or high $[K^+]_0$, but neither of these block the SG-induced pHi rise (Hirohashi and Vacquier, 2003b). Therefore, the pathways by which FSP and SG induce pHi increases are different; the receptor for SG remains unknown. The combined pHi increase induced by both molecules produces maximal AR. In addition to SG, speract may also play a role in the AR. In low pH seawater (pH< 7.6), speract potentiates the FSP-induced AR by contributing to the rise in pHi.

Moreover, two other channels have been detected that contribute to the AR. Teraethylammonium (TEA^+), which is a blocker of K^+ channels, inhibits the egg jelly-induced AR (Schackmann, 1989)

and TEA^+-sensitive K^+ channel activities have been measured from sperm membranes (Lievano *et al*., 1985). An anion channel blocker 4,4'-diisothiocyanostilbene disulphonic acid (DIDS) blocks the AR and a DIDS-sensitive Cl channel may be important either to maintain the membrane potential prior to AR induction or to contribute directly to ion fluxes during the AR (Morales *et al*., 1993).

Upon encountering the jelly coat, starfish sperm undergo for the AR. They stop swimming immediately after extruding a long acrosomal process (10-25 μm). Starfish sperm do not have to swim through the EJ; their long acrosomal tubule reaches the egg plasma membrane. Three EJ components are involved in AR (Hoshi *et al*., 1994). ARIS induces the AR in cooperation with Co-ARIS or asterosap in normal seawater, whereas ARIS alone induces it in high-Ca^{2+} or high pH seawater (Matsui *et al*., 1986). Thus, ARIS is regarded as the major acrosome reaction-inducing molecule. An anti- asterosap antibody drastically reduces the capacity of the egg jelly to induce the AR (Nishigaki *et al*., 1996). Furthermore, sperm did not undergo the AR in response to the EJ if the asterosap-induced changes are blocked by the pretreatment of sperm only with asterosap (Kawase *et al*., 2004). Thus, it is clear that, besides ARIS, asterosap is essential for the EJ-induced AR.

The sustained $[Ca^{2+}]_i$ elevation occurs via the SOC-like channel when sperm is simultaneously treated with ARIS and asterosap. The sustained $[Ca^{2+}]_i$ elevation depends on the asterosap-induced increase in pHi and is prerequisite for the AR (Kawase *et al*., 2005). Starfish sperm had a significant PKA activity and upon binding to the EJ, produce a small amount of cAMP. This author has cloned a regulatory subunit of PKA from starfish testis and found a kinase activity in the sperm (unpublished data). It was also found that PKA-mediated phosphorylation and elevation of Ca^{2+} induced by the EJ have a share in AR. PKA-mediated phosphorylation and elevation of Ca^{2+} significantly inhibited by the H89 (Spungin and Breitbart, 1996) and KT5720 (de Jonge *et al*., 1993), selective inhibitor for PKA. Within few seconds at least four proteins (75, 150, 200, 220 kDa) are phosphorylated by PKA when sperm contact to the EJ (unpublished data of this author).

Conclusions

AR is important prerequisites of the fertilization process. Upon encountering to the jelly layer a variety of ion channels of the spermatozoa is a characteristic event associated with AR. In this review, authors have attempted to highlight current advances to explain mechanisms underlying sperm-egg interaction and induction of the AR. I hope that some of these advances will allow new strategies to regulate these events and alter sperm function.

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