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Protein-Carbohydrate Interactions during Fertilization

Abstract

Interaction between gametes during fertilization is at least in part regulated by carbohydrate moieties of the zona pellucida (ZP) and carbohydrate binding proteins of the sperm surface. This review focuses on the protein-carbohydrate interactions during the primary binding of the sperm to the ZP in different species. Synthesis, structure and composition of the ZP are summarized. The functional significance of carbohydrate residues of the ZP as sperm receptor is discussed. Sperm surface proteins known to have specific ZP and carbohydrate-binding sites including the mouse β1,4-galactosyltransferase and sp56, the rabbit protein Sp17, a human mannose-binding protein and several members of the sperm-adhesin family are presented.

Key Words

Protein
Carbohydrate
Fertilization
Zona pellucida
Sperm receptor

Introduction

Fertilization is a process of fundamental biological significance. It proceeds as a well-choreographed cascade of cell-cell interactions between the gametes of opposite mating type [Yanagimachi, 1994; Snell and White, 1996]. The haploid egg and sperm fuse, initiating the development of a new diploid organism containing genetic information derived from both parents. Fertilization has been studied intensely for many years. Most of the early studies concentrated on fertilization in marine organisms particularly in invertebrates, like the sea urchin. In this species large numbers of female as well as male gametes are released into the local environment. Due to technical advances in biotechnology, biochemistry and molecular biology [reviewed by Dunbar et al., 1991; Wassarman, 1991, 1992] a better understanding of mammalian fertilization has now been achieved.

In mammalian species, fertilization is internal and the fertilization process has been adapted to this environment. At insemination several hundred millions of spermatozoa are released into the female genital tract. During their transport through the uterus and oviduct spermatozoa capacitate and subsequently undergo a change in motility called hyperactivation, a prerequisite for the sperm's ability to fertilize the oocyte. Fully capacitated spermatozoa pass through several layers of somatic cells of the former cumulus oophorus before reaching the zona pellucida (ZP), an acellular glycoprotein coat which surrounds the oocyte (fig. 1). The first contact between the spermatozoon and the ZP (attachment) is a loose, nonspecific association between the gametes and appears to be a disconcertingly random affair [Aitken, 1995]. It is followed by a relatively firm binding, which is species-specific and mediated by complementary receptors on the ZP (sperm receptor) and on the sperm surface. Once bound, sperm undergo the acrosome reaction, by which hydrolytic enzymes are released from the acrosome of the sperm head. This permits the spermatozoa to degrade and penetrate the ZP matrix by a combination of enzymatic digestion of the ZP glycoprotein and vigorous propulsion of the spermatozoan tail. During

Abbreviations used in this paper:
COC = Cumulus-oocyte complex; Gal = galactosyl; GalTase = β1,4-galactosyltransferase; GlcNAc = N-acetylglucosaminyl; TCM 199 = tissue culture medium 199; ZP = zona pellucida.

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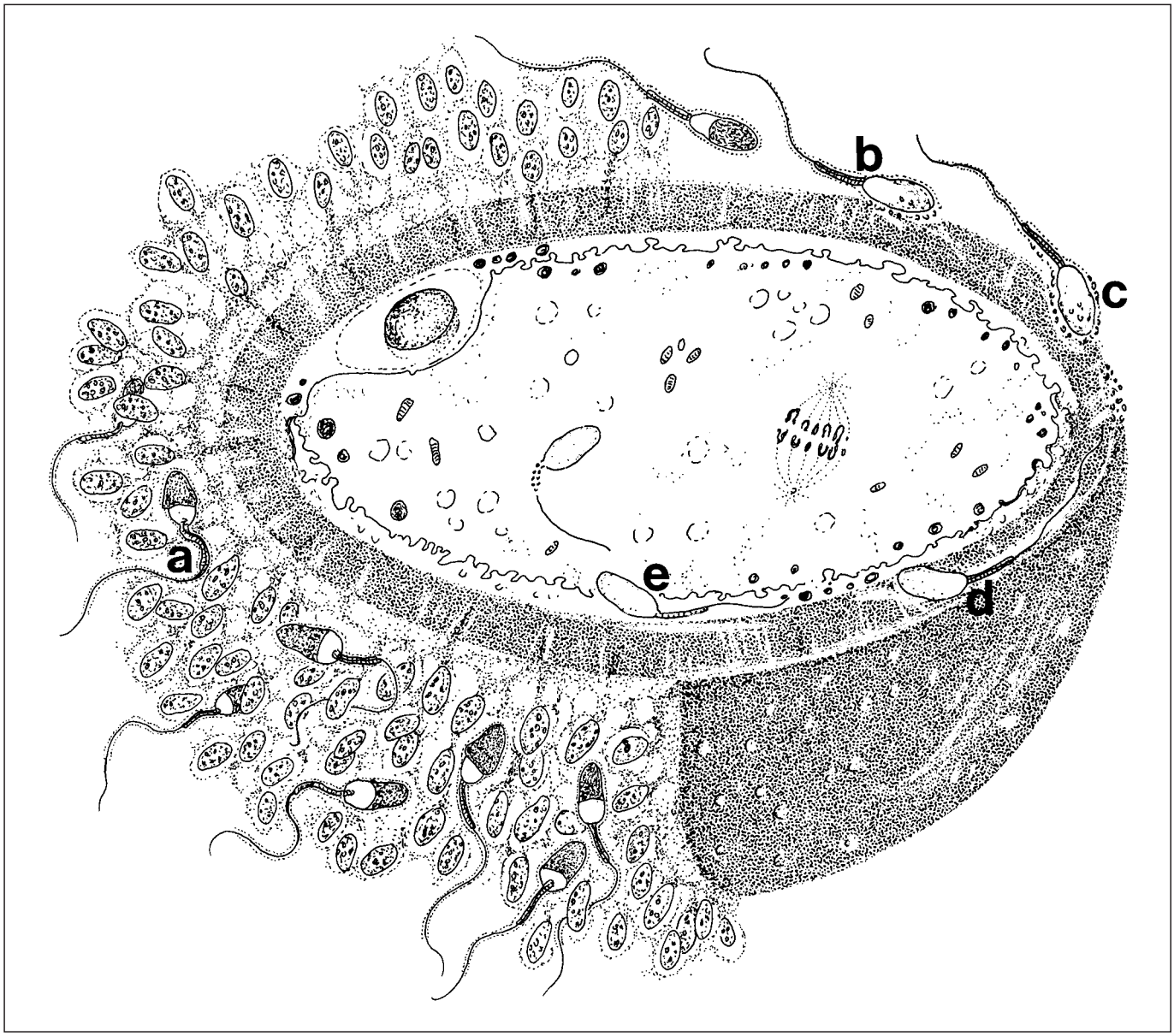

Fig. 1. Overview of the fertilization pathway. Fully capacitated spermatozoa pass through several layers of somatic cells of the former cumulus oophorus before reaching the ZP (a). After a first loose contact between the spermatozoon and the ZP, species-specific binding is mediated by complementary receptors on the ZP and on the sperm surface (b). Once bound the sperm undergoes the acrosome reaction (c). Penetration of the ZP (d). Eventually, the sperm reaches the perivitteline space and fuses with the plasma membrane of the oocyte and is then taken up into the cytoplasm of the oocyte (e).

this process acrosome-reacted spermatozoa are temporarily bound by the ZP glycoproteins via secondary binding mechanisms (secondary sperm receptors). Eventually a sperm reaches the perivitteline space and fuses with the plasma membrane of the oocyte, thereby fertilizing the oocyte [Burks et al., 1995]. Immediately after the first spermatozoon fuses with the oocyte, exocytosis of a set of secretory granules, the cortical granules of the oocyte, is triggered (cortical reaction). The contents of these granules modify the oocyte membrane and the ZP. Consequently any further penetration is prevented and the so-called block to polyspermia is established.

A number of recent studies has shown that carbohydrate moieties of the ZP and corresponding carbohydrate-binding

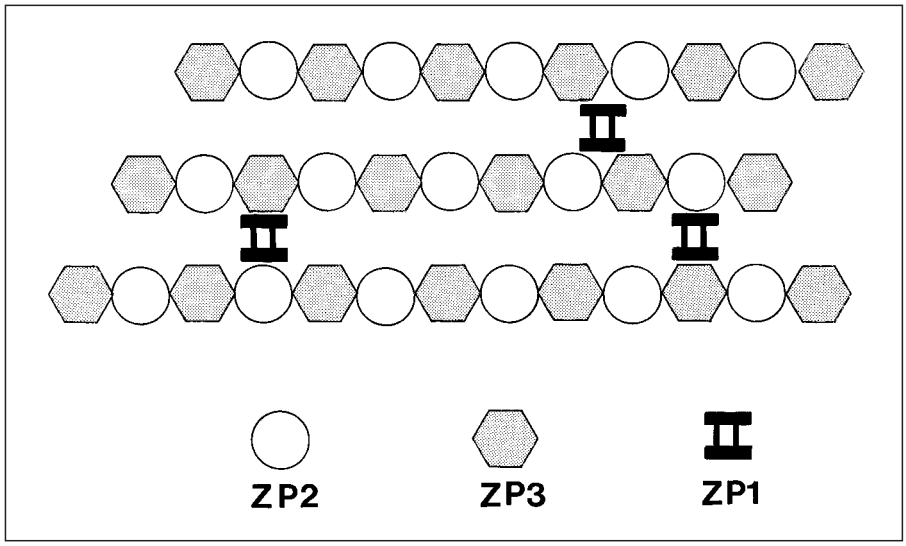

Fig. 2. Structure of the ZP of the mouse according to Wassarman and Mortillo [1991]. Heteromeric units of ZP2 and ZP3 are periodically arranged in filaments, which are interconnected by ZP1.

proteins on the sperm surface [Gabius, 1987; Sinowatz et al., 1989; Töpfer-Petersen et al., 1995a, b; Benoff, 1997; Shalgi and Raz, 1997; Sinowatz et al., 1997] regulate the interaction between the gametes. This review will focus on the protein-carbohydrate interactions during the primary binding of the sperm to the ZP.

Structure of the ZP

The ZP of mammalian oocytes is a porous extracellular matrix (fig. 1), constructed from only three glycoproteins, which build its typical fibrogranular structure [Wassarman and Mortillo, 1991]. The most obvious interspecies difference is the size of the ZP with a thickness varying from 1–2 µm in the opossum to 27 µm in the cow [Dunbar et al., 1994]. In many species the ZP is morphologically segregated in layers and there is a distinct asymmetry between the inner and outer layers [Philips and Shalgi, 1980; Ahuja and Bolwell, 1983; Shalgi and Raz, 1997]. Studies in the mouse provided insight into the ultrastructural organization of the ZP matrix. According to their electrophoretic mobility the glycoproteins of the ZP are denoted as ZP1, ZP2 and ZP3. These heterodimeric units are periodically arranged in filaments (fig. 2). These filaments appear to be interconnected by ZP1, the largest mouse ZP protein [Wassarman and Mortillo, 1991].

The glycoproteins of the ZP are the products of three major gene families referred to as ZPA, ZPB and ZPC, according to the size of their cDNAs [Harris et al., 1994]. The genes encoding ZP1, ZP2 and ZP3 of several mammalian species have been cloned. A relatively high degree of conservation of the gene sequences was found. Also, in different species the primary polypeptide structure including the number and position of most cysteine residues and potential N-glycosylation sites is highly conserved [Harris et al., 1994]. The diversity in molecular mass between different species is caused by high glycosylation of the ZP glycoproteins [Shalgi and Raz, 1997].

Synthesis of ZP Glycoproteins

The origin of the mammalian ZP glycoproteins is still controversial, Three theories have been developed proposing that glycoproteins are synthesized (1) by the oocyte only, (2) by the follicle cells only, (3) by both the oocyte and the follicle cells. There has been recent evidence that the contribution of follicle cells in the production of zona components is species-specific. Thus, in mice possessing only a thin zp, zona glycoproteins and their transcripts are exclusively synthesized by the oocyte [Haddad and Nagai, 1977; Fléchon et al., 1984; Wassarman et al., 1989; Kimura et al., 1994; Epifano et al., 1995]. In vitro studies confirmed that mouse oocytes are capable of synthesizing all zona proteins [Bleil and Wassarman, 1980].

In contrast, investigations in other species such as human [Grootenhuis et al., 1996], monkey [Grootenhuis et al., 1996; Martinez et al., 1996], rabbit [Dunbar et al., 1994; Grootenhuis et al., 1996], dog [Tesoriero, 1981], pig [Sinowatz et al., 1995a, b; Kölle et al., 1996] and cow [Kölle et al., 1998] have shown that both the oocyte and the follicle cells contribute to the synthesis of zona glycoproteins. Characteristically these species possess a well-developed

zona. As the rate of protein synthesis by the oocyte is too low to account for the oocyte's weight plus that of its zona, follicle cells have to be involved in the synthesis of the proteins [Schultz et al., 1979a, b]. These findings have been substantiated by in vitro studies on granulosa cells which were able to secrete ZP components [Maresh and Dunbar, 1987]. Moreover, some ZP proteins can be produced in the absence of oocytes in vivo [Skinner et al., 1984].

In situ hybridization studies and immunohistochemical investigations done in our laboratory demonstrated a change in the synthesis of ZP proteins and their mRNA in the course of ontogenesis. During follicular development of the porcine and bovine ovary, the protein ZPC and its transcript are mainly localized in the oocytes of primordial and primary follicles [Sinowatz et al., 1995a; Kölle et al., 1996, 1998]. In secondary follicles, both the oocyte and the follicle cells contribute to the synthesis of ZPC and its mRNA. In tertiary and preovulatory follicles ZPC and its transcripts are mainly found in the cytoplasm of the corona radiata cells. Whereas the bovine oocyte is involved in ZP synthesis during all stages of follicular development, porcine oocytes of tertiary follicles stop synthesizing ZPC. This result intimates a species-specific differential expression of these ZP proteins in the adult ovary [Kölle et al., 1996, 1998]. Moreover, investigations in fetal ovaries have shown that zona proteins are synthesized by follicle cells already during prenatal development. In the bovine fetus, ZPC is localized in oocytes of primordial and primary follicles and in both oocyte and follicle cells of secondary and tertiary follicles [Totzauer et al., 1998].

The medium essentially influences synthesis of ZPC in follicle cells during in vitro maturation of bovine cumulus-oocyte complexes (COCs). Thus, the cumulus cells of COCs maturated in tissue culture medium 199 (TCM 199) supplemented with bFSH, bLH and estrus cow serum show high amounts of ZPC in their cytoplasm. In contrast, in COCs maturated in TCM 199 without supplements ZPC synthesis is reduced. As FSH, LH and estrus cow serum have been shown to enhance the frequency of fertilization and to support blastocyst development [Schellander et al., 1990], the enhanced viability of the COCs maturated in the presence of these supplements seems to be correlated to an increased ability of synthesizing ZPC [Kölle et al., 1998].

Carbohydrate Composititon of Zona Glycoproteins

Similar to histochemical studies presented in this issue [Brinck et al., 1998; Danguy et al., 1998; Plendl and Sinowatz, 1998; Zschäbitz, 1998] plant and invertebrate lectins have been employed as tools to characterize the oligosaccharide chains of the ZP proteins. As outlined by Gabius [1998] and in this issue by von der Lieth et al. [1998], these sugar receptors frequently select a low-energy conformation for ligand binding. The wide panel of commercially available lectins with narrow sugar specificity allows the identification of distinct carbohydrate structures and particularly their in situ localization [Shalgi et al., 1991; Aviles et al., 1994; Maymon et al., 1994; Skutelsky et al., 1994]. Comparative cytochemical studies manifest species-dependent variations in the expression and distribution of sugar moieties throughout the ZP. Thus, the organization of the ZP in distinct layers, which have been demonstrated by microscopic studies in various species, appears to be accompanied by a spatial distribution of defined sugar structures throughout the width of the ZP [Shalgi et al., 1991]. The lectin-binding pattern in different species is highly specific. In rodents, this pattern varies only in the expression of ligand sites for α-galactose and/or β-N-acetylgalactosamine-recognizing lectins. Interestingly, binding of these lectins is not detectable in the ZP of the human, dog, cat, and pig. In general, an increasing variation of lectin-binding pattern correlates with evolutionary distance of species [Shalgi and Raz, 1997]. Some carbohydrate structures are found in all species examined. These are preferentially sugar residues such as mannose and N-acetylglucosamine, which are usually part of the core region of N-linked oligosaccharides, structural depictions for N-glycans being given in this issue by Geyer and Geyer [1998]. The lectin histochemical studies demonstrate that most variations appear to affect the terminal nonreducing region of the ZP sugar chains supporting the idea that even small structural differences may contribute to the establishment of the species-specific nature of gamete interaction.

All ZP glycoproteins studied so far posses both N-linked and O-linked oligosaccharides [Noguchi and Nakano, 1992; Wassarman, 1992; Hokke et al., 1993; Nagdas et al., 1994]. Galactosyl (Gal) in α-linkage was found on the O-linked oligosaccharide chain of mouse ZP3 [Bleil and Wassarman, 1988], as well as N-acetylglucosaminyl (GlcNAc) residues [Miller et al., 1992]. Mouse ZP2 and ZP3 also possess N-linked high-mannose hybrid oligosaccharide chains [Tulsiani et al., 1992], N-linked polyl-N-acetyllactosamine glycans and O-linked trisaccharide with the structure GlcNAc-β1,3Galβ1,3GalNAcol [Nagdas et al., 1994]. In addition, N-linked oligosaccharides of mouse ZP2 and ZP3 are mainly fucosylated with tri- and tetra-antennary complex-type chains with a similar structure [Noguchi and Nakano, 1992].

Due to the development of methods for large scale isolation of ZP from pig ovaries the structure of the ZP oligosaccharides has been thoroughly investigated in this species. Porcine ZP glycoproteins can be resolved electrophoretically into two components, with molecular masses of about 55 and 90 kDa [Hedrick and Wardrip, 1987]. The 55-kDa component, representing about 80% of the total protein content of the porcine ZP, consists of two distinct polypeptides, referred to as pZPB and pZPC. Sperm receptor activity has been correlated to pZPB, whereas pZPC is the homologue of the mouse ZP3 protein, a member of the ZPC family. Both proteins can be separated only after partial deglycosylation with endo-β-galactosidase [Sacco et al., 1989]. According to the deduced cDNA-encoded amino acid sequence, each of the proteins may possess up to five potential N-glycosylation sites [Harris et al., 1994]. Carbohydrate composition analysis, however, suggested that the pZPB and pZPC proteins contain three or four N-linked oligosaccharide chains and additionally three (pZPB) and six (pZPC) O-linked glycan chains [Yurewicz et al., 1992].

The N-linked oligosaccharides, released by hydrazinolysis from the 55-kDa family of porcine ZP glycoproteins, were separated into neutral (28%) and acidic (72%) carbohydrate chains by anion exchange HPLC [Noguchi et al., 1992]. Further details on methodological aspects of glycoconjugate analysis and its strategy are outlined in this issue by Geyer and Geyer [1998]. A competition assay revealed that the mixture of neutral chains acts as sperm receptor, while acidic chains have no activity. More than 30 different structures of N-linked oligosaccharides have been identified in the neutral fraction. The most abundant neutral structure is a biantennary oligosaccharide which is commonly found in other glycoproteins. Some structures lack terminal nonreducing galactose residue at the α1,3- or man α1,6-arms of the trimannosyl core or they contain linear N-acetyllactosamine repeats in their outer chains [Mori et al., 1989; Noguchi and Nakano, 1992]. The acidic N-linked sugar chains of the 55-kDa components occur as di-, tri-, and tetra-antennary structures. They can be sialylated and/or, as in most cases, sulfated at the poly-N-acetyllactosamine units. The major acidic nonsulfated components are sialylated (with Neu5Gc/Ac residues), biantennary and 4,2-branched, triantennary acidic chains. The sulfate groups are exclusively 1,6-linked to the N-acetylglucosamine residues of the N-acetyllactosamine repeats [Noguchi and Nakano, 1992].

The acidic poly-N-acetyllactosamine backbone of the O-linked oligosaccharides exhibits the same structure as in the N-linked glycans extending from the Galβ1,3GalNAc disaccharide core. Interestingly, the longer chains predominantly terminate with Neu5Gc/Ac residues, which are α2,3-linked to a galactose residue of the outermost lactosamine unit. Shorter chains with no or only one N-acetyllactosamine unit are also α2,6-sialylated at the proximal N-acetylgalactosamine residue. Neutral structures with α-galactose and β-N-acetylglucosamine residues and minor components with α-galactose and β-N-acetylgalactosamine residues at their nonreducing termini have also been identified [Hirano et al., 1993; Hokke et al., 1993, 1994; Sinowatz et al., 1997]. The amount of sulfated N-acetlyllactosamine repeats and the degree of sialylation contribute to the enormous heterogeneity of the carbohydrate portion of the ZP matrix. More than 30 different O-linked structures have been identified in porcine ZP glycoproteins and the diversity of the acidic N-linked structures appears to be even larger.

Carbohydrate Residues of the ZP as Sperm Receptor

Several forms of evidence refer to different carbohydrate residues present on the ZP as key molecules in the process of sperm-egg interaction. In mouse and pig, the sperm receptor activity has been attributed mainly to a certain class of O-linked oligosaccharides [Florman and Wassarman, 1985; Yurewicz et al., 1991]. At present, this interpretation is not easily reconcilable with the possibility of a contribution of neutral N-linked carbohydrates in the binding event [Noguchi et al., 1992; Töpfer-Petersen et al., 1995a]. In both species, the clustering of the biologically active carbohydrate moieties appears to be necessary to achieve high affinity binding. In the mouse, the active sperm receptor region has been mapped to the C-terminal 28 kDa peptide of mZP3 [Rosiere and Wassarman, 1992]. In the pig, trypsin digestion of isolated pZPB and pZPC glycoproteins produced a single O-glycosylated domain from each glycoprotein containing three to five O-linked glycosylations sites, respectively. The O-glycosylated domain of the pZPB protein only retained sperm receptor activity, indicating that both ZP glycoproteins may have a different O-glycosylation pattern. The poly-N-acetyllactosamine units, however, do not appear to play a role in sperm binding [Yurewicz et al., 1992]. The amino acid sequences of the O-glycosylated regions are highly conserved within the ZPB and ZPC protein families.

In species other than the pig it was suggested that various carbohydrates function as sperm receptor. Inhibition assays of sperm ZP binding revealed a role for D-man on human and rat ZP. Pretreatment of human spermatozoa

with D-man inhibited sperm penetration through the ZP [Mori et al., 1989]. In the rat α-methyl mannoside and D-man were the most potent inhibitors [Shalgi et al., 1986]. *L*-Fucose and the sulfate algal fucan fucoidin were shown to be involved in sperm ZP recognition in guinea pig, hamster, rat and human oocytes [Huang et al., 1982; Shalgi et al., 1986].

Sperm-Associated ZP and Carbohydrate Binding Proteins

Several criteria have to be met by sperm proteins to qualify them as candidates for zona binding proteins [Snell and White, 1996].

(1) ZP binding proteins should be on the surface of live, acrosome-intact sperm and they should bind to ZP. (2) ZP binding proteins should display species specificity in binding; antibodies to them should interfere with binding. (3) The inactivation of ZP glycoproteins that accompanies the major block to polyspermy should prevent binding of sperm to ZP of fertilized oocytes. (4) Confirmation as a bona fide ZP binding protein should involve a combination of gene disruption and gene transfer experiments.

Sperm proteins with affinity for ZP glycoproteins have been identified in a number of mammalian species by (1) incubating Western blots of sperm proteins with labeled ZP proteins, (2) by inhibiting sperm-zona binding with the help of specific polyclonal or monoclonal antisera, or (3) by using cross-linking reagents. Moreover, fluorescent neoglycoproteins are versatile tools to assess the capacity of spermatozoa to bind to carbohydrate ligands [Sinowatz et al., 1988, 1989]. However, only some sperm proteins have been characterized biochemically or structurally. Furthermore, to complicate the matter, carbohydrate-binding ability has been demonstrated only in few cases. Therefore, considering the wide variety of putative ZP-binding molecules identified even in a single species, the assumption is reasonable that fertilization is most likely mediated by multiple complementary receptor-ligand systems [Sinowatz et al., 1997].

Classification of the actual sperm lectins acting as primary gamete adhesion molecules is still in its infancy. Moreover, little is known about the type of binding (carbohydrate-protein or protein-protein), the actual hierarchy of interactions, and cross-talking mechanisms between different zona binding proteins, which may collectively govern the species specificity of gamete recognition. Several recent reviews have described extensively the various zona-binding proteins [Benoff, 1997; Shalgi and Raz, 1997; Sinowatz et al., 1997]. In the following the most likely candidates of putative zona binding proteins which use carbohydrate-protein interactions for their binding will be summarized.

To date only few proteins have emerged as candidate ZP adhesion molecules on acrosome-intact spermatozoa. The most thoroughly characterized sperm surface proteins known to have specific ZP and carbohydrate-binding sites are the mouse β1,4-galactosyltransferase (GalTase) [Miller et al., 1992] and sp56 [Bookbinder et al., 1995], the rabbit protein Sp17 [Richardson et al., 1994; Yamasaki et al., 1995], a human mannose-binding protein [Benoff, 1993] and several members of the spermadhesin family [Calvete et al., 1995; Töpfer-Petersen et al., 1995b]. Mouse GalTase, rabbit Sp17, and human mannose-binding protein are integral membrane proteins of spermatozoa [Miller et al., 1992; Benoff, 1993], whereas mouse sp56 and the spermadhesins are peripherally associated proteins which are at least partly derived from the male genital duct system and accessory sex glands [Cheng et al., 1994; Dostàlovà et al., 1994; Sinowatz et al., 1995b].

Mouse Sperm GalTase

The GalTase has been found on all mammalian sperm tested so far. It appears to be confined to the plasma membrane [Miller et al., 1992]. GalTase has been reported to mediate sperm egg recognition in mice by binding to GlcNAc residues present on ZP glycoconjugates. The following facts support a functional role for GalTase [Snell and White, 1996]. (1) Sperm GalTase is found on the surface of live sperm. It selectively interacts with mouse ZP3, as documented by its ability to transfer ^{3}H-galactose to ZP3 exclusively but not to the other mouse ZP proteins ZP1 and ZP2. (2) Purified GalTase, inhibition of GalTase, and antibodies to GalTase can block sperm egg binding [Shur and Neely, 1988]. (3) Overexpression of sperm surface GalTase in transgenic mice leads to production of sperm with an increased binding capacity for ZP3 [Youakim et al., 1994]. (4) Modifications of the GalTase binding site on ZP3 by an N-acetylglucosaminidase (released from the oocyte cortical granules upon oocyte activation) account for the absence of sperm binding activity of the fertilized oocyte and the early embryo.

Participation of GalTase in sperm binding to the ZP is a matter of vivid debate since a set of oligosaccharides terminating in galactose, either in α- or β-linkage, has been shown to inhibit binding of mouse sperm to oocyte, whereas oligosaccharides with β-N-acetylglucosamine at the nonreducing end did not significantly affect the number of

sperm bound to oocytes [Litscher et al., 1995]. Although this result does not rule out a biological role for GlcNAc residues or other sugar residues as well, it provides further support for the proposal that galactose at the nonreducing end of ZP O-linked oligosaccharide(s) may play an essential role in sperm binding [Bleil and Wassarman, 1988].

Mouse sp56

A second candidate for a zona binding protein in the mouse is sp56 [Bleil and Wassarman, 1990]. This 56-kDa protein was localized at the head of acrosome-intact but not acrosome-reacted mouse sperm. It was radiolabelled preferentially by the photoactivatable heterobifunctional Denny-Jafee cross-linker covalently linked to purified mouse ZP3. Purified sp56 eluted from size exclusion columns as a homodimer. Immunohistochemical studies demonstrated that it is a peripheral membrane protein located on the sperm head plasma membrane. There sperm binding to the ZP is initiated [Cheng et al., 1994]. More recent studies showed that sp56 is a peripheral membrane protein unique to testis. Its mRNA is exclusively found in spermatids and testicular spermatozoa. A highly related sp56 homologue is present in hamster but not in guinea pig and human [Snell and White, 1996]. Analysis of the deduced amino acid sequence of sp56 indicates that it contains multiple consensus repeats of 60 amino acids termed Sushi domains. Even though sp56 is a lectin that binds oligosaccharides on ZP3 it does not have regions with significant homology to carbohydrate recognition domains of previously characterized lectins [Bookbinder et al., 1995]. It was suggested that sp56 may recognize galactose by a novel mechanism [Snell and White, 1996].

Human Sperm Mannose-Binding Lectin

It has been suggested that mannose ligands on the human ZP are involved in sperm egg recognition [Mori et al., 1989]. Recent studies have provided direct evidence for the presence of receptors for mannose-containing glycoligands on the surface of human spermatozoa subjected to capacitating procedures [Benoff, 1993]. Moreover, a reduced expression of these receptors in specimens failing to fertilize oocytes has been demonstrated by Benoff [1993]. This author has proposed the following model of action of a human sperm mannose binding protein: freshly isolated motile spermatozoa contain a store of mannose-specific receptors in the subplasmalemmal space underlying the rigid, cholesterol-stabilized plasma membrane. Following capacitation the cholesterol content of the plasma membrane falls to <0.001 µmol/10^9 cells and mannose binding sites are translocated to the sperm surface. The receptors concentrate in the plasma membrane overlying the acrosome. Cooperative interaction of multiple carbohydrate recognition domains may lead to high-affinity mannose-ligand binding. The receptors subsequently aggregate and move to the equatorial/postacrosomal segment of the sperm head, activating a mechanism culminating in acrosomal exocytosis.

Human sperm mannose-receptors require calcium ions to bind tightly to mannose containing ligands [Benoff et al., 1993]. Surface expression of mannose receptors appears to be highly correlated to the level of zinc ions in seminal plasma. As transition metal ions support positioning of amino acid residues and thereby regulate carbohydrate binding without a direct contact to the ligand, e.g. concanavalin A, it was suggested that the sperm mannose receptor is a mannose-binding lectin. The structure of the carbohydrate-binding regions of the human sperm mannose lectin was characterized by Benoff [1993, 1997] using monospecific antibodies and by cDNA sequence analysis. The putative zona acceptor of the lectin shows the amino acid sequence and ligand-binding characteristics of the conserved carbohydrate recognition domain common to a family of membrane-bound and soluble mannose-specific lectins [Taylor and Drickamer, 1993; Drickamer, 1997; Gabius, 1997]. Results on the structure of the human sperm mannose-binding lectin also provide a basis to speculate on its role in sperm-zona binding. Sperm mannose-binding lectin, as other C-type lectins, may recognize terminal sugars as well as those within the core of the oligosaccharide. This implies that in human sperm zona-binding mannose residues do not need to be in a terminal position on the oligosaccharides of the zona glycoproteins. Mannose residues of their core region could also play an important role in species-specific primary binding of human gametes.

Spermadhesins

Spermadhesins are a group of 12- to 16-kDa polypeptides found in the seminal plasma and/or associated with the spermatozoal surface of several mammalian species, i.e. human, pig, bull, horse, and dog [Calvete et al., 1995; Töpfer-Petersen et al., 1995a,b]. They appear to be multifunctional proteins in that they exhibit lectin-like binding of carbohydrates, heparin-binding activity and serine protease inhibitor-binding capabilities [Sanz et al., 1992; Calvete et al., 1995].

Accumulating evidence indicates that members of the spermadhesin family might play a role in sperm capacitation and initial gamete interaction [Dostàlovà et al., 1995a, b].

Boar spermadhesins AQN-1, AQN-3, PSP-I, PSP-II, AWN-1, and AWN-2 are major secretory products of the seminal vesicle epithelium. Their concentration in the seminal fluid ranges from 0.6 to 7 mg/ml [Dostàlovà et al., 1994]. AWN-1 is also synthesized by the cells of the rete testis and the tubuli recti [Sinowatz et al., 1995b]. It is the only member of its family present on epididymal sperm. Following ejaculation, 12–60 million molecules of each AQN-1, PSP-I, AQN-2, AQN-3, AWN-1 and AWN-2 are coated to the apical third of the acrosomal cap. As a result of their interaction with phosphorylethanolamine, a major phospholipid of the sperm plasma membrane, the absorbed molecules are retained to the sperm surface. Most of the coating material is released during in vitro capacitation. The same may be true in vivo. Remaining spermadhesin molecules on capacitated sperm may act as primary ZP binding proteins. Whereas AQN proteins may play a role in secondary binding of acrosome reacted spermatozoa to ZP2, AWN-1 molecules could be involved in primary sperm binding. AWN-1 binds to a minor fraction of O-linked carbohydrate chains of porcine ZP glycoproteins [Dostalova et al., 1995a, b]. The basic structure of the preferred ligand is Gal-GalNAc^{+}-NeuAc. Whether AWN-1 functions merely to anchor spermatozoa to the ZP as a prerequisite to enable other sperm membrane proteins to find their ligands, or whether AWN-1 also participates in coupling sperm binding to the acrosome reaction-induced mechanisms remains to be established.

Purified stallion seminal plasma AWN, like its porcine homologue, binds to isolated equine zonae pellucidae. This result and the fact that frozen stallion epididymal spermatozoa possess fertilizing capacity [Barler and Gandier, 1957] suggest that spermadhesin AWN may be one of the factors contributing to the reproductive ability of horse epididymal sperm. Stallion and boar AWN molecules differ only in three amino acids. Since perissodactyls (i.e. horse) and artiodactyls (i.e. pig) have a common ancestry over 50 million years ago, the unusually low mutational rate might suggest that the AWN structure is under a strong selective pressure. This indicates a highly conserved conformation and most probably a biological function of spermadhesin AWN in both species.

The carbohydrate-binding site of spermadhesin AWN-1 and AQN-3 has tentatively been located around a conserved asparagine residue at position 50 [Calvete et al., 1993a, b]. The amino acid sequence of spermadhesins, however, does not show any discernible similarity with known carbohydrate recognition domains, indicating that spermadhesins may belong to a novel group of animal lectins. A sequence pattern-search analysis revealed that spermadhesins belong to a family of 16 functionally diverse proteins, many of which are known to be involved in developmental processes [Bork and Beckmann, 1993]. The structure of these proteins is built by a combination of several modules and all of them share the CUB domain, whose presence in a heterodimer of two spermadhesins has been verified by X-ray analysis [Romero et al., 1997].

Conclusion

Numerous studies of the last years have revealed a plethora of potential zona binding molecules in different species. It has to be taken in account that sperm-egg or sperm-zona binding is an operational definition that varies between laboratories. In future it needs to be worked out which of these potential candidates for gamete binding eventually will turn out to be of major or exclusive importance for the binding mechanism. It has to be kept in mind that a redundance of several zona binding proteins in a species would be plausible to ensure that the most important intercellular binding event for a species, i.e. fertilization, occurs without error.

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