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Morphology of Mammalian Sperm Membranes During Differentiation, Maturation, and Capacitation

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Abstract

The mammalian spermatozoon is a highly polarized cell whose surface membrane can be divided into five functionally, structurally, and biochemically distinct domains. These domains are formed during spermatogenesis, continue to be modified during passage through the epididymis, and are further refined in the female reproductive tract. The integrity of these domains appears to be necessary for the sperm to perform its function—fusion with the egg and subsequent fertilization. The domains can be identified morphologically by their surface contours and texture, the content, distribution, and organization of intramembranous particles after freeze-fracture, and by the density of surface and cytoplasmic electron-dense coatings in thin sections. By using a variety of labels that stain carbohydrates (lectins), lipids (filipin and polymyxin B), and monoclonal antibodies to specific membrane constituents, the biochemical composition of these contiguous membrane regions has also been partly elucidated. We review here what is known about the structure, composition, and behavior of each membrane domain in the mature sperm and include some information regarding domain formation during spermatogenesis. The sperm is an excellent model system to study the creation and maintenance of cell polarity, granule exocytosis, and fertilization. Hopefully this review will provide impetus for future studies aimed more directly at addressing the relationship of its morphology to its functions.

Keywords

Mammalian spermatozoon; Surface membrane; Spermatogenesis

INTRODUCTION

The morphologically mature mammalian sperm is constructed with an apparent minimal amount of accoutrements with which to perform its function, i.e., swim to the egg, recognize it, penetrate its investitures, and fuse with its membrane, thus introducing the male pronucleus into the egg cytoplasm to join that of the female (Eddy, 1988; Fawcett, 1975; Yanigimachi, 1988a,b). Its surface can be divided into domains, each of which plays a separate and specific part in this series of events which leads to fertilization (Aguas and Pinto da Silva, 1983; Cowan et al., 1987; Friend, 1984; Holt, 1984; Kan and Pinto da Silva,

1987; Koehler, 1985; Primakoff and Myles, 1983; Villarroya and Scholler, 1986; Yanagimachi, 1988b). These membrane domains originate during the elongation of round cells (spermatids) (Figs. 1–5) and appear to be conserved from one species to another. These five domains are anterior head (acrosomal segment), equatorial segment, posterior head (postacrosomal segment), midpiece, and principal piece (Fawcett, 1975). Each of these contiguous domains is separated by a structural and functional barrier, and each has unique structural and biochemical characteristics that must contribute to its physiology, although only in rare instances has a direct correlation yet been made. The head's equatorial segment, which marks the caudal extent of the acrosome and is the initial site of sperm-egg membrane fusion, is itself a boundary between the anterior and posterior head. A striated ring separates the posterior head from the tail's respiratory midpiece, which contains circumferentially stacked mitochondria. An annulus separates the midpiece from the fibrous sheath-encased flagellum (principal piece).

After the mammalian sperm achieves its terminal form and leaves the seminiferous tubule, it still must undergo a series of physiological changes in order to become capable of fertilizing an egg (Yanagimachi, 1988a). These changes begin in the epididymis (sperm maturation) and continue in the secretions of the female reproductive tract or in vitro (capacitation). These functional changes are often accompanied by alterations in the structure of the plasma membrane, which can be observed by electron microscopy of freeze-fracture and surface replicas, and with the use of lectins or sperm-specific antibodies in conjunction with replicas and thin sections, or with the use of the immunofluorescence microscope.

Morphological aspects of capacitation have been extensively observed and described, although the biochemical basis for these changes remains mysterious and their physiological significance can only be inferred (Peterson et al., 1987). Signs of capacitation occur in each of the five domains separately as each prepares to participate in the steps leading to fertilization. Some of these are decrease in thickness of the surface coating of the anterior head (Friend et al., 1977), relocation of sperm surface antigens from one plasma membrane domain to another or from the surface of the intact sperm head to the surface of the inner acrosomal membrane after the acrosome reaction (Flaherty and Olson, 1988; Myles and Primakoff, 1984; Myles et al., 1987; Phelps and Myles, 1987; Saxena et al., 1986), and increases in intramembranous particles in the post-acrosomal membrane as it prepares for sperm-egg membrane fusion (Toshimori et al., 1985). Other structures, such as the zipper in the principal piece of the tail, remain unchanged (Enders et al., 1983).

At the end of epididymal maturation and capacitation, sperm can undergo the acrosome reaction in the presence of calcium (Fléchon et al., 1986; Garbers, 1988; Yanagimachi and Usui, 1974), during which event the outer portion of the acrosomal granule membrane fuses with the anterior head portion of the plasma membrane and exocytoses the granule contents (Figs. 6–9). The acrosomal and plasma membranes become confluent in the equatorial region. After the acrosome reaction, the speed of the tail movements increases and there is a change in beat pattern; nucleotide hydrolysis also increases accompanied by an increased respiratory rate. Only after the acrosome reaction can the sperm plasma membrane fuse with that of the egg (Yanagimachi, 1988b).

Recently, direct correlation between certain membrane changes and biochemically identified molecules has become possible through the advent of immunogold labeling of fractured or thin-sectioned material, and by the use of ultrathin frozen sections for post-embedding staining. The use of biochemical techniques that select for specific sperm functions, such as the receptor for the zona pellucida sperm-binding glycoprotein, coupled with ultrastructural observations using immunogold labeling, may make it possible to approach such questions as where these strategic molecules come from, how they appear in the sperm plasma membrane, how they are marshalled into domains, and how these domains are reorganized during capacitation and following the acrosome reaction. Particularly interesting questions include the timing of the plasma membrane domain establishment in relation to nuclear condensation and the migration of nuclear pores (Figs. 1, 3), how membrane components are segregated during spermatogenesis, and what the role of cytoskeleton is in establishing or modulating domains.

This paper will concentrate on selected aspects of the morphology of each of these five domains of the mammalian sperm plasma membrane and outline what is known about the creation of these structures, and how they change during maturation, capacitation, and the acrosome reaction. We have organized the figures to show the chronological sequence of differentiation, maturation, capacitation, and the acrosome reaction. We have arranged the discussion into sections that pertain to this entire sequence of events but each of the five domains is discussed separately.

THE PLASMA MEMBRANE ACROSOMAL SEGMENT (ANTERIOR HEAD)

In describing the structure of this membrane, it is good to keep in mind that its two main functions are to bind to the zona pellucida and to fuse with the underlying acrosomal granule, thus permitting the exocytosis of the granule contents and the presentation of the inner portion of the granule membrane as a new surface (Fig. 9). The acrosomal segment of the plasma membrane overlies the acrosomal granule and is shaped with it during late spermatogenesis (Figs. 2, 3, 5, 6). The granule itself is created in a manner analogous to the formation of pancreatic zymogen granules, by the successive fusion of multiple vesicles derived from the Golgi apparatus, some of which are clathrin coated (Pelletier and Friend, 1983) (Figs. 1–3). As the large, single granule enlarges, the overlying plasma membrane acquires its mature contours. In the final stages of spermiogenesis, the Golgi apparatus migrates to the midpiece and is shed along with the endoplasmic reticulum in the cytoplasmic droplet, which is sloughed from the midpiece after nuclear condensation. Hence, the mature sperm has no mechanism for synthesizing either secretory or membrane proteins (Fawcett, 1975). However, further modifications of this membrane accompany both passage through the epididymis (Fleuchter et al., 1988; Suzuki and Yanagimachi, 1986; Toshimori et al., 1987; Voglmayr and Sawyer, 1986) and capacitation (Aguas and Pinto da Silva, 1989; Friend, 1984; Friend et al., 1977; Myles et al., 1987; Okabe et al., 1986; Saxena et al., 1986) either in the female reproductive tract or in vitro. Some of these modifications are the appearance in the epididymis and disappearance in the female reproductive tract of a surface coat (the quilt) readily observed in electron microscopy of tannic acid-stained thin sections, and as a hexagonal array in replicas in the fracture plane of freeze-fractured guinea pig sperm (Figs. 5, 7). This coating is also visible in surface replicas and can be removed by

either trypsin or neuraminidase to reveal the true membrane surface beneath (Bearer and Friend, 1982). When cauda epididymal sperm are treated with filipin (a sterol-binding polyene antibiotic), filipin-sterol complexes, visualized as 20-nm bumps in replicas, form over the acrosomal cap, excluded only from the membrane regions in which the hexagonal array is imprinted (Elias et al., 1979; Friend, 1984). This suggests either that there are microdomains within the expanse of the acrosomal segment, or that the filipin is excluded or prevented from forming its diagnostic membrane deformations by the quilt-like coating. The acrosomal cap portion of the guinea pig sperm plasma membrane does not have a discernable cytoskeletal undercoat stabilizing it in this region.

Whether the extracellular coat is added during passage through the epididymis or arises exclusively during testicular development and is reorganized to acquire its distinctive hexagonal pattern is unclear. However, if it is adorned during epididymal passage, it must originate from an exogenous source since epididymal spermatozoon would no longer have the necessary machinery for de novo synthesis. The possibility that epididymal fluid provides the molecules that constitute the quilt is supported by studies using lectins, cationic ferritin, and surface-specific monoclonal antibodies, which have shown that the sperm increases its negative surface charge and adds to its composition as it passes through the epididymis (reviewed in Yanagimachi, 1988a). Thus, it appears likely that there are additions to the sperm surface after testicular differentiation. Whether any of these cytochemically identified molecules are components of the quilt in the guinea pig, or indeed, if they play any role in its appearance, are open questions. In mouse, where no quilt pattern has been described, numerous membrane-bound vesicles adhere to the head of the sperm as they pass along the epididymis, while guinea pig sperm adhere to each other during passage. This adherent substance or vesicular material is apparently lost when sperm swim individually in the female reproductive tract. Again, direct correlation between the glycocalyx, specific antigens, and sperm-sperm adherence has not yet been made. However, taken together, these observations show that the sperm surface membrane is modified in function and composition as well as in structure during epididymal passage.

During capacitation in guinea pig sperm, the glycocalyx is lost from the acrosomal cap, as evidenced by a smoother surface appearance in both surface replicas and thin sections and by changes in lectin- and cationic ferritin-binding properties (Cross and Overstreet, 1987). In support of its association with the external glycocalyx, the imprint of the quilt pattern observed in freeze-fracture replicas also disappears from the fracture face of the membrane, and a compensatory expansion of filipin-sterol complexes throughout the acrosomal cap occurs (Friend, 1984). Finally, the acrosomal cap membrane becomes sensitive to Polymixin B, a polypeptide antibiotic that reacts with membranes exposing anionic lipid in the outer leaflet (Bearer and Friend, 1982), suggesting either a change in total membrane lipid content or a change in partitioning of anionic lipid between the bilayers. A measurable change in diffusion coefficients of lipid analogs accompanies these structural and compositional changes (Wolf, 1987; Wolf et al., 1986) as well as changes in the diffusion of Fab fragments of monoclonal antibodies to sperm surface components (Myles et al., 1981; Primakoff and Myles, 1983; Saxena et al., 1986).

Physiologically, capacitation ends with a dramatically increased “fusability” of the plasma membrane in the presence of calcium. In mouse, it has been demonstrated that loss of acrosomal surface antigen is a necessary prerequisite to the acrosome reaction (Okabe et al., 1986), and in ram, Voglmayr and Sawyer (1986) have shown that particular surface glycoproteins are lost while others are gained during incubation of ejaculated sperm in uterine or oviductal fluid. Hence, further modification of the sperm membrane with respect to structure, function, and composition appears to be an important part of both in vivo and in vitro capacitation. Again, correlation between the molecules gained and lost during this final step of sperm maturation, changes in the surface structure, and the functional changes necessary for granule exocytosis have not yet been fully made. However, the suggestion has been advanced, but not proven, that biochemical changes such as changes in lipid composition/distribution and the loss or gain of surface glycoproteins result in an increase in the permeability of the membrane to calcium by mediating conformational changes in a voltage-sensitive calcium channel (Shapiro, 1987). In mouse, an 83-kD glycoprotein, one of the three glycoproteins that constitute the zona pellucida, has been shown to mediate both sperm adherence to the zona as well as the acrosome reaction (Wasserman, 1988). Identification of the sperm receptor for this glycoprotein, probably also localized structurally to the acrosomal cap region of the plasma membrane, may help to elucidate the relationship between loss of the glycocalyx and changes in lipids and glycoprotein composition with the functional parameters of zona binding and acrosomal granule exocytosis. A zona binding protein (ZBP) recognized by a rabbit anti-sperm autoantibody has been isolated from rabbit sperm membranes and shown to bind to an 87-kD protein from the zona pellucida of rabbit eggs (O’Rand et al., 1988). This antigen is localized over the acrosomal cap of rabbit sperm (O’Rand and Romrell, 1981). The 87-kD zona pellucida constituent may be analogous to the mouse 83-kD zona pellucida glycoprotein, which mediates sperm binding and induces the acrosome reaction in capacitated sperm.

THE EQUATORIAL SEGMENT

Although the major function of the equatorial segment is to provide a membrane capable of fusing externally with the egg plasma membrane, it also appears to play the major role of a barrier to diffusion of membrane constituents of the acrosomal and post acrosomal regions of the plasma membrane which lie on either side of it (Figs. 4, 5, 8). The equatorial segment is not capable of fusing with the egg membrane prior to the acrosome reaction, but little structural change has been noted preceding that process except for the observation of circular clearings in freeze-fracture replicas, which are devoid of intramembranous particles, poor in sterols (Friend, 1984; Toshimori et al., 1985, 1987), and depleted in anionic lipid (Bearer and Friend, 1982). Such changes are not regularly observed in non-cryoprotected, rapidly frozen sperm samples. When seen, these clearings also correlate in size to fenestrations observed in the surface coat overlying the posterior head portion of the plasma membrane and in the densely packed filament system beneath it, the postacrosomal sheath, which adheres tightly to that portion of the membrane (Olson et al., 1987). It is intriguing to continue speculation that these clearings predict sites of membrane-membrane fusion.

Recently, a sperm-surface specific monoclonal antibody has been isolated and shown to recognize a PI-anchored antigen on the posterior portion of the guinea pig sperm head

(Phelps et al., 1988). Fab fragments of this antibody prevent fertilization. Hence, a sperm surface antigen present in this portion of the head appears to mediate fusion by way of the external plasma membrane leaflets of two adjacent cells. Immunofluorescent localization of this antigen does not show it to be localized in submicroscopic circular clearings. Guinea pigs immunized with the sperm head protein PH-2 indeed experience fully effective contraception (Primakoff et al., 1988). A mouse sperm-specific monoclonal antibody that binds to the equatorial segment of all mammalian species tested also apparently blocks the membrane fusion step of fertilization (Saling et al., 1983).

While some of the components of the acrosomal segment of the plasma membrane change during epididymal passage and capacitation, others remain the same. Furthermore, loss of components does not appear to be mediated by their free diffusion from this part of the membrane to contiguous regions. In addition, components specifically localized in the adjacent post acrosomal segment do not appear to flow up into the acrosomal membrane and mix with its inhabitants. How is the topographic integrity of the plasma membrane maintained? Apparently, surface coatings can be lost without relieving the constraint to diffusion of the more permanent membrane molecules (Bearer and Friend, 1982; Primakoff and Myles, 1983). In addition, different components of the membrane have different diffusional coefficients—arguing against a lipid phase model's reducing diffusion rates, and keeping molecules in place (Wolf, 1987). Some appear to move very rapidly and thus, slow diffusion rates cannot explain their localization (Myles et al., 1984). Although all three major cytoskeletal proteins have been identified in sperm—actin, tubulin, and intermediate filaments (Olson et al., 1987; Virtanen et al., 1984)—none have been found to underlie the acrosomal cap, although a filamentous structure has been described in the mouse (Olson et al., 1987). Antivimentin antibodies stain the equatorial segment—the boundary between the acrosomal cap and the post acrosomal portion of the plasma membrane—and filaments suggestive of intermediate filaments have been observed there (Friend, 1989; Virtanen et al., 1984). Hence, the integrity of the acrosomal segment membrane composition might be maintained by either attachment of membrane components to the filaments underlying the membrane whose protein composition is as yet unknown, or by a barrier to diffusion created by an intermediate filament ring attached to the plasma membrane at the equatorial segment and perhaps tacking it down to the nuclear membrane beneath. Beneath the post acrosomal membrane is a sheath of tightly packed, 9 nm diameter filaments with a left-handed helix (Fig. 8). In hamster sperm, actin antibodies label this area (Olson et al., 1987). The integrity of the acrosomal portion of the plasma membrane might be maintained only because the post acrosomal membrane is so tightly adherent to this sheath that very little can diffuse in or out of it. However, it is quite likely that all three mechanisms for maintaining the compositional integrity of this membrane—extracellular glycocalyceal attachments, intramembranous lipid phase diffusional parameters, and intracellular cytoskeletal attachments—are operative, each regulating the movements of different components and thus, all acting in concert to produce the mosaic of domains in the head of the sperm.

Although sperm have a highly specialized polarity, which they maintain in the absence of cell-cell junctions, certain analogies to epithelial cells can be made. In the polarized epithelial cell, W.J. Nelson and Veshnock (1987) have shown that spectrin oligomers play a role in localizing the ATPase ion channel to specific regions of the membrane, while other

experiments suggest that cell-cell junctions restrict the diffusion of other membrane constituents (Gumbiner and Louvard, 1985). These junctions are rich in many cytoskeletal components, including intermediate filaments. Hence, the spectrin attachment type of localization may be analogous to the post acrosomal sheath, while the junctional complexes may parallel the intermediate filaments of the equatorial segment. As yet, for all cell systems, the molecular mechanism by which membrane domains are maintained is unknown.

THE MIDPIECE

The midpiece contains the pair of centrioles, from one of which project the flagellar microtubules, a neck rich in intermediate filaments and actin, and the circumferentially arranged mitochondria. It is separated from the head by the striated ring (Fig. 10A, D), a membrane specialization of unknown composition. Many organized quasi-crystalline structures of distinctive morphology have been described in the neck at this junction of nucleus and tail, including the particle array of the implantation fossa, the paracrystalline packing of the clustered nuclear pores, and apparent filamentous attachments between the nuclear envelope and the cytoplasmic connecting piece (Fawcett, 1975; Friend and Heuser, 1981; Holt, 1984; Pelletier and Friend, 1983). The plasma membrane is endowed with several differentiations not found anywhere else, including string-of-pearl-like necklaces of particles that encircle the midpiece over the mitochondria (Fig. 10B). In some sperm, the residuum of the cytoplasm is apparent. This retained cytoplasmic droplet frequently stains with markers otherwise characteristic of head regions and appears to have some of the same composition of that membrane. In most species, it is completely sloughed during maturation. It contains the disintegrated remnants of cytoplasmic organelles and a variety of vesicles whose content is unknown. In the guinea pig, a portion of it, without RER or Golgi apparatus, is retained. Indeed, these vesicles could be the source of surface components added during epididymal maturation or later, or simply vestigial autophagic vacuoles.

THE PRINCIPAL PIECE

The tail contains the motile element of the sperm that allows it to be propelled through the viscous cervical secretions, uterus, and fallopian tube (Fig. 11). Tail movements change after capacitation, becoming more rapid and whip-like. How the microtubules slide synchronously to produce a defined movement that results in forward motility is not completely defined, although the requirement for ATP and calcium is well established. The cytoplasmic and membrane components of the midpiece are sharply limited from the principal piece of the tail by an electron-dense band which appears to extend to the surface and is visible in freeze-fracture replicas and surface replicas as the annulus (Fig. 10C–E). The annulus has appeared to be a consistent barrier, preventing diffusion between membrane proteins of the posterior tail and the midpiece. However, recent studies (Friend, 1986; Myles et al., 1984) using the guinea pig sperm monoclonal antibody PT-1 have shown that this antigen, which is restricted to the posterior tail in mature sperm (Fig. 11D), also flows to the midpiece following capacitation. This suggests that there is a change in the permeability of the annulus as a flow barrier accompanying capacitation, although no structural change has yet been demonstrated. Rapid freezing of unfixed sperm does show a tighter, more orderly

array of circumferential particles in the annulus than is seen in freeze-fracture replicas of fixed cells. A subtle change in the organization of this region, therefore, may have been missed thus far—the slightly disordered state of the particles having been interpreted as its precapacitation architecture. Whether this change in diffusion plays a role in preparing the sperm for the faster tail movements that subsequently occur with the acrosome reaction is unknown. Clearly, the membrane over the tail must be attached to the axonemal complex in some way to prevent it being flung off like a discarded snake skin during the rapid phase of tail movements.

Similarly intriguing structures of the sperm tail principal piece include the zipper, a membrane modification overlying fiber number one (Enders et al., 1983; Friend and Fawcett, 1974; Friend and Heuser, 1981), and a pattern of distinctive intramembranous particles (Fig. 10C) that resemble, but do not function as acetylcholine receptors.

CONCLUSIONS

The mammalian spermatozoon is a streamlined cell with five contiguous individual parts, each of which contributes to a specific step in the process of fertilization. Ultrastructural descriptions of the head of the sperm have disclosed that regions that perform one function are structurally different from regions that perform another function. Biochemical studies using antibodies to surface antigens have shown that these structural and functional domains contain molecules specific to that domain. The next step is to investigate the direct relationship between the presence of a particular antigen, the ultrastructure of that membrane domain, and how that molecule participates in the function of that membrane domain during the series of events that result in fertilization.

While the sperm is highly specialized, much of its behavior is analogous to other cell systems. For instance, exocytosis of secretory granules occurs in a wide variety of cells and, therefore, studies of the acrosome reaction in sperm are useful in elucidating this process in other systems. The sperm has some advantages over other systems in that it is readily obtained in large numbers and in suspension and, hence, is accessible to drug manipulations or cytochemical probes. The vast expanse of the acrosomal cap region of fusible membrane makes this membrane easily monitored at the light microscope level. The triggering of the acrosome reaction may be analogous to other systems of cell-cell recognition, such as neutrophil granule exocytosis and, hence, the enzymatic events involved may also contribute to our understanding of stimulus-response coupling, such as the relationship of receptor binding, GTP hydrolysis, and phospholipase C, the role of protein kinase C as middleman, and the identity of the mysterious second messenger that triggers the exocytotic response.

Another area of inquiry to which the sperm model might contribute significantly is the question of cell polarity—how do cells create and maintain plasma membrane domains of differing composition such that each domain can perform its unique task? Obviously, sperm are capable of maintaining domains in the absence of junctions with other cells and with an apparent paucity of known cytoskeletal proteins. Which molecules mediate the stabilization of membrane constituents in sperm may tell us much about the maintenance of similar domains in polarized epithelia. Significantly, one of the first signs of malignancy is the loss

of structurally recognizable polarity. Therefore, understanding the mechanisms by which cells “know” and remember what is top and what is bottom is also crucial to our understanding of “differentiation.”

The sperm has a major disadvantage as a biological model system in today’s world of fast-paced molecular biology in that it is terminally differentiated and cannot be cultured or directly transformed. With the advent of transgenic mice, however, it has been possible to produce genetically altered sperm (Braun et al., 1989). By transforming with genes under the control of protamine promoters, haploid sperm can be induced to express genes post meiotically. An advantage of such a system is that simple gene copies can be more easily manipulated as in yeast or other haploid organisms. It may also be possible to alter the surface antigens crucial to fertilization to dissect their biochemical behavior in vivo.

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Fig. 1.

The sperm begins as a spermatogonium near the basement membrane of the seminiferous epithelium. Its nucleus has scattered nuclear pores; its cytoplasm, sparse organelles. The spermatogonium undergoes several mitotic and two meiotic divisions to become an early spermatid, bridged by cytoplasmic channels. N, nucleus; PM, plasma membrane. $\times 18,000$.

Fig. 2.

A: As a step 4 spermatid, when the nucleus condenses at its anterior pole, a smooth area underlies the acrosomal cap, as seen in **B**, and the nuclear pores begin to migrate distally. $\times 27,600$. **B:** The acrosome forms from the fusion and coalescence of Golgi-derived vesicles. Ac, acrosome. (From Pelletier and Friend, *Am J Anat* 167:119–141 (1983), by copyright permission from Alan R. Liss, Inc.) $\times 34,000$.

Fig. 3. The Golgi-apparatus (G) continuously adds content to the acrosomal membrane (Ac). The nuclear pores continue to migrate distally toward the tail. Beyond the leading edge of the acrosome development are clusters of intramembranous (pre-pore) particles, arrows. N, nuclear arrows. $\times 49,500$.

Fig. 4.

Upon release from the Sertoli cell, the sperm head is fully shaped. A faint band separates the acrosomal from the postacrosomal segment (PA). During development and later, during epididymal maturation, the number of particles in the postacrosomal segment (PA) increase.

Inset: A diagram of the head membrane domains, seen in freeze fracture. $\times 22,400$.

Fig. 5.

A: Surface replica of a fully mature, non-capacitated guinea-pig sperm head. The acrosomal cap portion of the plasma membrane is actually subdivided into three domains. The central crescent resembles the post acrosomal segment (PA), more with respect to antibody labeling, lectin labeling, and lipid perturbation, than it does the remainder of the acrosomal cap. Several circles are windows through the glycocalceal covering. Arrow indicates the equatorial segment. $\times 11,000$. **B:** Underlying the plasma membrane at the equatorial segment (arrow in A) are slender processes that point to the tip of the head. Their impression is often seen in freeze fractures and surface replicas of the sperm. $\times 59,200$.

Fig. 6.
A: Stacked acrosomes of epididymal sperm in the guinea pig. Stacked sperm are characteristic of several species. $\times 42,000$. **B:** After filipin sterol perturbation of the acrosomal membrane, the area of the acrosome underlying the crescent in the plasma membrane (Fig. 5A) also behaves differently, labeling less than the remainder of the outer acrosomal membrane. $\times 54,000$.

Fig. 7.

A: The acrosomal cap portion of epididymal sperm is characterized by a dense glycocalyx, seen here in thin section as well as in surface replica (Fig. 5A). $\times 80,000$. **B:** Freeze-fracture pattern through the glycocalyx-rich portion of the membrane. $\times 32,000$. **C:** A diagram of the major membranes of the anterior head of the sperm. **D:** Freeze-fracture after filipin perturbation of the acrosomal portion of the plasma membrane, lower right portion of figure, showing the 25-nm filipin sterol perturbation typical of the anterior head portion of this membrane. The post acrosomal segment (PA) of this particular sperm is not perturbed at all by the filipin treatment. N, nuclear envelope. $\times 80,000$.

Fig. 8.

Prior to fertilization, the major event in the life of the sperm is capacitation, the preparedness of the sperm to undergo the exocytic acrosome reaction. After maturation, but before the acrosome reaction, the sperm head maintains functional segregation of the acrosomal and post acrosomal domains. **A:** A thin section through the equatorial segment of the sperm head, showing the close apposition of the plasma membrane to the distal end of the acrosome. $\times 46,000$. **B:** Maclura pomifera lectin bound to 20-nm gold (preparation courtesy of W. Isenberg), showing a sharp demarcation of labeling between the acrosomal and post acrosomal regions. $\times 34,000$. **C:** A mouse monoclonal head antibody of Primakoff and Myles showing a labeling pattern similar to the lectin, only over the acrosomal segment of the plasma membrane. $\times 48,000$. **D:** A freeze-etched replica of the filaments underlying the plasma membrane of the post acrosomal segment. $\times 128,000$.

Fig. 9.

A: Diagram of the equatorial segment of a capacitated epididymal sperm based on freeze-fracture observations. Notice the separation of the plasma membrane (A,B) from the acrosomal membrane (D,E), A: Outer leaflet of the acrosomal membrane. B: Inner leaflet of acrosomal membrane. C: Theoretical cytoskeletal network between plasma and acrosomal membranes. D: Outer leaflet of outer acrosomal membrane. E: Inner leaflet of outer acrosomal membrane. F: Inner leaflet of inner acrosomal membrane. G: Outer leaflet of inner acrosomal membrane. H: Acrosomal content. The nucleus would be beneath (G). **B:** The equatorial region of the sperm head following the acrosome reaction. In the equatorial segment, there is now continuity between the plasma membrane, post acrosomal region, and the inner acrosomal membrane. A: Outer leaflet of the plasma membrane. B: Inner leaflet of plasma membrane now in continuity with F and G, which were the acrosomal membrane previously. C: Represents a change in the putative cytoskeletal elements between the two membranes. The only cytoskeletal protein identified in the guinea pig at this level of the sperm head has been vimentin, the intermediate filament protein. **C:** A sperm head before the acrosome reaction. $\times 28,000$. **D:** Portions of the acrosomal cap, equatorial, and postacrosomal segments of a sperm head after the acrosome reaction (from Shi and Friend, *Biol Reprod* 29:1027–1032 (1983), by copyright permission from the Society for the Study of Reproduction). $\times 34,000$.

Fig. 10.

A: Striated ring separating the head from the tail. Notice the clusters of particles immediately in front of the striated ring. In the guinea pig, each sperm cell is individually marked with different combinations and numbers of clusters in this region. Each species has its own variation on such markings, e.g., the rabbit uses lines. $\times 65,000$. **B:** Chains of intramembranous particles decorate the plasma membrane overlying mitochondria of the midpiece. $\times 80,000$. **C:** Freeze fracture of the annulus separating the midpiece, upper portion, and principal piece lower portion of the tail. These circumferential lines of particles seen here on E face overlie the fibrous plaque seen at this juncture on thin section as in (**E**). $\times 86,000$. **D:** Diagram of the juncture between the sperm head and tail and differentiations in the tail. Upper arrow indicates the position of the striated ring; lower arrow indicates the position of the annulus. **E:** E-face differentiation includes lines of large particles, which resemble previously described ion channels. $\times 67,000$.

Fig. 11.

Like the anterior and posterior parts of the head, the midpiece and principal piece of the sperm tail also differ in morphologic features, lectin binding, and antibody recognition. **A:** Thin section of the guinea pig sperm midpiece (From Friend and Heuser, *Anat Rec* 199:159–175 (1981), copyright permission obtained from Alan R. Liss, Inc.). $\times 110,000$. **B:** *Maclura pomifera* attached to 20-nm gold (preparation courtesy of W. Isenberg) labels the whole sperm tail uniformly. $\times 60,000$. **C:** Ruthenium red staining of the plasma membrane of the principal piece. Ruthenium red also stains the whole tail uniformly. $\times 64,000$. **D:** A 10-nm gold labeled anti-PT1 antibody of Primakoff and Myles. This antibody recognizes an antigenic determinant in the plasma membrane of the principal piece of the tail exclusively. After capacitation, this antibody and its label migrate across the annulus, throughout the

midpiece of the sperm tail, up to the striated ring at the juncture of the head and tail (see Friend, 1989). $\times 70,000$.

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