GAMETE SURFACE MOLECULES THAT MEDIATE MAMMALIAN FERTILIZATION

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SUMMARY

Fertilization is a highly programmed process by which two radically different cells, sperm and egg, unite to form a zygote, a cell with somatic chromosome numbers. The process is the net result of a complex sequence of molecular events that allow sperm to recognize and irreversibly bind to the egg's extracellular coat, the zona pellucida (ZP), undergo the acrosome reaction (AR), and fuse with the egg plasma membrane. The male gamete undergoes continuous morphological and biochemical modifications during sperm development in the testis, maturation in the epididymis, and capacitation in the female genital tract. Only the capacitated sperm are able to bind to the ZP, and undergo the signal transduction cascade that results in the exocytosis of acrosomal contents, i.e., induction of the AR. Accumulated evidence has helped consolidate the view that the carbohydrate recognizing receptor molecules (glycohydrolases, glycosyltransferases, and/or lectin-like molecules), present on the surface of capacitated spermatozoa, recognize and bind to the terminal sugar residue(s) of bioactive glycans (ligands) on the ZP. The carbohydrate-mediated adhesion event causes sperm to undergo the AR. The action of hydrolytic and proteolytic enzymes released during the AR, along with the enhanced thrust generated by the hyperactivated beat pattern of the bound spermatozoon, are important events that regulate sperm penetration through egg vestments. In this article, we have discussed extensive progress that has been made to enhance our understanding of molecules and molecular events that regulate fertilization.

Key words: Acrosome reaction, carbohydrates in fertilization, mammalian reproduction, sperm capacitation, sperm-egg interaction.

INTRODUCTION

Testicular spermatozoa are morphologically differentiated cells; however, they are neither progressively motile nor able to fertilize an egg (1). Although mammalian sperm acquire forward motility during epididymal maturation (2-4), the ability to bind to the egg's extracellular glycocalyx, the zona pellucida (ZP), is acquired in the female genital tract (1, 5-6). During residence in the female genital tract, spermatozoa undergo a series of biochemical and functional changes collectively referred to as capacitation (1). The changes during this event lead to sperm hyperactivation and their ability to bind to the ZP and undergo the Ca^{2+}-triggered acrosome reaction (AR) (1, 7).

Mammalian spermatozoa possess two parts: i) the head with the acrosomal (anterior head) and post-acrosomal (posterior head) regions, and ii) flagellum comprising of the middle, principal, and end piece (4, 6). Whereas, the molecules (receptors) responsible for binding to the complementary sugar residues on the ZP (ligands) are present on the surface of the anterior head of the capacitated spermatozoa, the hyperactivity is a result of molecular changes in the flagellum (1, 8, 9). Thus, sperm capacitation is a net result of biochemical and functional changes in the head and flagellum regions that activate the cell signaling pathway.

The recognition and binding of opposite gametes is an essential event in the process of fertilization (1, 5, 6, 10, 11). Sperm-egg interaction, at least in the mouse, is believed to take place in two stages. First, the capacitated sperm loosely and reversibly adhere to the surface of ZP followed by the species-specific irreversible binding (1). Both these bindings are thought to be due to the complementary molecules on the surface of opposite gametes. Although all potential molecules have not been identified, accumulated evidence from several investigations suggests that early events in the fertilization process are mediated by carbohydrates (1, 5, 6, 11). The glycan portion of several glycoconjugates present in the female genital tract and the ZP is believed to modulate cell-cell adhesion, including sperm-egg interaction, sperm-oviduct adhesion, and implantation of embryo. Several types of glycan moieties, including high mannose/ hybrid-type, sialylated, glucosaminylated, fucosylated, and galactosylated present on the ZP glycoconjugates have been implicated in sperm-egg interaction (for review, see 5, 6). We highlight here various molecules and discuss extensive progress that has been made to enhance our understanding of the molecular events that regulate fertilization.

Capacitation, a prerequisite event before sperm acquires fertilizing ability

Much of the knowledge on capacitation has come from the work by Austin (12) and Chang (13). Over five decades ago, the two investigators independently reported

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functional changes on spermatozoa during passage through the female reproductive tract. The precise site of capacitation may be different in various species; however, the *in vivo* process in several species is most efficient when spermatozoa pass through the uterus and oviduct (1). The oviductal secretions collected from the estrous females have been demonstrated to be most efficient in rendering functional changes in spermatozoa *in vitro*. Mammalian sperm can also be capacitated *in vitro* by incubating ejaculated or cauda epididymidal spermatozoa in a chemically defined medium supplemented with energy substances, such as glucose and pyruvate, and bovine serum albumin (BSA) or beta-cyclodextrins (14-16). The protein, a major component in the female genital tract, or beta-cyclodextrins is believed to facilitate capacitation by efflux of sterols, mainly cholesterol, fatty acids, and phospholipids from the sperm plasma membrane (PM). The precise mechanism as to how the loss of cholesterol/phospholipids regulates the physiological priming of spermatozoa is not fully understood; however, it is generally accepted that the efflux of sterols increases fluidity and permeability of the sperm PM, making it fusogenic (1). The current knowledge on the dynamics and physiology of capacitation has been discussed in a review article (17) and a book chapter (18) and will not be repeated here. However, we will summarize recent advances that seem to suggest that capacitation involves physiological priming of sperm PM that exposes acrosomal contents on the sperm surface over the acrosome (19).

Biology of capacitation

As capacitation proceeds, a number of changes occur on spermatozoa. The known changes include: i) efflux of sterols, mainly cholesterol, ii) increased adenylyl cyclase activity and increased levels of cAMP, iii) protein tyrosine phosphorylation of a subset of sperm components, iv) elevated intra-sperm pH, v) Ca^{2+} influx, vi) loss of sperm surface molecules, vii) modification/alteration of the sperm PM, viii) changes in the lectin-binding patterns, ix) hyperactive motility, and x) membrane priming. However, with the exception of cholesterol efflux (20), the sequence of other changes and their significance in sperm capacitation remains evasive.

An important cell surface alteration in the capacitating/capacitated spermatozoa is a change in the lectin binding patterns, a result consistent with the suggestion that the cell surface glycan units are altered during this event (21). One possible explanation for this change could be that uncapacitated sperm cells lose surface-coating molecules that expose other glycoproteins with different glycan chains. A second possibility could be that the existing sperm surface glycoconjugates are modified *in vivo* by glycosyltransferase activities reported by us to be present in the female reproductive tract secretions (22). Since the levels of these synthetic enzymes are regulated during estrous cycle, it is reasonable to assume that they have a role in modifying the existing sperm surface glycan moieties. A third possible explanation for the changes in the lectin binding properties *in vivo* could be the association of an oviductal glycoprotein on the surface of spermatozoa (23, 24). Finally, the acrosomal contents, including glycohydrolases, present within the acrosome of uncapacitated spermatozoa, could become accessible on the cell surface over the acrosome as capacitation proceeds (19). Many intra-acrosomal glycohydrolases are glycoproteins (7). Their exposure to the surface of capacitating spermatozoa will be expected to alter their lectin-binding patterns.

The fact that only the capacitated sperm bind to the ZP and undergo the Ca^{2+}-dependent AR suggests that major changes occur on the anterior head (peri-acrosomal) region of spermatozoa, an area believed to be involved in the sperm-egg binding (25). Since the acrosome acts in concert with the PM overlying the organelle, a brief discussion on its organization will contribute to our understanding of the physiological priming of membranes during capacitation. More details on the formation and organization of the sperm acrosome is presented in our review article (26).

A well-developed acrosome is a sac-like structure with an inner acrosomal membrane (IAM) and an outer acrosomal membrane (OAM) covering the anterior portion of the nucleus. In mammals, the size and shape of the acrosome varies from species to species and depends on the morphology of the sperm head which generally falls into two categories, a sickle-shaped in rodents and a skull-cap/paddle-shaped (spatulale) in several larger species, including man. The sperm acrosome is a Golgi-derived secretory organelle that resembles the cellular lysosome in many ways; however, it is considered analogous to a secretory granule. The important features of this secretory vesicle, as reported by Burgess and Kelly (27), are: i) the secretory contents are stored over an extended period of time and are present in a concentrated form, ii) the contents form a dense structure and are stored for several weeks during sperm development in the testis and subsequent maturation in the epididymis, and iii) the organelle undergoes secretion as a result of an external stimulus.

The interior of the acrosome proper is thought to be biochemically and morphologically compartmentalized with specific components present in discrete regions of the organelle. Two sets of components with different solubility are present in the acrosome, a set of readily soluble components and a set of insoluble (particulate) matrix components. The soluble antigens include glycohydrolases, many proteases, and CRISP-2, a cysteine-rich secretory protein (28), whereas the insoluble components are a part

of acrosomal matrix proteins. The well-characterized matrix components are AM50 (28), AM67, the guinea pig orthologue of the mouse sperm protein Sp56 (29), proacrosin and proacrosin-binding protein Sp32 (28).

In a recent report, Kim et al. (30) demonstrated that the mouse sperm component Sp56, a protein initially immunolocalized on the surface of capacitated spermatozoa, was actually a component of the acrosomal matrix. The protein was reported to be absent from the sperm surface unless the OAM and PM have begun fusing or have ruptured. To explain how Sp56 protein could have been mistaken as a cell surface molecule, the investigators hypothesized that sperm capacitation represents a transitional state whereby the membranes are modified by destabilization or initial fusion events (30). These initial phases could allow the antibody access to the intra-acrosomal matrix protein Sp56 that becomes exposed to extracellular milieu and, under conditions used for immunolocalization, the molecule appeared to have a surface localization. The proposed explanation is consistent with our biochemical and immunocytochemical approaches which have provided evidence strongly suggesting that mouse spermatozoa, incubated in a medium that favors capacitation, undergo membrane changes in a time-dependent manner. The net result is the progressive accessibility of acrosomal glycohydrolases on the surface of capacitating/capacitated (acrosome-intact) spermatozoa (19).

Is the surface exposure of acrosomal contents functionally significant?

As stated above, the capacitating/capacitated spermatozoa undergo changes in the peri-acrosomal region of the head which enable them to recognize and bind to the ZP, a result consistent with the suggestion that the process brings about major changes on the surface of sperm head. It is plausible that some of the exposed acrosomal contents, in addition to the carbohydrate-binding molecules (receptors) on the sperm PM (6), participate in sperm-zona interaction. The growing list of acrosomal molecules suggested to be exposed during sperm capacitation includes acrosomal matrix protein SP56 (29, 30), proacrosin/acrosin (31), hyaluronidase (32), beta-D-galactosidase and beta-D-glucuronidase (26), an acrosomal protein SP10 (33), and acrosomal arylsulfatase A (34, 35). The reported presence of several intra-acrosomal antigens on the sperm surface of capacitated spermatozoa is consistent with our suggestion that capacitation-associated exposure of these molecules is functionally significant.

Sperm capacitation: Common components and potential similarities with early events of Ca^{2+}-triggered membrane fusion in somatic cells and viruses

Accumulated evidence suggests that, as in regulated secretion in somatic cells, Ca^{2+} is required for capacitation as well as for the induction of the AR. Calcium exerts its effect on sperm functions through calmodulin (CaM), a 17 kDa acidic protein tnat regulates many signaling pathways in somatic cells by modulating activities of enzymes and ion pumps. Sperm cells contain high levels of CaM in the head and flagellum regions, localizations consistent with its reported role in sperm function (36). In addition to CaM, the sperm acrosome contains synaptotagmins, a family of trans-membrane proteins suggested to have a regulatory role in membrane fusion events (37). The two calcium sensor proteins (CaM and synaptotagmin) interact with SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptor), a large family whose members have been found on either the target membrane, the t-SNAREs or the transport vesicles that shuttle between pairs of communicating membranes, the v-SNAREs (38). This implies that SNARE proteins may play a vital role in assembling complexes that form a bridge between the fusing membranes.

A formed transport vesicle (v-SNARE) is likely to inspect many potential targets before it finds a complementary target membrane (t-SNARE). This crucial step is thought to be controlled by Rab proteins, a family of small GTPase, that enable v-SNARE and t-SNARE to find a correct match. After the vesicle (v-SNARE) encounters the correct target membrane, the physical attachment of the v-SNARE and t-SNARE (tethering) occurs. The attachment event is thought to be the earliest known event that precedes the formation of a trans-SNARE complex. The vesicles remain bound allowing the Rab proteins to exchange GTP for GDP that enables tight adhesion (docking) of the vesicle to the target membrane prior to their fusion. This implies that the docking of the transport vesicle to the target membrane and their subsequent fusion are two separate events. For instance, it is possible to prevent fusion while permitting docking by keeping cytoplasmic concentration of Ca^{2+} low. This results in an accumulation of vesicles attached to (but not fused to) the target membrane and may represent the end-point of sperm capacitation before sperm-egg binding and influx of additional Ca^{2+} which signals membrane fusion and acrosomal exocytosis.

The priming of membranes and their actual fusion during secretory and endocytotic pathways can be broken down into four steps. First, there is formation of vesicles from the donor membrane. Second, the vesicles are transported to their destination. Third, tethering and docking of vesicles with the target plasma membrane occurs. Finally, the vesicles fuse with the plasma membrane. It raises a logical question: Does the priming of membranes during sperm capacitation represent the first three steps of secretory pathway? As stated above, the end-point of capacitation, a process unique to the male gamete, is the hyperactive motility and the responsiveness to undergo the agonist-induced fusion of the outer

acrosomal membrane and the plasma membrane. Thus, it is reasonable to argue that sperm capacitation and the AR utilize many of the molecules that regulate the membrane fusion among eukaryotes. The common molecules in these pathways include N-ethylmaleimide-sensitive factor (NSF), which is suggested to have an important role in intracellular fusion, soluble NSF attachment proteins [SNAPs] and a large family of SNARE proteins (39, 40). At the functional level, SNARE proteins take part in bringing fusing membranes closer to each other. Other molecules, such as NSF, SNAPs, and large-sized molecules (>250 kDa) are thought to participate in the transport of cargo proteins during tethering, an event representing the earliest known step in membrane targeting and fusion. Interestingly, all these molecules, in addition to Ca^{2+} sensor proteins (calmodulin and synaptotagmin), and Rab3A have been identified in the sperm acrosome (for review, see 41).

The above discussion seems to suggest potential similarities between sperm capacitation and early stages of Ca^{2+}-triggered membrane fusion in somatic cells and viruses. For instance, prior to the fusion there is a contact and merger of the two phospholipid bilayers of the fusing membranes, hemifusion of the bilayers at the site of membrane contact followed by formation of fusion pore (42). This implies that the sperm capacitation, like early events of membrane fusion, represents progressive membrane priming and not an all-or-nothing change. While additional evidence in support of the proposed similarities between sperm capacitation and early events of the membrane fusion is awaited, the time-dependent appearance of several immunopositive patterns in our original report (see Figs 3-5 in 19) favors our argument. The reported presence of several intra-acrosomal antigens on the surface of capacitating/capacitated spermatozoa (see above) is consistent with this suggestion.

Although relatively little is known about the mechanism(s) underlying physiological priming of spermatozoa, the end-point of this process is responsiveness of cells to undergo the AR (20). Thus, it is reasonable to suggest that the OAM and the sperm PM, the two membranes that will ultimately fuse during the AR, realign in capacitating/capacitated cells in a manner similar to the step-wise preparation of fusing membranes. First, the two membranes come together by a progressive evagination of the OAM (see Fig. 3 in 43). Second, complementary molecules on the fusing membranes allow their close contact (tethering) and tight adhesion when facing leaflets of the two lipid bilayers intermingle (docking). Finally, pores develop in distinctive phases (destabilization), some of which may have features typical of membrane channels. The dynamic aspects of the fusion pores, thought to be the priming steps prior to the membrane fusion among eukaryotes and among stations of secretory pathways (44), may occur in capacitating spermatozoa (see Fig. 4 in 43). The intra-acrosomal contents and/or antibodies could diffuse through the fusion pores revealing them on the surface of capacitating/capacitated spermatozoa.

Sperm-zona (egg) binding

There is overwhelming evidence that the recognition and binding of male and female gametes is a carbohydrate-mediated receptor-ligand binding event that is initiated by interaction of carbohydrate-binding molecules (glycosyltransferases, glycohydrolases or lectin-like sugar binding proteins) on the surface of the capacitated spermatozoa (receptors) and their complementary glycan (ligand) moiety(ies) on homologous ZP (1, 5, 10, 11). Such a mechanism is analogous to adhesion events in the lower kingdom such as the binding of bacteria, viruses, and other pathogens to their host cells. In mammals, the carbohydrate-mediated event of sperm-egg binding is best understood in the mouse, although there is some information in other species, including man (1, 5). In the mouse, capacitated spermatozoa interact with the zona-intact eggs in a highly precise manner. They loosely and reversibly adhere to the ZP, which is followed by a tight irreversible binding (1).

Zona pellucida glycoconjugates

All mammalian eggs are surrounded by an extracellular glycocalyx, the ZP. The ZP in many species is a relatively simple structure composed of three glycoproteins designated ZP1, ZP2 and ZP3; the pig and human have a fourth form as well (for review, see 5). In the mouse, two of the glycoproteins, mZP2 and mZP3, interact non-covalently to form long filaments which are interconnected by mZP1 forming a three-dimensional network of cross-linked filaments, the extracellular matrix (45). Such a structure may explain the elasticity of the ZP and the relative ease of its penetration by acrosome-reacted spermatozoa. The ZP mediates many events, including, (i) relative species-specificity, (ii) sperm activation (induction of the acrosome reaction (iii) block to polyspermy, and (iv) protection of the growing embryo from fertilization to implantation (1).

In recent years, considerable progress has been made in understanding structure-function of various zona components. In particular, work on mZP has resulted in identification of primary (mZP3) and secondary (mZP2) binding sites for homologous spermatozoa (45). Several lines of evidence listed in previous review articles (5, 45) strongly suggest that glycan units of mZP3 provide the primary ligand site(s) for the sperm receptors. Two of these evidences are highly specific and are worth repeating. First, sperm binding activity of mZP3 is sensitive to trifluoromethane sulfonic acid, an acid known to break glycosidic bonds between monosaccharide residues of

N-linked and *O*-linked oligosaccharides without altering the protein backbone (46). Second, the ability of mZP3 to competitively inhibit sperm-egg binding is unaffected by the treatment with pronase, a protease that digests the protein backbone of mZP3; however, the resulting glycopeptides (*N*-linked and *O*-linked glycopeptides ranging in size from 1.5-6.0 kDa) are still able to inhibit sperm-egg binding *in vitro* in a dose-dependent manner (46).

It should be noted that the binding of capacitated spermatozoa starts a cascade of signaling events resulting in acrosomal exocytosis. The ability of mZP3 to serve as the primary ligand mainly depends on glycan chains; however, its ability to induce the AR depends on glycan units as well as the protein backbone. Consistent with this possibility is the finding that the pronase generated ZP3 glycopeptides retain bioactivity, but do not induce the AR unless the bound glycopeptides (glycan units) are cross-linked on the sperm surface by anti-ZP3 IgG (47). Studies published from another laboratory also suggest that binding of multiple glycan units of mZP3 to the sperm surface galactosyltransferase causes its aggregation and triggering of the AR (48). We have recently demonstrated that specific sugar residues (mannose, *N*-acetylglucosamine, and *N*-acetylgalactosamine) can induce the AR, but only when covalently conjugated to a protein backbone (49). The potential implication of multiple monosaccharide residues in initial sperm-egg binding is consistent with a report demonstrating that initial molecular interaction between sperm and mZP3 is a complex binding process which reflects multiple sperm surface receptors with multivalent ZP3 (50). The implication of several sugar residues in initial sperm-egg binding and induction of the AR is also consistent with this possibility. The sugar residues suggested to have a role in initial binding of the opposite gametes are alpha -D-galactose, beta-D-galactose, beta-*N*-acetylglucosamine, mannose, and sialic acid. Although a terminal fucosyl residue has not been implicated in initial binding of opposite gametes in the mouse, its presence appears to be obligatory for an oligosaccharide to bind to spermatozoa with high affinity. Taken together, these studies suggest a possible interaction between receptors on the surface of capacitated sperm and terminal sugar residues on mZP3 or neoglycoproteins prior to the induction of the AR. The ZP3 or protein conjugated sugar residues are thought to induce the AR by cross-linking or aggregating receptor on the sperm PM (see above).

Work from our group and others suggest that *N*-linked (asparagine-linked) glycans may be bioactive molecules that are recognized by the sperm surface receptor(s). It is interesting to emphasize that *N*-linked glycans contribute nearly half of the molecular mass of mZP3 and over 40% of mZP2 (51). Thus, a discussion on the complexities in the structure of *N*-linked glycans will contribute to a better understanding of their role in sperm-egg interaction. The *N*-linked glycans may either be of high mannose, hybrid or complex (bi-, tri, tetraantennary) structures (6). The three types of glycans contain the basic structure composed of a branched trimannose region to an *N*, *N*'-diacetylchitobiose, which is attached to the amide nitrogen of an asparagine residue on the protein. In the high mannose oligosaccharide the core structure is substituted by á-linked mannosyl residues whereas in complex structures the core structure is elongated by the presence of trisaccharide (sialic acid-galactose-*N*-acetylglucosamine). The hybrid type glycan is a combination of high mannose and complex type where one antenna of the core structure contains only mannosyl residues and the other antenna contains one or two trisaccharide units on alpha-1,3 branch (6).

In addition, many glycoproteins, including mZP2, mZP3, and porcine ZP3 contain N-linked poly-N-acetylglucosaminyl glycans. These glycans contain repeat units of disaccharide (3Gal beta1, 4Glc*N*Acbeta1) present on complex-type tri- and tetraantennary structures. The fact that these glycans were demonstrated by us to contribute 23 kDa and 16 kDa to the molecular mass of mZP2 and mZP3, respectively (51), suggests that the two zona components may contain a variety of structurally variable polylactosaminyl chains. Indeed, current evidence indicates that polylactosaminyl glycans present in many cell surface glycoconjugates may contain four variable terminal sugars, suggesting that polylactosaminyl glycan chains on mZP2 and mZP3 may also be quite complex structures with many variables. Thus, the number of *N*-linked glycan units in glycoproteins is very large and can run into hundreds. However, although individual cells are capable to synthesize many *N*-linked glycan chains, the process is highly specific and controlled in such a way that the glycan chains at a particular glycosylation site have a small number of closely related structures. Nonetheless, the fact that a large number of glycan structures are possible makes it difficult to identify and chemically characterize the bioactive glycan residue(s). The efforts are further hampered by the small amounts of ZP glycoproteins that can be purified and subjected to structure-function studies.

It is noteworthy that, whereas some studies suggest that *O*-linked glycan units are the bioactive molecules, our own studies have provided evidence suggesting that *N*-linked high mannose/hybrid type glycans on the mouse and rat zona-intact eggs may be recognized by sperm surface mannosidase (for reviews, see 5, 6). The enzyme is a glycosidase. Its catalytic mechanism of action has been discussed in the above reviews. The catalytic mechanism includes the formation of an enzyme-substrate (carbohydrate) intermediate before cleavage of the sugar residues. Since purified sperm surface

mannosidase cleaves negligible amounts of [^{3}H] mannosyl residues from [^{3}H] mannose-containing glycoproteins after 4 h of incubation at 37°C, it is surmised that an intermediate of sperm (enzyme)-zona (substrate) is formed that leads to the next step in fertilization before a significant amount of mannosyl residues is cleaved. The evidence for the presence of high mannose/hybrid-type oligosaccharide on mZP2 and mZP3 (52) is consistent with this suggestion.

It should be noted that, like mZP3, the porcine ZP glycoprotein (pZP3) has been reported to contain sperm binding activity. The 55 kDa molecule is also highly glycosylated, containing *N*-linked , *O*-linked , and poly-*N*-acetyllactosaminyl glycans. A recent report by Noguchi et al. (53) presented evidence suggesting that a mixture of neutral *N*-glycan chains is important in sperm-egg recognition and binding in the pig. Taken together, data from various laboratories provide evidence suggesting that both *N*-linked and *O*-linked glycan units may be bioactive and important in sperm-egg interaction.

Sperm molecules (receptors) with affinity for the ZP

As stated above, the ZP is a relatively simple structure containing 3 or 4 glycoproteins. However, the sperm PM overlying the acrosome (see above) is a complex structure consisting of dozens of proteins/glycoproteins. For nearly three decades, investigators have used multiple approaches to identify and isolate the complementary receptor molecules in several species. Their efforts have resulted in the recognition of several putative receptors on spermatozoa. Since the putative sperm receptors, along with their complementary molecules on the ZP, have been described in two previous reports (for reviews, see 5, 6) and will not appear here.

Why are there so many putative receptors on the sperm plasma membrane? The following factors may have contributed to the long list of proposed receptor and ligand molecules. First, several receptor-ligand interactions may occur between spermatozoa and zona-intact egg before a committed sperm-egg binding. The multiple receptors may participate either individually or as multimeric receptor complexes. The experimental evidence, suggesting that the initial molecular mechanism between spermatozoa and ZP is a complex binding event that reflects interaction between multiple sperm proteins with multivalent ZP3 (50), is consistent with the above possibility. Second, since sperm-egg interaction is relatively species-specific, it is possible that different molecules are functionally significant in different species. Alternatively, multiple sperm surface molecules may interact with complementary ligands in a well-programmed manner; the precise order of these interactions or the dominant receptor-ligand interaction may vary among species and may contribute to the species-specificity during fertilization. It is not yet known whether the multiple receptors act individually or form multimeric receptor complexes.

Exocytosis of acrosomal contents

The reversible binding of capacitated spermatozoa to the terminal sugar residue(s) of the bioactive glycan moiety(ies) is thought to trigger at least two distinct signaling cascades: a pertussis toxin-sensitive G protein cascade and activation of ion channels (25) that result in an influx of Ca^{2+}. The transient rise in intracellular Ca^{2+} ions and other second messengers, such as cyclic adenosine monophosphate and inositol triphosphate, initiates a cascade of signaling events that elevate the intrasperm pH and induce the AR. The order in which the activation of various molecules fits in the puzzle of a signal transduction pathway prior to the AR is not yet known.

Morphologically, the AR occurs in several steps. First, there is a swelling of the acrosome, followed by the fusion of the sperm PM and the OAM at multiple sites (for review, see 26). Second, there is formation of hybrid vesicle (vesiculation) and a time-dependent release of acrosomal contents. Finally, there is disappearance of acrosomal contents and vesicles that are held together by the acrosomal matrix. The hydrolytic action of enzymes (glycohydrolases, proteinases, phosphatases, etc.) released at the site of sperm-zona (egg) binding makes it possible for the hyperactivated sperm to penetrate the ZP and fertilize the egg.

The signal that triggers the AR reaction in the mouse is believed to be the recognition and irreversible binding of multiple sugar residues of mZP3 by complementary receptor molecules on the sperm PM (Fig. 1). The ability of mZP3 to be the natural agonist depends on the glycan moieties as well as the polypeptide portion of the molecule; the latter facilitates the aggregation of the sperm surface receptors (Fig. 2). Our studies with synthetic glycoproteins (neoglycoproteins) demonstrated the need for the sugar residues as well as the protein backbone in the induction of the AR (49).

The fusion and vesiculation of the sperm PM and OAM at multiple sites allow the acrosomal contents to be released, thereby exposing the inner acrosomal membrane. The exposed membrane reveals a new set of binding sites specific for mZP2 which hold the acrosome-reacted (hyperactive) sperm bound to the egg before it penetrates the ZP relying on hydrolytic and proteolytic enzymes along with the enhanced thrust generated by the hyperactivated beat pattern of the bound sperm flagellum (7, 26). Although the molecules thought to be potentially important in secondary binding events and sperm-egg fusion will be of interest to many readers, they are beyond the scope of this article. Interested readers are referred to a review article by McLeskey et al. (54).

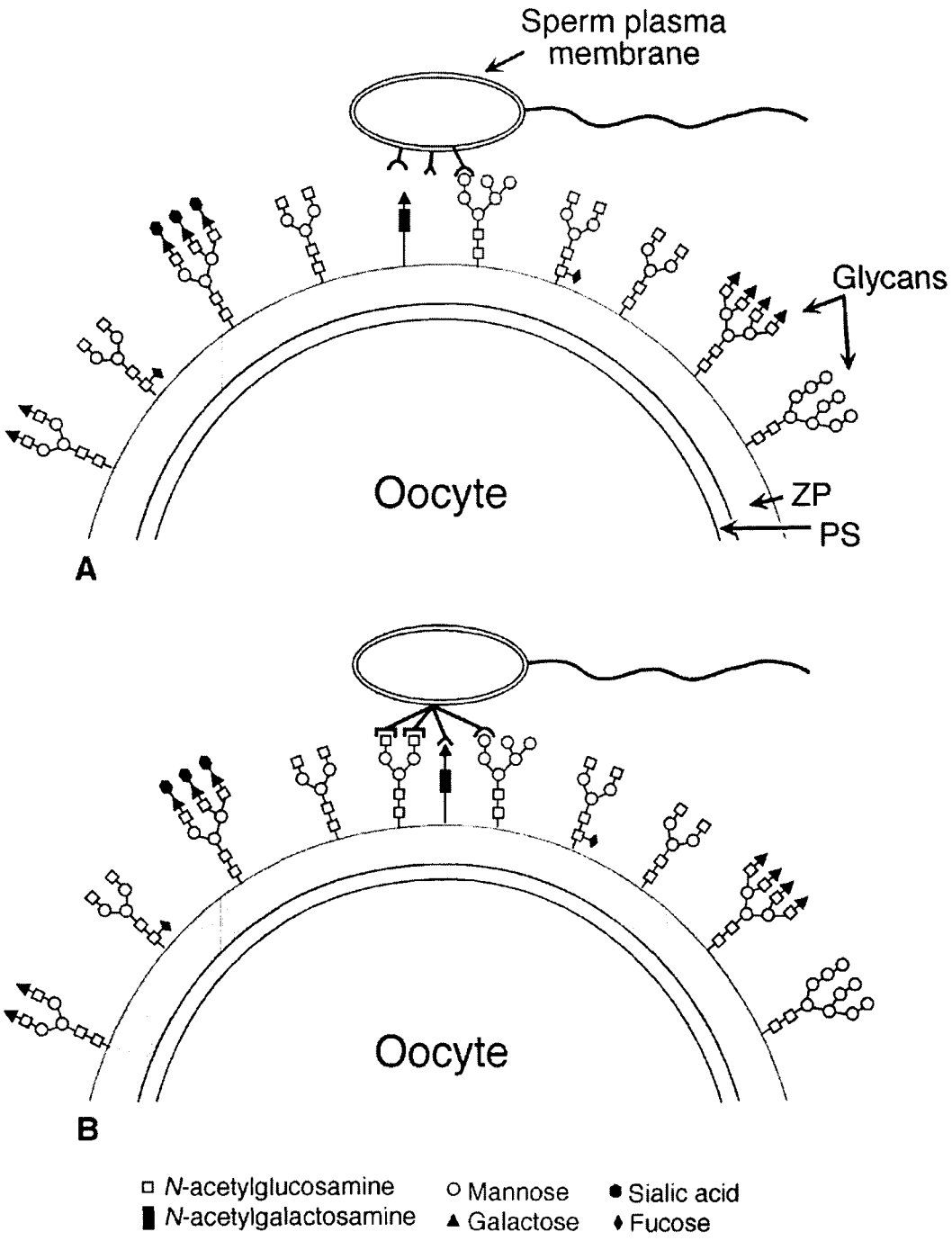

Fig. 1. Model illustrating sperm-zona (egg) interaction. A, a single receptor on the sperm plasma membrane recognizes its complementary oligosaccharide chain (ligand) on the zona-intact egg; B, multiple receptors on the surface of capacitated sperm recognize multiple oligosaccharide chains (ligands) for the tight and irreversible binding. ZP, zona pellucida; PS, perivitelline space.

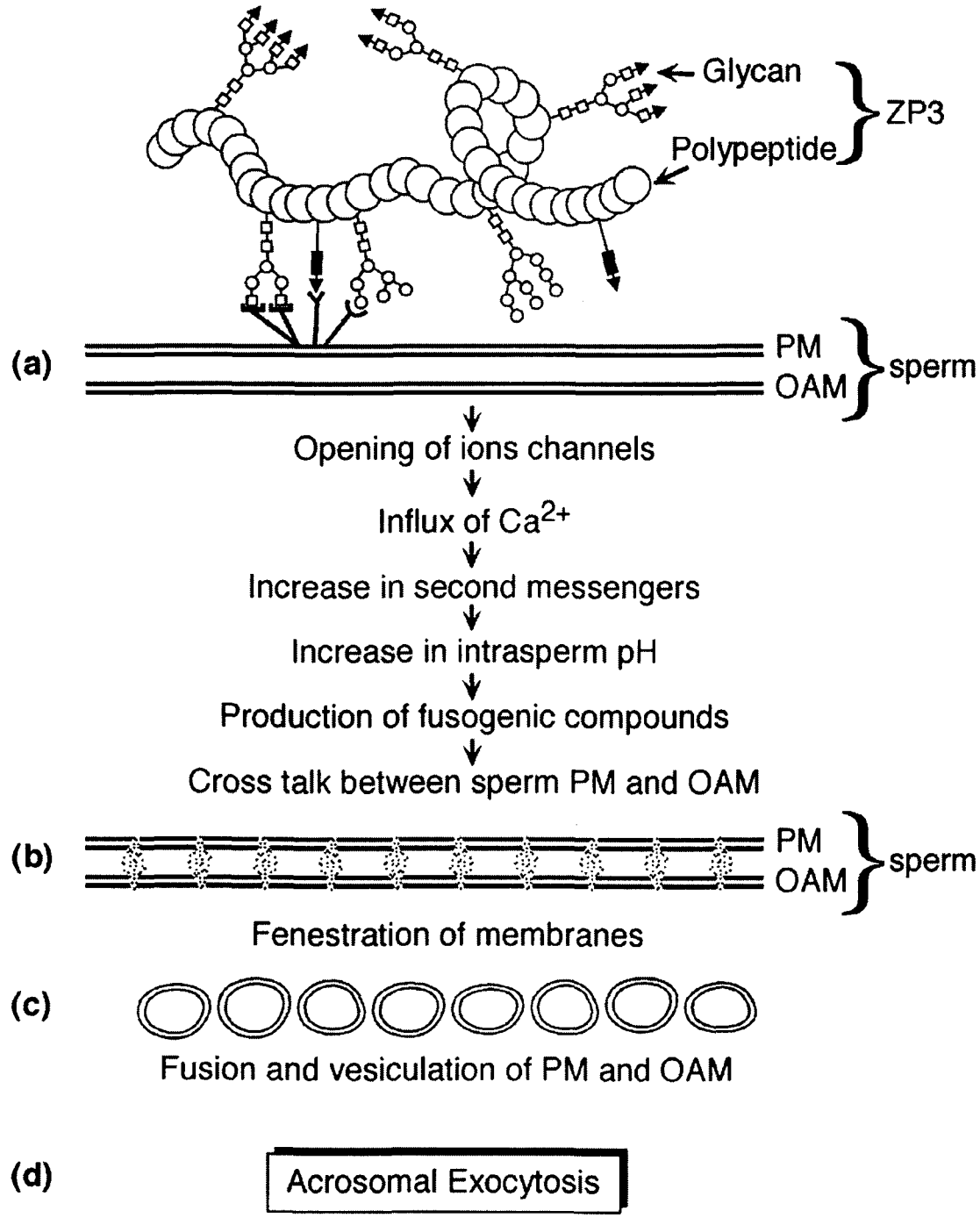

Fig. 2. Model illustrating a possible sequence of events leading to acrosomal exocytosis and release of acrosomal contents. a, multiple sugar residues (ligands) on ZP3 recognize and bind to complementary receptors on the sperm PM and start a signal transduction cascade; b, in response to increased Ca^{2+} and pH, the F-actin which provides a physical barrier between the PM and OAM depolymerizes to form soluble monomeric actin (G-actin which disperses bringing the PM closer to OAM); c, the rise in Ca^{2+} also activates phospholipase A_2, an enzyme that cleaves fatty acids from phospholipids to form lysophospholipids promoting fusion and vesiculation of the sperm membranes; d, the formation of PM and OAM vesicles (hybrid vesicles) allow acrosomal contents to be released at the site of sperm-oocyte binding. PM, plasma membrane; OAM, outer acrosomal membrane; IP3, inositol triphosphate.

CONCLUSIONS

Mammalian fertilization is the net result of a complex set of molecular events in which capacitated spermatozoa bind to homologous zona-intact egg and undergo a complex series of programmed events before a single sperm fuses with the egg PM. The article has focused on molecules thought to be functionally significant at various stages leading to fertilization. We have discussed accumulated evidence strongly suggesting that sperm-egg (zona) interaction is a carbohydrate-mediated receptor-ligand binding event which depends on glycan-recognizing molecules on the surface of capacitated sperm (receptors) and their complementary glycan moiety(ies) on ZP. The interaction of opposite gametes initiates a signal transduction pathway resulting in the exocytosis of acrosomal contents, i.e., induction of the AR. Special attempts have been made to discuss the involvement of various sperm components that likely modulate capacitation and potential cross-talk between the sperm PM and OAM during the assembly of the membrane fusion machinery in capacitating sperm. We are hopeful that the article will provide an understanding of the complex process of mammalian fertilization.

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