J. Membrane Biol. 165, 1–10 (1998)

The Journal of
Membrane
Biology

Topical Review

Lipid Heterogeneity and Membrane Fluidity in a Highly Polarized Cell, the Mammalian Spermatozoon

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Received: 17 November 1997/Revised: 9 February 1998

Introduction

The mammalian spermatozoon is a highly polarized cell consisting of two main sections, the head and the tail. These sections are enclosed by a differentiated plasma membrane to give five specialized regions: acrosome (AC), equatorial region (EQ), postacrosome (PA), midpiece (MP) and the principle piece (PP) (Fig. 1) (Bedford & Hoskins, 1990). Each region has a specialized function; its associated membrane has a lipid and protein composition that is tailored to provide a suitable environment for that function (Holt, 1984; Peterson & Russell, 1985). The membrane lipid infrastructure and fluidity develops during maturation to ensure that the genetic material carried by the sperm cell is joined with that of the ovum for fertilization.

Mammalian spermatozoa, upon leaving the testis, pass through the epididymis, where they undergo a process of maturation. The sperm transits from the caput to the cauda via the corpus, during which it undergoes various morphological, physiological and biochemical changes (Bedford & Hoskins, 1990). The plasma membrane especially undergoes considerable reorganization to provide the potential for capacitation (Cooper, 1986; Fraser, 1995; Harrison & Gadella, 1995). The process of capacitation prepares the plasma membrane for the acrosome reaction, and is essential if the spermatozoon is to fertilize an egg (Fraser, 1995).

The acrosome is a membrane limited vesicle that is located in the anterior head region, between the nuclear envelope and the plasma membrane. It consists of an inner and outer membrane, which in the equatorial region, form a stable parallel arrangement. During the acrosome reaction enzymes are released from this vesicle. The outer acrosomal membane fuses with the overlaying plasma membrane of the anterior head region. The hybrid membrane is shed, before the spermatozoon can penetrate the egg. The inner membrane and the equatorial region of the acrosome remain intact after completion of the reaction (Bedford & Hoskins, 1990). Energy for the cell is provided by mitochondria that are located in the midpiece region. The highest concentration of membranous structures is therefore located in the anterior head (AC) and the midpiece regions.

The literature regarding lipid composition and fluidity of mammalian spermatozoan membranes is fragmentary and often species-specific. However, this review summarizes current knowledge of how the membrane organization of the mammalian spermatozoon is determined by its complex mixture of lipids. Where necessary aspects such as the role of the lipids in cryopreservation (Parks & Graham, 1992), peroxidation (Aitken, 1995; Lenzi et al., 1996) and protein regionalization (Cowan & Myles, 1993; Cowan et al., 1997) will be mentioned but have been adequately dealt with by the reviews cited.

Spermatozoan Membranes are Composed of Complex Mixtures of Lipids

Spermatozoan membranes are composed of a heterogeneous mixture of phospholipids, glycolipids and sterols

Key words: Spermatozoa — Lipid composition — Membrane fluidity — Membrane domains

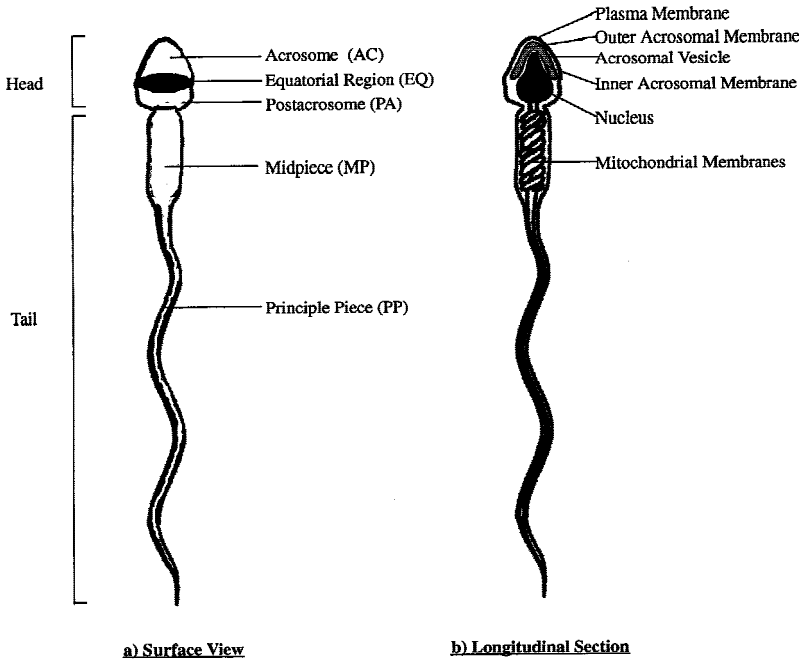


Fig. 1. Diagram showing the different regions of the mammalian spermatozoon (adapted from Alberts et al., 1989).
(*a*) Surface view
(*b*) Longitudinal section

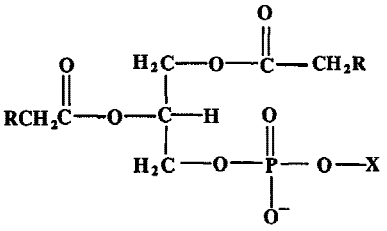
(Parks & Lynch, 1992). Attempts have been made to fractionate subcellular membranes and to define their lipid composition (Parks, Arion & Foote, 1987). The plasma membrane is of special interest because of its direct role in the fertilization process, and consequently has been the focus of several studies. Its lipid composition is similar to that of whole sperm but with differences in distribution (Holt & North, 1985; Hinkovska, Dimitrov & Koumanov, 1986).

The majority of the compositional studies were carried out in the 1970s and 1980s, and Lin et al (1993) is one of only a very few that used modern analytical techniques. Although all these studies give a good insight into the composition of the membrane, variations in extraction and analysis methods make quantitative comparisons between investigations difficult. Information on the precise lipid molecular structures present in spermatozoa is rare, and there is a need for more data in this area using modern, standardized procedures.

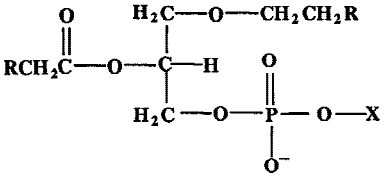
The organization of the lipids in spermatozoan membranes (Cardullo & Wolf, 1990) is much more complex than that suggested by the original Fluid Mosaic Model of membrane structure of Singer & Nicolson, 1972. They envisaged lipids and proteins freely diffusing within a simple bilayer structure. It is now widely recognized that membranes are not homogenous but that lateral asymmetry (Curtain, Gordon & Aloia, 1988; Tocanne et al., 1989, 1994; Glaser, 1993) and transmembrane asymmetry (Devaux, 1993; Zachowski, 1993) are the rule rather than the exception. Lipids and proteins are arranged in a mosaic of domains (Thompson, 1993). These domains represent small-scale heterogeneities in composition, shape and fluidity within the plane of the membrane, over the range of hundreds of nanometers to a few micrometers (Edidin, 1997). They arise from the complex interactions of the heterogeneous mixtures of phospholipids, sterols, and proteins that make up all biological membranes. For example, cholesterol preferentially associates with spingomyelin, and it is proposed that lateral assemblies form platforms that encourage particular cellular events (Simons & Ikonen, 1997; Harder & Simons, 1997). A more detailed explanation of membrane domains can be found in some thorough and excellent reviews presented recently (Thompson, 1993, 1997).

Phospholipids of the Spermatozoan Membrane Have a High Proportion of *sn-1* Ether-Linked Fatty Acyl Groups

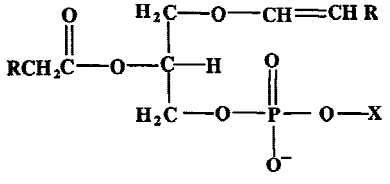
The major phospholipids of the spermatozoan membrane are phosphatidylcholine (PC), phosphatidylethanoline (PE) and sphingomyelin (SPM). Phosphatidylserine (PS), Phosphatidylinositol (PI), phosphatidic acid, diphosphatidyl glycerol (DPG) and lysophospholipids are present as relatively minor components. Analysis of the aliphatic moieties linked to the phospholipids shows that the major saturated fatty acids are palmitic acid (16:0), and stearic acid (18:0). The major polyunsaturated fatty acids are arachidonic acid (20:4, n-6), docosapentanoic (22:5, n-6) and docosahexonic (22:6, n-3) acid (Horrocks & Sharma, 1982; Parks & Lynch, 1992). A high proportion of the saturated fatty acyl moieties are linked via an



1, 2 diacyl phospholipid



1-alkyl-2-acyl phospholipid



1-alk-1'-enyl-2-acyl phospholipid

Fig. 2. Structure of the three subclasses of phospholipids in the spermatozoa membrane.
R = fatty acid group
X = choline or ethanolamine

ether bond in the PC and PE fractions at position 1 of the glycerol moiety (*sn-1* linked). This ether bond can be either an alkylether or an alk-1′-enylether (plasmologen). The *sn-2* position in these ether lipids is predominately occupied by polyunsaturated fatty acids attached via the normal ester bond to the glycerol moiety of the phospholipid (Horrocks & Sharma, 1982). The manor of attachment gives rise to three subclasses of phospholipids in sperm membranes: 1,2 diacyl, 1-alkyl-2-acyl and 1-alk-1′-enyl-2-acyl (Evans, Weaver & Clegg, 1980; Horrocks & Sharma, 1982) (Fig. 2).

Using modern analytical techniques, Lin et al. (1993) investigated the molecular species of phospholipid in rhesus monkey spermatozoa. Using the PE they showed that the different fatty acid moieties linked to this fraction alone gave rise to thirteen molecular species. A remarkable feature of the ether linked PE and PC fractions isolated from spermatozoa of a number of species is that the predominant molecular configuration is the one with 16:0 as the ether linked group at the *sn-1* position and 22:5 (n-6) or 22:6 (n-3) as the ester linked group at the *sn-2* position. The diacyl PC and PE have a more complex fatty acid composition. As a result of the metabolic stability of the ether linkage, ether lipids in the membrane may form stable structural components while the diacyl lipids undergo degradation and resynthesis (Selivonchick et al., 1980; Paltauf, 1994).

Spermatozoa from a number of species have a high proportion of polyunsaturated fatty acids linked to the phospholipid fractions (Parks & Lynch, 1992; human, Lenzi et al., 1996; hamster, Awano, Kawaguchi & Mohri, 1993; ram, Jones & Mann, 1976; Parks & Hammerstedt, 1985; Hinkovska et al., 1986; rat, Agrawal, Magargee & Hammestedt, 1988; Aveldano, Rotstein & Vermouth, 1992; boar, Nikolopoulou, Soucek & Vars, 1985, 1986; bull, Selivonchick et al., 1980; rhesus monkey, Lin et al., 1993). However, in goat spermatozoa the phospholipids have a much larger proportion of saturated fatty acid moieties, with little 22:5 (n-6) or 22:6 (n-3) (Rana et al., 1991); the major unsaturated fatty acids are

18:1 (n-9), 18:2 (n-6) and 20:4 (n-6). In addition, goat epididymal sperm plasma membranes contain wax esters and 1-O-alkyl-2, 3-diacyl glycerols (Rana et al., 1992).

Sphingomyelin from rhesus monkey (Lin et al., 1993), goat (Rana et al., 1991), and boar (Nikolopoulou et al., 1985, 1986; Evans et al., 1980) spermatozoa has a low polyunsaturated fatty acid content. Saturated and monounsaturated fatty acids predominant in SPM. Sphingomyelin forms 10–15% of the total lipids in spermatozoa. Polyenoic very long chain fatty acids (VLCFA -22 carbons or more) have also been found associated with sphingomyelin in ram, bull, rat, boar and human spermatozoa (Poulos et al., 1986, 1987; Robinson, Johnson & Poulos, 1992); the amount increases during maturation to form 5% of the total fatty acid content in a number of mammalian species (Robinson et al., 1992). In addition, a novel molecular species of sphingomyelin containing 2-hydroxylated polyenoic VLCFA has also been isolated (Robinson et al., 1992).

The phospholipids PS and PI comprise approximately 4 and 3% respectively of the total phospholipids. In studies where analysis has been carried out, the fatty acids associated with these lipids are largely 16:0 and 18:0 (Nikolopoulou et al., 1985, 1986; Lin et al., 1993). However, hamster (Awano et al., 1993) and rat (Aveldano et al., 1992) spermatozoa have appreciable amounts (30%) of polyunsaturated fatty acids (20:4 n-6, 22:5 n-6, 22:6 n-3) bound to SPM, PS and PI fractions.

Phospholipids are Asymmetrically Distributed Between the Inner and Outer Leaflets of the Plasma Membrane

In spermatozoa, compositional asymmetry between the outer and inner leaflet of the plasma membrane (transmembrane asymmetry) is well established, with clear species-specific patterns. Compositional analysis reveals that in ram (Hinkovska et al., 1986) and goat (Rana et al., 1993) the choline phospholipids (PC and SPM) are mainly found in the outer leaflet. PS, PE and phosphatidylglycerol (PG) are equally distributed between the two leaflets of the membrane in ram. PE forms the major lipid of the inner leaflet of goat membranes whereas in ram PI and DPG predominate. Transmembrane asymmetry is established and maintained by an ATP-dependent protein, aminophospholipid translocase (Nolan et al., 1995; Muller et al., 1994, 1996). This enzyme rapidly translocates PS and PE from the outer leaflet to the inner leaflet of the membrane. It may be important to the functioning of the leaflets that transmembrane asymmetry provides each side with a different fluidity and domain structure.

For example, from compositional analysis, the phospholipid molecular species that is likely to predominate in the outer leaflet is the *sn-1* ether-linked 16:0/*sn-2* ester-linked 22:6-PC. Molecular modeling of the diacyl equivalent of this phospholipid (*sn-1* ester linked 16:0/*sn-2* ester linked 22:6-PC) reveals that the high degree of unsaturation of the acyl moieties in the *sn-2* position reduces the effective chain length of these moieties and increases the overall cross-sectional area of the phospholipid. The interaction with cholesterol is compromised, thereby reducing the rigidifying effect on the membrane (Stubbs & Smith, 1994). The high degree of unsaturation, the weaker interaction with cholesterol and the larger cross-sectional area all indicative of a highly fluid outer leaflet. The localization of aminophospholipids in the inner leaflet favors the formation of nonbilayer structures (hexagonal II phases) particularly when interacting with calcium (Verkleij, 1984; Quinn, 1989; Allen, Hong & Papahadjopoulos, 1990; Chernomordik, 1996). This may lead to the coexistence of bilayer and nonbilayer structures in the membrane as a whole, which will promote destabilization of the bilayer and facilitate the acrosome reaction.

Sterols are Concentrated in the Acrosomal Region

The second major lipid component of sperm membranes is sterol of which cholesterol is the most abundant found in a variety of species (Parks & Lynch, 1992; boar, Nikolopoulos et al., 1985; goat, Rana et al., 1991; rat, Agrawal et al., 1988). Sterol sulfates and sterol esters form minor components. However, in rhesus monkey spermatozoa desmosterol is the major sterol (59%) and in mature hamster cholesta-7, 24-dien-3β-ol together with desmosterol form the main sterols (48% and 45% respectively) (Lin et al., 1993). The sterol to phospholipid molar ratio varies from 0.21 in hamster (Awano et al., 1993), to 1.0 in human (Darin-Bennett & White, 1977).

Sterol distribution in the membrane has been determined using the polyene antibiotic filipin (Robinson & Karnovsky, 1980). Filipin forms complexes with sterols that can be assayed in freeze fracture replicas. The highest density of these complexes is observed in the plasma membrane covering the acrosome (Friend, 1982; Toshimori, Higashi & Oura, 1985; Tesarik & Flechon, 1986; Suzuki, 1988; Lin & Kan, 1996). The density of these complexes also increases during epididymal maturation in the plasma membrane covering the acrosome. In the hamster sterol is present mainly in the outer leaflet of the PM covering the acrosome (Suzuki, 1988) whereas in the guinea pig (Friend, 1982) and mouse (Lin & Kan, 1996) it resides in the inner leaflet. The distribution of sterol is of considerable importance because its depletion is an important step of capacitation (Lin & Kan, 1996).

A lower cholesterol/phospholipid ratio in human spermatozoa has been shown to correlate with faster capacitation time (Hoshi et al., 1990). This observation may be explained by an increase in membrane fluidity

(Wolf, Scott & Millette, 1986*a*) which facilitates fusion with the underlying outer acrosomal membrane (Ehrenwald, Parks & Foote, 1988*a,b*). The acrosome reaction can be reduced by artificially increasing the levels of cholesterol in the plasma membrane (Cross, 1996) or by dietary manipulation of both serum cholesterol and polyunsaturated fatty acids (Diaz-Fontdevila & Bustos-Obregon 1993). The higher concentration of cholesterol may alter the lipid domain organization and decrease the fluidity of the acrosomal region which would slow down membrane fusion during the acrosome reaction. Similarly, sterol sulfates also inhibit the acrosome reaction by inhibiting proteolytic enzymes involved in the reaction (Roberts, 1987). These sterol sulfates are found in high concentrations in the epididymis, where they are taken up and localized in the plasma membrane overlying the acrosomal and midpiece regions (Langlais et al., 1981). They can be hydrolyzed via sulfatase activity in the female tract to reverse the inhibition, promote the acrosome reaction and enable fusion with the ovum (Roberts, 1987).

Anionic Glycolipid Distribution Changes During Capacitation

Glycolipids form approximately 8% of the total polar lipids. Two glycolipids that have been characterized in spermatozoa are 1-O-alkyl-2-O-acyl-3-β-D-galactosyl-sn-glycerol and 1-O-alkyl-2-O-acyl-3-β-D- (3′-sulfo)-galactosyl-sn-glycerol (SGalAAG) (Ishizuka, Suzuki & Yamakawa, 1973; Murray et al., 1980). The latter is often referred to as seminolipid and is the major glycolipid; it is not found in other mammalian cell membranes. The molecular species that predominates contains 16:0 as both the ether-linked alkyl and ester-linked acyl moieties in the structure (Selivonchick et al., 1980; Nikolopoulou et al., 1985). Using the fluorescent probe polymycin B, the distribution of anionic lipids has been mapped and found to be concentrated in the acrosomal region (Bearer & Friends, 1980; 1982). Since polymycin B is membrane impermeable it only detects anionic lipids in the outer leaflet of the plasma membrane. The probe therefore reflects the surface distribution of SGalAAG which is the most predominant anionic lipid in the outer leaflet (Gadella et al., 1994). Indirect immunofluorescence labeling and detection with digital imaging fluorescence microscopy shows that in freshly ejaculated boar sperm the SGalAAG is primarily located at the apical ridge of the plasma membrane of the sperm head. The possible physiological function of SGalAAG at this location on the membrane is to prevent premature initiation of the acrosome reaction. It may then contribute to the final fusion event between the sperm and the egg when it migrates to the equatorial region during capacitation, as evidenced by experiments with zona coated coverslips (Gadella et al., 1995).

Membrane Fluidity Changes During Epididymal Maturation

Vijayasarathy (1982) first showed that in bull spermatozoa the lipid phase of the plasma membrane is more fluid than the outer acrosomal membrane. This correlates with a threefold higher cholesterol content of the outer acrosomal membrane. The majority of studies since then have attempted to correlate the changes in plasma membrane composition during epididymal maturation with membrane fluidity.

Membrane fluidity properties can be measured by a number of methods, including ^{2}H-NMR, ESR, fluorescence recovery after photobleaching (FRAP) and time-resolved or steady-state fluorescence anisotropy (Stubbs & Smith, 1984). Each method determines a different aspect of membrane organization, although they are all referred to by the general term ‘membrane fluidity’. For example, ^{2}H-NMR, ESR, time-resolved and steady-state fluorescence anisotropy determine rotational motion of lipid in the membrane, whereas FRAP measures lateral motion in the plane of the membrane (Stubbs & Smith, 1984). The distinction between these measurements is important; measurements of rotational diffusion provide information about molecular motion on a molecular length scale; measurements of lateral diffusion provides information about molecular motion on a much larger scale. Thus the latter is sensitive to the presence of barriers to diffusion, which may be present within the membrane and result in domain formation. In addition to direct measurement of membrane fluidity, lipid compositional data is also commonly used to indicate changes in membrane fluidity. The key chemical indices used are sterol/phospholipid, PE/PC, SPM/PC and saturated/unsaturated molar ratios (Shinitzky, 1984; Stubbs & Smith, 1994). An increase in any of these ratios indicates a reduction in fluidity and an elevation of the phase transition temperature.

During epididymal maturation many of these indicators of fluidity change. The total lipid content of spermatozoa decreases markedly and in most species there are specific changes in fatty acids, (bull, Poulos, Voglmayr & White, 1973; boar, Nikolopoulou et al., 1985; ram, Poulos et al., 1975; Hammerstedt et al., 1979; Parks & Hammerstedt, 1985; goat, Rana et al., 1991; rat, Aveldano et al., 1992). There is a reduction in the saturated and/or monounsaturated fatty acids, with a concomitant increase in the proportion of polyunsaturated fatty acid groups. The consequence of all these changes is a lowering of the PE/PC and saturated/unsaturated ratios during maturation, potentially increasing the fluidity of the membrane. In studies that have measured the ether lipid concentrations (1-alkyl-2-acyl and 1-alk-1′-enyl-2-acyl PE and PC) there are marked alterations relative to other phospholipids during maturation. However, in absolute

terms the concentrations remain unchanged or even slightly decrease.

Remodeling of sterols also occurs during maturation, the extent of which is species dependent. In boar the total concentration of sterols remains unchanged, but with a decrease in the cholesterol concentration and an equivalent increase in desmosterol and cholesterol sulfate (Nikolopoulou et al., 1985). There is no change in the sterol-to-phospholipid molar ratio. In ram (Parks & Hammerstedt, 1985) the concentrations of sterols decrease whereas in goat they increase (Rana et al., 1991). In both ram (Parks & Hammerstedt, 1985) and goat (Rana et al., 1991) the sterol and phospholipid remodeling results in an increase in the sterol-to-phospholipid molar ratio.

Dissecting the changes between the inner and outer leaflets of goat spermatozoa plasma membranes during maturation shows that there is a decrease in unsaturated fatty acids in the inner leaflet and an increase in the saturated fatty acids in the outer leaflet (Rana et al., 1993). The marked increase in the two chemical indices of fluidity, cholesterol/phospholipid and saturated/unsaturated molar ratios, does indeed correlate with a significant decrease in measured fluidity of the plasma membrane (Rana & Majumder, 1990). In rat spermatozoa, decreases in the chemical indices of fluidity correlate with an increase in local polarity, fluidity and molecular disordering (Kumar, 1993). The lateral diffusion of mouse spermatozoa plasma membrane lipids changes little but there is a significant increase in the nondiffusing fraction (Wolf, Hagopian & Ishijima, 1986*b*). In ram, however, there is an increase in both the nondiffusing fraction and the lateral diffusion (Wolf & Voglmayr, 1984; Wolf, 1995). The nondiffusing fraction is thought to be due to the presence of gel phase domains within the plasma membrane lipid (Wolf, Lipscomb & Maynard, 1988). In guinea pig spermatozoa there is a decrease in both the nondiffusing fraction and the lateral diffusion (Cowan et al., 1997). In human spermatozoa measurements indicate a high state of fluidity of the lipid domains (Sinha, Kumar & Laloraya, 1994). In contrast, spermatozoa from patients with oligospermia show more rigid lipophilic membranes domains which may be the cause of their infertility.

The Phase Behavior of Lipids May Lead to the Coexistence of Gel and Liquid Crystalline Domains

The phase behavior of the lipids can lead to domain formation (Quinn, 1989; Vaz & Almeida, 1993). Lipids in artificial bilayers undergo phase changes at a temperature (thermotropic phase transition temperature—T_m) determined by the composition of the bilayer. Below T_m the lipids are in the gel phase whereas above they enter a more fluid liquid crystalline phase. The presence of cholesterol tends to mask the phase changes because it acts as a 'buffer' of fluidity, stiffening the membrane at temperatures above T_m and making it more fluid below. In the complex lipid environment of the mammalian membrane, different lipids have a different T_m, and gel and liquid crystalline phases coexist (Wolf, 1994).

Implicit in the transmembrane asymmetry model is the notion that phase transition behavior and fluidities of the outer and inner leaflets are different. Isolation of membranes may result in the loss of mechanisms that maintain the asymmetry. Therefore measurement of these parameters in isolated membranes or extracted lipid components may not reflect the situation in intact spermatozoa and at best will reflect an average of the membrane properties (Devaux, 1993). Nevertheless, from these experiments one can infer the phase behavior of the sperm membrane *in situ.* Using differential scanning calorimetry (DSC), experiments with extracted phospholipids show that major phase transitions are centered around 26°C in a number of species (Wolf et al., 1990; Parks & Lynch, 1992). However, extracted glycolipids show higher phase transitions centered around 35°C. Phase transition as high as 60°C have also been detected and tentatively attributed to the presence of disaturated phospholipids. Fluidity measurements in isolated spermatozoa plasma membranes of boar reveal two phase transitions at 32°C and 6°C (Canvin & Buhr, 1989). Similar experiments in ram reveal three phase transitions at 37°C, 26°C and 17°C (Holt & North, 1986), but in rat only one phase transition is detected at 21°C (Hall, Hadley & Doman, 1991). The multiple phase transitions are thought to arise from the existence of different lipid domains. Using Fourier transform infrared spectroscopy (FTIR) complex lipid phase transitions in intact, live boar spermatozoa occurred over a range of 25°C, centered around 18°C (Drobnis et al., 1993). This may be attributable to multiple phase transition events involving membrane lipids from different regions or from different classes with different phase transition temperatures. The latter possibility, in the complex lipid mixture of live spermatozoa membranes, will lead to the coexistence of liquid-crystalline and gel-phase lipid domains at physiological temperatures during cooling, when some lipids reach their T_m and enter the gel phase before others.

However, in intact, live human spermatozoa no thermotropic phase transition in the plasma membrane is detected using the generalized polarization technique (Palleschi & Silvestroni, 1996). This argues against the coexistence of gel- and liquid-crystalline phase domains in the case of humans, which could be due to the relatively high cholesterol content of these membranes compared to spermatozoa of other species (Darin-Bennett & White, 1977). Similarly, it has been demonstrated that in preparations made of mixed phospholipids with cholesterol greater than 15 mol% the coexistence of gel- and liquid-crystalline phase domains cannot be detected

(Parasassi et al., 1994). The importance of the thermotropic phase transition effects during cryopreservation of spermatozoa is well established (Parks & Graham, 1992), with a close relationship between cold shock sensitivity and lipid composition (Watson & Morris, 1987; De Leeuw et al., 1990; Buhr et al., 1994).

Membrane Lipid Domains are Reorganized During Capacitation and Acrosome Reaction

Changes in domain structure of the plasma membrane of spermatozoa is detected during capacitation and acrosome reaction. Exposure of boar spermatozoa to effectors of capacitation results in a decrease in the packing order of membrane lipids and is accompanied by an increase in the permeability of the plasma membrane (Harrison, Ashworth & Miller, 1996). Following the acrosome reaction, rearrangement of the membrane lipids in bovine and human spermatozoa is also detected, by fluorescent lipid vesicles (Arts et al., 1993). These vesicles require the presence of anionic lipids in the liposomal bilayer to fuse with the spermatozoa membrane. The fusion is found to be restricted to the EQ region and lateral lipid diffusion from this region is inhibited (Arts, Jager & Hoekstra, 1994). Similarly, acrosome-reacted guinea pig spermatozoa show a marked reduction in the lateral diffusion of plasma membrane lipids over AC and PA regions (Cowan, Myles & Koppel, 1987).

Functional Barriers Between Regions Determine the Composition and Lateral Organization of Membrane Lipids

The distribution of phospholipids within the membrane has been investigated using several fluorescent lipophilic probes. These reveal a variety of labeling patterns, depending on the condition and epididymal development of the spermatozoa. Two types of labeling patterns occur most commonly: even across all regions, and preferential regionalization into the acrosome and midpiece (Ladha et al., 1997). This regionalization is observed with both cationic (1,1′-dihexadecyl-3,3,3′3′-tetramethylindocarbocyanine; DiI) Wolf & Voglmayr, 1984) and anionic dyes such as ODAF (5-N-(octadecanoyl) aminofluorescein) (Ladha et al., 1997) or merocyanine (bis-(1,3-dibutylbarbiturate)trimethineoxonol) (Schlegel et al., 1986; Sivashanmugam & Rajalakshmi, 1997). The preferential localization of cationic dyes may be due to their attraction by anionic lipids of relatively higher concentration within these regions (Gadella et al., 1994). Similarly, lipids that are packed loosely into domains, believed to be formed within regions enriched in anionic phospholipid, may be being detected by merocyanine (Stillwell et al., 1993). These domains in mouse (Schlegel et al., 1986) and rhesus monkey (Sivashanmugam & Rajalakshmi, 1997) develop during epididymal maturation, being fully formed after transit through the corpus. In ram sperm they are present throughout the entire epididymal maturation process (Schlegel et al., 1986).

It cannot be mere coincidence that the two regions that have the highest concentration of membraneous structures are the most intense when labeled with lipophilic dyes. An alternative explanation put forward recently suggests that the sperm cell plasma membrane is very sensitive to mechanical and chemical stresses. Since it is so easily compromised, it is possible that there is some degree of labeling of internal membranes as well as the plasma membrane (Ladha et al., 1997). In this case, regions with the highest membranous structures, the acrosome and midpiece, then have relatively more dye associated with them.

Although GP, DSC and FTIR provide information on overall or 'bulk' properties of a cell membrane, they lack the precision and resolution necessary to investigate specific regions within it. FRAP on the other hand, is a microscopic technique that permits the interrogation of a specific area within a region, defined by a laser beam. Fluorescent lipophilic probes incorporated into the outer leaflet of the spermatozoa plasma membrane have been used with this technique to give information on the lipid lateral diffusion—the diffusion coefficient (D) and the mobile fraction (%R) (Tocanne et al., 1989). It is important to determine the labeling pattern prior to measuring the membrane lipid lateral diffusion. Using the ODAF probe, bull spermatozoa with regionalized labeling have a large nondiffusing fraction; in contrast, evenly labeled spermatozoa show little nondiffusing lipid fraction. It is concluded that regionalized labeling is due to permeabilization of the membrane (as explained above), leading to substantial changes in the physical state of the membrane lipid (Ladha et al., 1997). Lateral diffusion coefficients in evenly labeled cells exhibit regionalized differences, with D decreasing from the AC to the PP region (AC > PA > MP > PP). The values of D are significantly higher than those obtained by another probe DiI. This was used to measure lipid lateral diffusion in ram (Wolf & Voglmayr, 1984), mouse (Wolf et al., 1986*b*) and guinea pig (Cowan et al., 1997). The higher D values with ODAF are in keeping with the indices of fluidity suggested by the chemical composition of the plasma membrane.

The DiI probe also indicates a significant nondiffusing lipid fraction in ram and mouse but not in guinea pig spermatozoa. It has been suggested that DiI labels permeabilized or damaged spermatozoa more efficiently than live intact spermatozoa (Ladha et al., 1997). If the labeling pattern is not determined prior to the diffusion coefficient, it is not possible to differentiate between live and damaged spermatozoa, and an average value for D will be obtained that is lower than the true value. It is also possible that because the two probes have different structures they may be localized in different lipid do-

mains. Despite these problems the D for DiI shows regionalized differences in ram (Wolf & Voglmayr, 1984) and mouse (Wolf et al., 1986*b*) but not in guinea pig spermatozoa (Cowan et al., 1997). The lipid lateral diffusion data suggest there are functional barriers to diffusion between regions in some species, preventing randomization of the plasma membrane lipids. These will maintain region-specific membrane compositions which are required for associated specialized functions.

Summary

The membranes of mammalian spermatozoa are composed of a complex mixture of lipids which is modified during epididymal maturation. Although there are many species-specific changes in the membrane, a common characteristic is the introduction of highly unsaturated phospholipid acyl moieties and an increase in the relative content of ether-linked phospholipids at this time. The predominant phospholipid molecular species is *sn-1* ether-linked 16:0/*sn-2* ester-linked 22:6-PC and PE. This membrane lipid remodeling is thought to provide the correct infrastructure and fluidity for the membrane-mediated events that lead to fertilization. In the plasma membrane, the lipids are organized into five specialized regions; acrosome (AC), equatorial (EQ), postacrosome (PA), midpiece (MP) and the principle piece (PP). Each region has a distinct lipid composition, as required for associated specialized functions. Experiments suggest that functional barriers to diffusion exist between the regions preventing mixing and randomization of membrane components. There is compelling evidence to support the existence of both lateral and transmembrane lipid heterogeneity in the plasma membrane of spermatozoa.

The author is supported by funding from the BBSRC. Many thanks to Dr. A.C. Pinder for helpful discussions and critically reading the manuscript. I would also like to thank Peter James, Paul Gunning and the library staff for their help and patience in dealing with my numerous requests.

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