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Special Review

Egg-jelly signal molecules for triggering the acrosome reaction in starfish spermatozoa

MOTONORI HOSHI*, TAKUYA NISHIGAKI, AKIRA USHIYAMA, TATSUYUKI OKINAGA, KAZUYOSHI CHIBA and MIDORI MATSUMOTO

Department of Life Science, Tokyo Institute of Technology, Yokohama, Japan

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***Address for reprints:** Department of Life Science, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227, Japan. FAX: 81-45.924.5777.

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Discovery of the acrosome reaction

In 1947 J.C. Dan revisited her home country, the USA, for the first time after difficult years in Japan during World War II (Fig. 1). She took the first commercial Bausch and Lomb phase contrast microscope back to Japan the following year (Fig. 2) and set up a research program on fertilization of marine invertebrates at Misaki Marine Biological Station, which had been re-opened after being used as a tiny portion of navy facilities during the war (Colwin and Colwin, 1979). Appreciating the great technological advantages of the phase-contrast microscopy, she first explored an old problem of sperm entrance into eggs (Dan, 1950). Then she concentrated on the changes in the sperm tip upon fertilization, for which she also applied electron microscopy, another great technological innovation. In fact, she was a pioneer in examining such material with the electron microscope. She discovered the biological significance of the changes, for which she introduced the term *acrosome reaction*, detected through careful observation of living spermatozoa under the phase microscope at Misaki, and intensive examination of spermatozoa immediately after fixation in sea water suspension with an electron microscope at Tokyo Institute of Technology (Dan, 1952, 1954; for a review see Dan, 1967). The electron microscope that she used was a very primitive model, although it was one of the latest in those days (the fourth machine of Hitachi Electron Micrograph), and was operated by a specialist. It was a curious yet fruitful collaboration between a biologist who had never touched an electron microscope and an electron microscopist who had never touched a biological specimen (Akabori, personal communication).

Her discovery of the acrosome reaction, for which she was given the award of the Zoological Society of Japan in 1958, fundamentally changed the approach to studying fertilization (Tilney, 1985). It reminded scientists of the simple, yet important and long overlooked fact that the spermatozoon itself should be studied as a cell to understand the process of fertilization. Although it was well known that spermatozoa are cells, they had been much neglected as such and treated rather as if they were simply a complex of chemicals that activated eggs. Now it is firmly established in most animals that the acrosome reaction is the mechanism for spermatozoa to ensure a spatio-temporally matched exposure of devices essential for penetration through the egg coat and for subsequent fusion with the egg plasma membrane.

Although it is well established in sea urchins and other marine invertebrates that dissolved jelly substances trigger the acrosome reaction in homologous spermatozoa, there has been much argument about the site where fertilizing spermatozoa undergo the acrosome reaction. In the starfish, however, drawings in a classical paper by Fol in 1877 show, even though the author did not know the acrosome reaction, that fertilizing spermatozoa undergo the acrosome reaction upon encountering the jelly coat, for which we now have conclusive evidence (Dale *et al.*, 1981; Ikadai and Hoshi, 1981a). We therefore attempted to isolate and identify a signal molecule(s) for triggering the acrosome reaction from the jelly coat in two species of starfish, *Asterias amurensis* of the order Forcipulata and *Asterina pectinifera* of the Spinulosa. Although *Asterias is* much better understood than *Asterina,* basically similar features are obtained in both species.

This paper summarizes our knowledge at the moment on the signal molecules in the jelly coat of starfish eggs for triggering the acrosome reaction.

Starfish egg jelly and its components

ARIS

Spawned eggs of starfish are encased in two layers of acellular coats, the vitelline coat and the jelly coat, which is a transparent and gelatinous outer layer. Although chemical analysis of the jelly coat of sea urchin eggs dates back to the 1940s (for a review see Monroy, 1965), such information was not available in starfish until Muramatsu (1965) obtained "a sulfated mucopolysaccharide" from the jelly coat of *Asterias amurensis* eggs. It is now known that starfish egg jelly consists of three groups of organic molecules: glycoproteins, sulfated steroid saponins and oligopeptides (Hoshi *et al.*, 1986, 1988, 1990a,b, 1991).

The jelly coat of *A. amurensis* eggs contains two glycoproteins. One is a high mannose glycoprotein of molecular mass about 80 kDa with unique saccharide structures (Endo *et al.*, 1987). However, this component does not appear to be directly involved in the acrosome reaction. The other is a highly sulfated, fucose-rich glycoprotein of an extremely high molecular mass (>10^7 kDa), presumably the same as or closely related to Muramatsu's mucopolysaccharide. Since this unique glycoprotein plays a key role in triggering the acrosome reaction, it is named acrosome reaction-inducing substance (ARIS) (Uno and Hoshi, 1978; Ikadai and Hoshi, 1981a,b). The activities of ARIS are species-specific, resistant to S-carboxymethylation and to pronase digestion which destroys about half of the protein moiety, and are susceptible to periodate oxidation and to solvolytic desulfation. These results suggest the importance of sulfated saccharide chains for the activities (Ikadai and Hoshi, 1981b; Matsui *et al.*, 1986a,b). This allows us to substitute a pronase digest of ARIS (P-ARIS) for intact ARIS, which makes experiments easier to run. Sugar compositions of ARIS/P-ARIS are significantly different in the two species of starfish. P-ARIS of *A. amurensis* contains fucose, galactose, xylose, N-acetylgalactosamine and N-acetylglucosamine in a molar

Fig. 1. A portrait of J.C. Dan.

ratio of 8.8:3.1:2.7:0.64:1.0. A unique saccharide structure, Xyl1→3Gal1→(SO_3^-)3,4Fuc→, is identified as a part of ARIS saccharide chains in *A. amurensis* (Okinaga *et al.*, 1992).

In sea urchins, the jelly component primarily responsible for eliciting the acrosome reaction is a subject to be debated (briefly reviewed in Keller and Vacquier, 1994). Sea urchin jelly contains two groups of macromolecular components, a fucose sulfate polymer (FSP; or fucose sulfate glycoconjugate, FSG) and a sialoglycoprotein (reviewed by Suzuki, 1990b); fucose and other sugars were found in the jelly in the 1940s (for a review see Monroy, 1965) while sialic acid was first detected by S. Isaka from the jelly of Japanese urchins in 1969 (reviewed by Hotta, 1977). Although the activity of ARIS in sea urchin jelly was first attributed to an FSP (SeGall and Lennarz, 1979), the evidence for the importance of protein portion is accumulating. For example, the activity of jelly, FSG and other jelly-fractions is significantly susceptible to proteases (Ishihara and Dan, 1970; Yamaguchi *et al.*, 1989; Mikami-Takei *et al.*, 1991), the activity of FSG fractions depends on the content of protein (Garbers *et al.*, 1983) or of bound glycoprotein (Shimizu *et al.*, 1990), and FSP becomes less active the more it is purified (DeAngels and Glabe, 1985). It has been reported by Keller and Vacquier (1994) that purified FSP has no significant ARIS activity. Instead, the activity is associated only with two glycoproteins with approximate relative subunit masses of 82 and 138 kDa. It is also suggested that the egg jelly is a network composed of an FSP superstructure to which glycoproteins are bound (Bonnell *et al.*, 1994).

Co-ARIS

The second jelly component involved in the induction of acrosome reaction in starfish is a group of sulfated steroid saponins. Although saponins are rare in animals, two echinoderms are exceptionally rich in saponins: asteroids with steroid saponins called asterosaponins and holothurians with triterpenoid saponins called holothurins (for a review, see Burnell and ApSimon, 1983). The jelly coat of starfish eggs also contains asterosaponins, some of which are known to agglutinate spermatozoa in *A. amurensis*. (Uno and Hoshi, 1978). Some others are found to serve as a co-factor for ARIS to induce the acrosome reaction in normal sea water, and are therefore named Co-ARIS. Three asterosaponins shown in Fig. 3 are identified as principal Co-ARIS molecules in *A. amurensis* (Fujimoto *et al.*, 1987; Nishiyama *et al.*, 1987b). The action of Co-ARIS is not much species-specific and a Co-ARIS fraction of *Asterina pectinifera* is effective as the co-factor for *Asterias amurensis* ARIS, and *viceversa* (Hoshi *et al.*, 1990a; Amano *et al.*, 1992c). For the activity of Co-ARIS, the sulfate group and the side chain of the steroid are important, while the saccharide chain of Co-ARIS appears unnecessary to be strictly specific (Nishiyama *et al.*, 1987a).

The acrosome reaction is well studied also in the sea cucumber, in which the spermatozoa form a very long acrosomal process within seconds (reviewed by Tilney, 1985). Since they are rich in triterpenoid saponins, namely holothurins, it will be interesting to test whether any holothurins serve as Co-ARIS in the sea cucumber.

SAP

The third component in starfish jelly is a group of peptides that diffuse out of dialysis membrane. It is well established in sea urchins that the jelly coat contains sperm activating peptides (SAPs) that restore the depressed rate of sperm motility and respiration at low pHs to the level in normal sea water. The work initiated by S. Isaka and developed mostly by his students is an important contribution from Japan (Ohtake, 1976; Suzuki *et al.*, 1981; reviewed by Suzuki, 1989, 1990a,b), though we owe much to American colleagues on the molecular mechanisms of the action of SAPs (for a review see Garbers, 1989). The diffusate of starfish jelly also has a SAP-like activity susceptible to pronase digestion. Long before we had purified starfish SAPs, we obtained substantial evidence, to be described in the following section, that they also

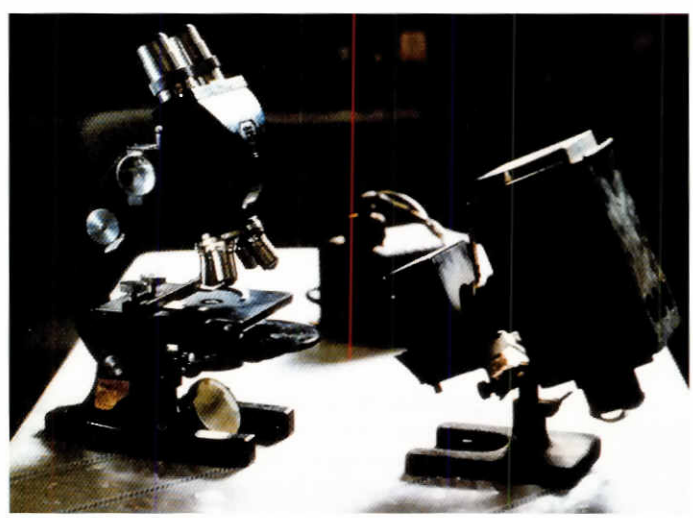

Fig. 2. The phase contrast microscope that J.C. Dan took from the USA to Misaki with the aid of a grant from the American Philosophical Society. *She expressed her thanks in a paper as follows: "The writer wishes to express sincerest thanks to the Society, in particular to Prof. E.G. Conklin, and to the Bausch and Lomb Optical Company, for their exceedingly kind cooperation in making the instrument available ahead of their production schedule, at the expense of considerable inconvenience and extra effort on the part of their staff". (Dan, 1950).*

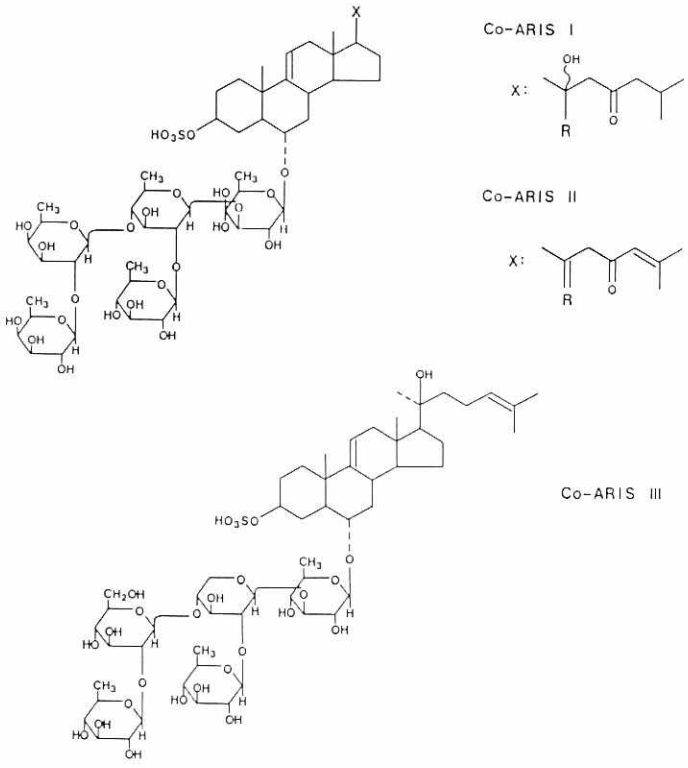

Fig. 3. Structures of Co-ARIS I, II and III. *Co-ARIS I and II are expressed in the hydrated form at 6-deoxy-xylo-hexos-4-ulose. (From Fujimoto et al., 1987).*

play an important role for the induction of acrosome reaction (Matsui *et al.*, 1986a,c; Hoshi *et al.*, 1986, 1988).

Very recently, we have finally purified twelve SAPs from the jelly coat of *A. amurensis* eggs. All of them are identified as glutamine-rich tetratriacontapeptides with a 25-residue ring formed by a disulfide linkage (Nishigaki *et al.*, submitted). They are much larger than sea urchin SAPs and do not show any significant similarities to the known sequences so far as we searched. Purified SAPs indeed play an important role in the induction of acrosome reaction as expected, and the ring structure appears essential for the activity. Somehow similar peptides have been partially purified as the sperm attractant in another forcipulate starfish (Punnett *et al.*, 1992), although in tests so far, the purified SAPs have not shown such activity.

Since there is no reason to believe that spermatozoa are exposed to a low pH upon contact with or during passage through the jelly coat in the sea, we have suggested that the main physiological function of sea urchin SAPs is also to trigger the acrosome reaction in co-operation with ARIS (Hoshi *et al.*, 1986, 1988). Actually, there is experimental evidence for the idea (Yamaguchi *et al.*, 1989).

Induction of acrosome reaction

The acrosome reaction is basically an event of exocytosis by which spermatozoa expose the devices essential for penetration through the egg coats and for fusion with the egg plasma membrane, and it is therefore essential for fertilization. In general, the acrosome reaction is species-specific and triggered by signals from the eggs or their appendices (for a review see Tilney, 1985). In sea urchins and some marine invertebrates, the acrosome reaction is induced simply by increasing the concentration of Ca^{2+} or pH of sea water (Dan, 1952; Collins and Epel, 1977; Decker *et al.*, 1976). These conditions are not sufficient to induce the acrosome reaction in starfish spermatozoa, but if both Ca^{2+} concentration and pH are increased, they spontaneously undergo the acrosome reaction (Ikadai and Hoshi, 1981a; Matsui *et al.*, 1986a). Similarly, they undergo the acrosome reaction under the conditions that increase both intracellular Ca^{2+} (Ca^{2+}_i) and intracellular pH (pH_i) (Matsui *et al.*, 1986a,b; Amano *et al.*, 1993).

Starfish egg jelly induces the acrosome reaction species-specifically, dose-dependently and quickly. The jelly-induced acrosome reaction depends very much upon external cations, especially Ca^{2+} and H^+, susceptible to Ca^{2+}-channel antagonists, and facilitated by an increase in either Ca^{2+}_i or pH_i. It is accompanied by an abrupt uptake of external Ca^{2+}, a Na^+-dependent transient increase in pH_i, and a transient but remarkable increase in cAMP (Matsui *et al.*, 1986 a,c). These features are quite similar to those found with sea urchins in which it is generally accepted that the egg jelly triggers the acrosome reaction by stimulating Ca^{2+} channels and Na^+/H^+ exchangers in the sperm plasma membrane (Schackmann and Shapiro, 1981; reviewed by Darszon *et al.*, 1989; Garbers, 1989; Schackmann, 1989).

In *Asterina pectinifera* at least, the egg jelly also elicits the degradation of sperm histones, which may be a key reaction leading eventually to the decondensation of sperm nucleus (Amano *et al.*, 1992a). We have found that the acrosome reaction and histone degradation are independent events triggered by the same jelly components (Amano *et al.*, 1992a,b,c, 1993).

The ability of a jelly solution to induce the acrosome reaction decreases with dilution and reaches null at about 1 μg sugar/ml. A jelly solution of 0.8 μg sugar/ml remains inactive even after enriched with ARIS (or P-ARIS) up to 50 μg sugar/ml. When it is fortified with 20 μg/ml Fraction M_8, a diffusible fraction containing both Co-ARIS and SAP, the ability is remarkably improved. It is therefore concluded that diffusible components limit the capacity of a jelly preparation to trigger the acrosome reaction (Matsui *et al.*, 1986a).

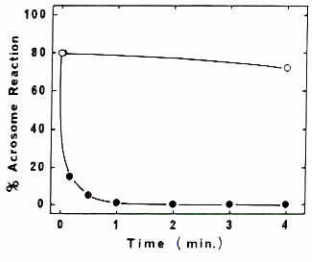

Fig. 4. Effects of the jelly in Ca^{2+}-deficient sea water on the acrosome reaction. *Spermatozoa were treated with jelly of 50 μg sugar/ml in Ca^{2+}-deficient sea water for a given period and then Ca^{2+} was added to give a normal concentration of 10 mM (—●—), or the spermatozoa were treated first with Ca^{2+}-deficient sea water, and then sufficient jelly and Ca^{2+} were added to give a concentration of 50 μg sugar/ml and 10 mM, respectively (—○—). Points are means for two experiments. (From Matsui et al., 1986b).*

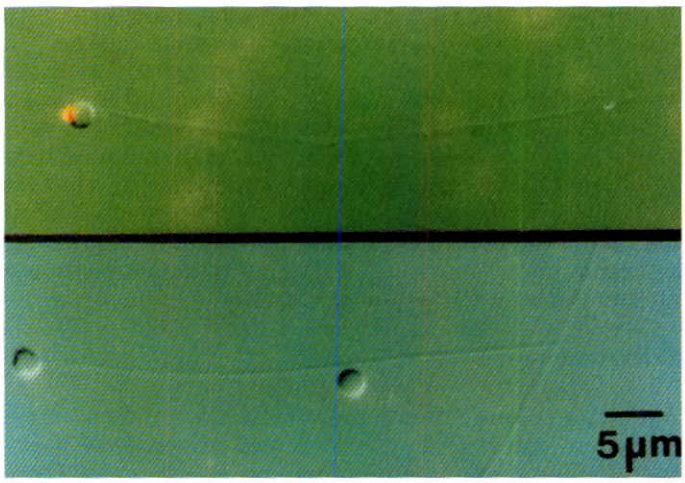

Fig. 5. Localization of ARIS receptors in *A. amurensis* spermatozoa. *These images were taken by double exposure with a Nomarski microscope and a fluorescence microscope at a magnification of x1000. Spermatozoa were incubated with P-ARIS conjugated with FluoSpheres (fluorescent polystyrene beads with a mean diameter of 30 nm) in the absence (top) or presence (bottom) of unlabeled ARIS in excess.*

In normal sea water, combinations of ARIS (or P-ARIS) and Fraction M_8 trigger the acrosome reaction in spermatozoa to a similar extent in a similar fashion at a similar rate as the jelly does (Hoshi *et al.*, 1988). Similarly, combinations of ARIS (or P-ARIS) with adequate amounts of Co-ARIS trigger the acrosome reaction with all accompanying changes described above, except for the pH_i increase. However, individually they are not able to trigger any of these changes in spermatozoa. It should be stressed here that changes triggered by ARIS and Co-ARIS proceed significantly slower than those by the jelly (or by ARIS and Fraction M_8). Most of spermatozoa treated with the jelly complete the acrosome reaction within 30 seconds, whereas it takes 2 minutes if treated with ARIS and Co-ARIS (Hoshi *et al.*, 1988). A mixture of ARIS and SAP triggered the acrosome reaction even in the absence of Co-ARIS to an appreciable extent but much less than the jelly did (Nishigaki *et al.*, unpublished data).

ARIS, but no other jelly components, does induce the acrosome reaction in Ca^{2+}-enriched or pH-elevated sea water (Ikadai *et al.*, 1981a,b; Matsui *et al.*, 1986a). The acrosome reaction induced by P-ARIS in high Ca^{2+} sea water is also not accompanied by a transient increase in the pH_i so far as monitored by 9-aminoacridine as a probe. Conversely, it is possible to increase the pH_i without triggering the acrosome reaction by SAPs in Fraction M_8, but not by Co-ARIS saponins, in normal sea water, or even by the jelly if external Ca^{2+} is maintained very low (Hoshi *et al.*, 1986; Matsui *et al.*, 1986c). It is therefore concluded that the pH_i increase, at least the one detectable by 9-aminoacridine, is not sufficient nor obligatory for the induction of the acrosome reaction in starfish spermatozoa. Nevertheless, we think that SAPs play an important role in the induction of the acrosome reaction under natural conditions on the following account (Hoshi *et al.*, 1988, 1990a,b, 1991).

The amounts of ARIS and Co-ARIS required for inducing the acrosome reaction are much lower if Fraction M_8 containing both Co-ARIS saponins and SAPs is used instead of mixtures of, or each of, purified Co-ARIS saponins. Similarly, when Fraction M_8 is digested with pronase to which Co-ARIS is resistant, the apparent activity of Co-ARIS in the fraction is much reduced with a concomitant loss of sperm activating capacity (Matsui *et al.*, 1986b,c; Nishiyama *et al.*, 1987a). In fact, in the presence of a sufficient amount of P-ARIS, 5 μg Fraction M_8/ml, 90 μg Co-ARIS I/ml, or 24 μg Co-ARIS II/ml is required for 50% induction of the acrosome reaction (Hoshi *et al.*, 1990a). As described, combinations of P-ARIS with Fraction M_8, but not with CoARIS, perfectly mimic the jelly in the manner and way of inducing the acrosome reaction, further suggesting the importance of all three components.

Incomplete stimulation of spermatozoa

When starfish spermatozoa are treated in normal sea water with insufficient amounts of jelly to elicit the acrosome reaction, or conversely with sufficient jelly but in Ca^{2+} -deficient sea water, they lose the ability within a minute to undergo the acrosome reaction and accompanying ionic changes in response to newly added jelly or Ca^{2+} or both in excess (Fig 4). Furthermore, they seem to lose their capacity to react to the jelly coat *in situ*. Similarly, if they are treated with P-ARIS (or ARIS) and Fraction M_8 not simultaneously but sequentially in either order with an interval over minutes, they do not undergo the acrosome reaction and become incompetent to the jelly. Such effects of "pre-treatments" are found with ARIS, P-ARIS and Fraction M_8, but not with Co-ARIS or with pronase-digested Fraction M_8, suggesting that ARIS and an oligopeptide(s), presumably SAPs, have the ability to make spermatozoa incompetent to the jelly (Matsui *et al.*, 1986b). This effect of Fraction M_8 is not simply attributable to its ability to raise the pH_i, because spermatozoa are still responsive to the jelly even after the pH_i is significantly increased in alkaline sea water.

However, those spermatozoa undergo the acrosome reaction just like intact ones if $Ca^{2+}{}_i$ and pH_i are increased by the treatments with a calcium ionophore (A23187) and monensin, or with a Ca^{2+} channel activator (maitotoxin) and either Fraction M_8 or alkaline sea water (Matsui *et al.*, 1986b; Nishiyama *et al.*, 1986; Amano *et al*, 1992b). They appear therefore to keep intact the machinery that mediates ionic changes in the cytoplasm and exocytosis of the acrosome. The acrosome reaction induced by maitotoxin in combination with Fraction M_8 or alkaline sea water is susceptible to verapamil as jelly-induced acrosome reaction. These data suggest that the pretreatment effects are due to an irreversible change(s) in the steps not later than the stimulation of Ca^{2+} channels and Na^+/H^+ exchangers; presumably the signal transduction from the receptors to the channels and exchangers. It is also suggested that verapamil and maitotoxin act on the same channel.

The activity of ARIS (or P-ARIS) to render spermatozoa incompetent to the jelly by pre-treatment is parallel in many features to its activity to induce the acrosome reaction under favorable conditions (Matsui *et al.*, 1986b). Therefore it seems likely that the two apparently different effects of ARIS upon spermatozoa actually result from a single action of ARIS presumably to the sperm-membrane receptors. This may be true also for the effects of SAPs.

Receptors for the signal molecules

The extremely large molecular size and species-specific action of ARIS suggest the presence of a specific ARIS receptor in the sperm plasma membrane. In fact, *A. amurensis* spermatozoa have

the ability to bind ARIS, as well as P-ARIS, specifically (Ushiyama *et al.*, 1993). The binding is species-specific and almost reaches a plateau within a minute, which is comparable to the time course of jelly-induced acrosme reaction. The acrosome-intact (*un*reacted) spermatozoa have one class of ARIS receptor with a dissociation constant of 2 nM or less, and the total number of binding sites of about 200 per cell. Since the binding is significantly inhibited by a mixture of saccharide chain fragments liberated from ARIS, the receptor seems to recognize the sugar moiety at least partly. The site of ARIS binding was specifically localized to the anterior region of sperm heads (Fig. 5). By using colloidal gold conjugated ARIS and electron microscopy, it was further confined to a single, small domain of the plasma membrane just peripheral to the region occupied by the acrosomal vesicle and periacrosomal components (Longo *et al.*, submitted). In summary, starfish spermatozoa have a small number of a high-affinity receptor for ARIS in a restricted area of the plasma membrane.

In sea urchins, a WGA-binding glycoprotein of 210/220 kDa has been claimed to be the receptor for FSG or the acrosome reaction-inducing ligand (Trimmer *et al.*, 1987; Sendai and Aketa, 1989). However, it has yet to be proved directly that the glycoprotein specifically binds FSG or the ligand.

For the activity of Co-ARIS, the sulfate group and the side chain of the steroid are important, while the saccharide chain of Co-ARIS appears unnecessary to be strictly specific (Nishiyama *et al.*, 1987a), suggesting that spermatozoa do not have a specific receptor for Co-ARIS. Instead, it implies an interesting hypothesis to be tested, namely that the steroid ring and side chain of Co-ARIS infiltrate or are inserted into sperm plasma membrane and the sulfate group and sugar chain contribute to keep Co-ARIS in the right position and orientation to interact correctly with a component(s) in sperm plasma membrane. It is also consistent with this assumption that ARIS and SAP, but not Co-ARIS, show the pre-treatment effects.

The receptors for SAPs are identified in several sea urchins, a glycoprotein of 77 kDa for SAP-I (Dangott and Garbers, 1984), and a guanylate cyclase of 160 kDa for SAP-IIA (Shimomura *et al.*, 1986). In starfish, we have only indirect evidence for a SAP receptor (Matsui *et al.*, 1986b), and the isolation and identification of SAP receptors in *A. amurensis* spermatozoa are now in progress in our laboratory.

Conclusion and a brief prospect

ARIS binds to its receptor in a restricted domain of sperm surface, which in turn stimulates verapamil- and maitotoxin-sensitive Ca^{2+} channels and therefore increases $Ca^{2+}{}_i$. Co-ARIS may infiltrate or be inserted into sperm plasma membrane, and as a result it may modulate the binding of ARIS and/or the succeeding activation of Ca^{2+} channels. SAP stimulates Na^+/H^+ exchange systems and thus increases the pH_i. It may also contribute to some extent in increasing the $Ca^{2+}{}_i$ as suggested by Schackmann and Chock (1986). When starfish spermatozoa reach the jelly coat of a homologous egg, they rapidly increase the $Ca^{2+}{}_i$ and pH_i in response to the action of ARIS, Co-ARIS and SAP in concert, and therefore undergo the acrosome reaction within a minute or less.

Forty years have passed since J.C. Dan discovered the acrosome reaction, yet we are still a long way from understanding its molecular mechanisms. Now we have in hand the three signal molecules responsible for triggering the acrosome reaction in starfish spermatozoa; namely, ARIS, Co-ARIS and SAP. Although we know the precise structures of Co-ARIS saponins and SAPs, it is urgently required to figure out the molecular structure of ARIS, at least that of the sulfated saccharide chains essential for its binding to the receptor, and accordingly for its activity. Then we have to know much more about the spermatozoa; the receptors for ARIS and SAP, the plasma membrane components that interact with Co-ARIS, ion channels and exchangers, signal transduction systems and so on. For these, we have started gene analyses in our laboratory and collaboration with molecular physiologists and other specialists. We believe that we are to open a door to the next step for understanding gamete interactions.

Summary

It was in the early 1950s that J.C. Dan discovered the acrosome reaction in sea urchins, starfishes and several other marine invertebrates at Misaki Marine Biological Station on the Pacific coast of Japan. We now know that in many animals including mammals the acrosome reaction is an essential, and probably the most central, change in spermatozoa for fertilization. Starfish spermatozoa undergo the acrosome reaction upon encountering the jelly coat consisting of glycoproteins, steroid saponins, oligopeptides and inorganic components. To induce the acrosome reaction, three egg jelly components act in concert on the spermatozoa: a highly sulfated glycoprotein named acrosome reaction-inducing substance (ARIS), a group of sulfated steroidal saponins named Co-ARIS, and a group of glutamine-rich tetratriacontapeptides named sperm activating peptide (SAP). The action of ARIS is quite species-specific due to the specificity of ARIS-receptors in a restricted domain of the sperm surface and depends very much on sulfated saccharide chains. Co-ARIS is not much species-specific and its action depends on the sulfate group and steroid side chain. SAPs have a ring of 25 residues and increase the intracellular pH of spermatozoa. None of them can induce the acrosome reaction by itself in normal sea water, but ARIS does induce it in high Ca^{2+} or high pH sea water. Although a combination of ARIS and either Co-ARIS or SAP induces the acrosome reaction in normal sea water, all three are required to mimic the full activity of dissolved jelly coat.

KEY WORDS: *starfish, jelly coat, acrosome reaction, sperm activating peptide, glycoconjugate*

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