Formation and organization of the mammalian sperm head

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Summary. The formation and organization of a mammalian sperm head occurs through diverse cellular and molecular processes during spermiogenesis. Such cellular events include sequential changes in the nucleus and the acrosome—which is derived from the Golgi apparatus—in concert with prominent bundles of microtubules, the manchette. However, these complex processes are readily impaired by a variety of intrinsic and extrinsic factors, eventually causing various types of male infertility—such as teratozoospermia—which include the deformation of the acrosome and nucleus. In order to comprehend such idiopathic male infertility syndromes, it is important to clarify the mechanism involved in sperm head formation and organization. In addition to the manchette, two key structures in these events are the acroplaxome and the perinuclear theca. The acroplaxome forms the acrosome plate with periodic intermediate filament bundles of the marginal ring at the leading edge of the acrosome, and its nature has recently been characterized. The perinuclear theca, which is located in the perinuclear region in the sperm head, contains not only a cytoskeletal element to maintain the shape of the sperm head but also functional molecules leading to oocyte activation during fertilization.

This review discusses recent developments regarding the formation and organization of the mammalian sperm head in relation to its relevant functions.

Introduction

The formation and organization of mammalian sperm heads is a much studied subject (Longo and Cook, 1991; De Kretser and Kerr, 1994; Mujica et al., 2003), but the underlying cellular-molecular relations remain unclear due to an incomplete understanding of the processes at the subcellular and molecular levels. In order to understand the sequential changes in the structure and associated molecules of sperm head components, a considerable amount of experimental evidence has been accumulated by conventional electron microscopy, immunocytological analyses utilizing specific probes or antibodies against specific molecules and, more recently, by the aid of gene manipulation techniques. In particular, mutant animal models show a variety of phenotypes with morphological and functional abnormalities which have provided important clues to our understanding of the etiology of teratozoospermia (Vanables and Cooke, 2000; Escalier, 2001; Cooke and Saunders, 2002).

In humans, male infertility is induced by a number of factors such as the environment, behavior, genotoxicity and genetics, all causing impaired spermatogenesis at various stages of development (De Kretser and Baker, 1999). Among these, teratozoospermia is a common phenotype, which is mainly due to the disruption of events of the metamorphosis stage, i.e., spermiogenesis, in which cell organelles are reorganized into complicated structures of mature spermatozoa which lack mRNA. Thus, morphological abnormalities range from cellular to subcellular levels.

Based on this background, the formation and organization of the mammalian sperm head is discussed in light of recently accumulated experimental evidence for two key components—the acrosome and perinuclear theca (PT)—which surround the sperm head.
1. Main structures and organization of mammalian sperm heads

The mammalian sperm head is completely surrounded by a plasma membrane and is bordered from the tail by a characteristic zone, the posterior ring, which is the site of the plasma membrane anchoring to the nuclear envelope (Fig. 1, 2). The sperm head is composed of two main regions: the acrosomal region and the postacrosomal region. The acrosomal region is further subdivided into two regions: the anterior acrosomal region and the posterior acrosomal region, i.e., the equatorial segment.

The sperm head contains two main structures, one of which is the nucleus which is surrounded by the nuclear envelope, the other being the acrosome, which is surrounded by the outer acrosomal membrane and inner acrosomal membrane. The nucleus is almost entirely present in the head. The acrosome covers the anterior half of the head, whose covered area varies by species. The postacrosomal region is located in the range comprising the posterior end of the equatorial segment to the posterior ring region. The postacrosomal region contains the postacrosomal sheath (PAS) located between the plasma membrane and the nuclear envelope. The PAS is also called the "calyx" (Fawcett, 1970; Longo et al., 1987; Oko et al., 1990). In this review, we refer to this visible ladder-like structure at the postacrosomal region as the PAS in order to clearly differentiate it from other homogeneous substances, i.e., non-ladder-like PT substances (Fig. 1, 6). Except for the acrosome, the space between the nucleus and the overlying plasma membrane is collectively referred to as the PT, where a number of molecules have been identified to date. The molecules contained in the PT are thought to play significant roles not only during sperm head formation but also during fertilization, as shall be discussed later.

The fine structures and related molecules within the acrosome sac have recently been thoroughly reviewed (Olson et al., 2003; Yoshinaga and Toshimori, 2003) and therefore will not be discussed here. This review will focus on the instrumental structure, currently termed the "acroplaxome" (from the Greek words akros, topmost; platus, flat; soma, body) (Kierszenbaum et al., 2003), which is located at the leading edge of the acrosome sac (or the acrosome cap) in developing spermatids (Fig. 4).
2. Structures or components related to the elongation of the acrosome and nucleus

The acroplaxome

The elongation and formation of the acrosome occurs throughout spermiogenesis. The accumulation of acrosomal materials and flattening of the acrosome cap both occur in round spermatids (up to step 8 spermatids in the mouse and rat). The elongation and formation of the acrosome follows nuclear condensation; the phase of nuclear elongation precedes the principal condensation. During the flattening of the acrosome cap in the early round spermatid, the leading edge of the acrosome cap thickens to form an electron-dense complex, the marginal ring (Fig. 4, 5). This prominent marginal ring, the acroplaxome, has recently been structurally and biochemically characterized in the rat (Kierszenbaum et al., 2003).

The components of the acroplaxome are initially assembled as a complex of electron-dense materials in the narrow space between the nuclear envelope and inner acrosomal membrane of the nascent acrosome cap, and relevant materials then appear to accumulate within the sub-acrosomal space, forming the acrosome plate with periodic intermediate filament bundles of the marginal ring at the leading edge of the acrosome (Fig. 4; Kierszenbaum et al., 2003). The acrosome plate is connected to the nuclear plate which is seen as an electron-dense zone, the nuclear lamina, of the nuclear envelope (Fig. 4). The nature of the acroplaxome has recently been clarified and found to contain actin and keratin (Kierszenbaum et al., 2003). The keratin is composed of a glycine-rich domain homologous to human keratin 9 (K9). Thus, the acroplaxome marginal ring is a complex of filamentous actin (F-actin) and keratin, which is also called the F-actin-keratin-containing plate. The acroplaxome is therefore thought to play an important role in head shaping through acrosome elongation, possibly together with the perinuclear ring, from which bundles of microtubules emerge to form the microtubule-relating manchette. The perinuclear ring is seen as an electron-dense bulge at the proximo-lateral top of the cytoplasm in an elongating spermatid (Fig. 5).

Fig. 2. Transmission electron microscopic (TEM) image of mouse sperm head (sagittal view) showing the sperm head domain and components. Asterisk indicates the subacrosomal space (perforatoprium) filled with components of perinuclear theca.
In relation to F-actin and the perinuclear ring, one molecule, arc (i.e. activity regulated, cytoskeletal associated; also called Arg3.1), which was originally found localized in neurons, colocalizes with F-actin, yielding an acrosome (Maier et al., 2003). The arc is known to be present in the perinuclear region of round, elongating, and elongate spermatids. Detailed analyses are awaited that will permit an understanding of how the arc is related to acrosome elongation.

It has recently been found that KIFC1, a C-terminal kinesin motor, appears to be involved in the formation and elongation of the acrosome (Yang and Sperry, 2003); KIFC1 first appears on membrane-bounded organelles in the medulla of early spermatids and then localizes to the acrosomal vesicle. KIFC1 continues to be present on the acrosome of elongating spermatids as it flattens on the spermatid nucleus. Increasing amounts of KIFC1 are found at the caudal aspect of the spermatid head and in the distal cytoplasm. The KIFC1 motor is also found in the nucleus of very immature round spermatids just prior to its appearance on the acrosome. KIFC1 is localized just below the nuclear envelope and adjacent to the sub-acrosomal membrane. Interestingly, KIFC1 is associated with importin beta and colocalizes with this nuclear transport factor on curvilinear structures that are associated with the spermatid nucleus. Thus, KIFC1 appears to assist in the formation and/or elongation of the spermatid acrosome, presumably in association with nuclear components.

The manchette

The manchette, which consists of a bundle of microtubules, has long been presumed to play a role in the elongation of the sperm head or nucleus (for reviews, Clermont et al., 1993; Meistrich, 1993). Nuclear elongation occurs during late spermiogenesis (from step 9 spermatids in the mouse and rat). During this stage, the manchette is transiently formed in the disto-lateral region of the cytoplasm (Fig. 5). The timing of the development of the manchette is very precise; it appears in early elongating spermatids and disappears when the elongation and condensation of the spermatid nucleus approaches completion. Bundles of microtubules radiate from the perinuclear ring, which is closely opposed to the distal end of the acrosome sac (Fig. 5). The microtubules run centro-distally toward the neck region. Also found among the microtubules are many molecules that are presumed to anchor or harbor in or around the manchette (for a review, Kierszenbaum, 2002). One of these is particularly intriguing, being a small GTPase of the Ras superfamily, Ran; which is present in the cytoplasm and nucleus of round spermatids and in the manchette of elongating spermatids. Ran GTPase is presumed to control the traffic of nuclear proteins during the condensation of the spermatid nucleus (Kierszenbaum, 2002; Kierszenbaum et al., 2002). Thus, microtubules in the manchette apparently serve to convey a variety of molecules that are necessary for spermatid nuclear condensation and tail formation, although the detailed mechanisms of this process have yet to be elucidated.

The spermatid nucleus simultaneously condenses as the manchette develops. The acrosome sac also extends distally, and the posterior part of the acrosome, i.e., the equatorial segment, simultaneously becomes clearly visible. Eventually, the acrosome sac flattens, and the head region of the spermatid also elongates and flattens. Thus, the timing of the appearance and disappearance of the manchette is apparently related to the elongation of the acrosome and nucleus.
It has been reported that, in the case of the az/haz mutant mouse, an abnormal head shape is induced through the abnormal shaping of the nucleus by the ectopic positioning of microtubules of the manchette (Meistrich et al., 1990; Russell et al., 1991).

Another point to note here is that the manchette plays a role in trimming the residual cytoplasm of a spermatic, pulling the cytoplasm down to the distal side. This permits the residual cytoplasm to be discarded at spermiation. It would be also interesting to understand the relationship between the manchette and the reorganization of the residual cytoplasm of a spermatic, since this also appears to be an important event that is required for spermatic formation.

**Perinuclear theca (PT)**

The PT is also called the perinuclear substance or perinuclear matrix, and is located in the perinuclear region in the sperm head (Longo and Cook, 1991; Oko and Maravei, 1995; Mujica et al., 2003). The PT is essentially a cytoskeletal element, and some components of the PT are SDS-insoluble, as evidenced by the appearance of structures such as the ladder-like ones between the PAS and overlying the plasma membrane or by the PAS itself, at the postacrosomal region (Fig. 1). Thus, the PT is found as a condensed layer of cytoplasm that is sandwiched both between the nuclear envelope and the inner acrosomal membrane apically at the acrosomal region as well as between the nuclear envelope and the plasma membrane caudally at the postacrosomal region. Although the PT is structurally and compositionally subdivided into two regions, e.g., the sub-acrosomal layer and the PAS (Aul and Oko, 2002), we prefer to subdivide the PT into four spaces (or layers) based on both the spatial arrangement of molecules therein and the structural nature. This is because these regions (spaces) are functionally distinct and deserve further discussion, and because the number of molecules at this stage, which are steadily increasing, are restricted to these spatially distinct spaces.

One of the four subdivisions is the "sub-acrosomal space (or layer)", which is also called the "perforatorium", the space between the nuclear envelope and the acrosome sac at the acrosomal region (Fig. 2, 3). The sub-acrosomal space continues into the sub-PAS space lying between the nuclear envelope and the PAS at the postacrosomal region (Fig. 1, 3). Although the sub-PAS space is the second subdivision of the PT, some free substances in the sub-acrosomal space and sub-PAS space can move unimpeded between them because of the spatial continuity. The third subdivision in the "peri-acrosomal space", the space between the outer acrosomal membrane and the plasma membrane that overly the acrosome. The fourth subdivision is a restricted space between the PAS and the overlying plasma membrane at the postacrosomal region; we call this space the "peri-PAS space". A number of functional and structural components are housed or located in each of these four spaces (subdivisions).

Functionally, the ES and the proximal postacrosomal region are involved in the initial sperm-egg fusion (Bedford and Cooper, 1978; Toshimori, 1982, Toshimori and Oura, 1993; Yanagimachi, 1984) leading to oocyte activation (Kimura et al., 1998; Perry et al., 1999 and 2000). Therefore, the oocyte-activating factor is presumably located in either the distal peri-acrosome space or the proximal proximal peri-PAS space or both. In fact, one molecule, MN13 (an approximately 56 kDa protein), which is located distinctively in the peri-PAS space at the postacrosomal region (Fig. 6, Toshimori et al., 1991), has been reported to be involved in oocyte activation (Manandhar and Toshimori, 2003). This functional point is discussed below in more detail.

Concerning the role of acrosomal-nuclear docking and shaping (elongation) of the spermatic nucleus and acrosome, it has been reported that PT proteins are assembled during spermogenesis either in peripheral association with the developing acrosomic vesicle as it attaches to and caps the apical half of the spermatic nucleus to form the sub-acrosomal layer, or in the manchette to form the PT at the postacrosomal region, including the PAS itself (Oko, 1995, Oko and Matavei, 1995). We also have obtained similar results based on immunohistochemistry using a monoclonal antibody against MN13 (unpublished data). Eventually, MN13 is organized into the peri-PAS space during spermatic elongation (Fig. 6), suggesting the possibility that MN13 anchors the plasma membrane to the PAS, which is further connected to the nuclear envelope via a certain PT substance in the sub-PAS space.

Biochemically, well-characterized molecules that are located in the PT are as follows: calcin (Longo et al., 1987; von Bulow et al., 1995; Lécuyer et al., 2000), cyclin I and II (the Greek word c et a n lambda l zeta for the cup or beaker; Hess et al., 1993, 1995; Rousseaux-Prevost et al., 2003), testis-specific capping protein (CP), heterodimer formed by the association of testis-specific isoform subunits, CP13 (Tanaka et al., 1994; Hurst et al., 1998) and CPβ3 (von Bulow, 1997), PERF15 (Oko and Morales, 1994; Korley et al., 1997), cytoplasmic histone, subacrosomal H2B variant, SubH2Bv, or formerly PT15 (Oko and Maravei, 1995), signal transducer and activator of transcription, STAT4 (Herrada and Wolgemuth, 1997) and actin-related protein family members (Arp), Arp-T1 and Arp-T2 (Heid et al., 2002).

Before addressing the question of acrosome-nucleus interactions, it should be noted that the sub-acrosomal
Fig. 4. TEM images showing the acroplaxome marginal region (MR). A: MR is localized at the leading edge of the acrosome in a round spermatid (step 7). B: Higher magnification of another acroplaxome marginal ring, showing the components in more detail; periodic intermediate filament bundles (•) at the acrosome plate (AP) connect the acrosome to the nuclear plate (NP) of the nuclear envelope (NE).
space of mammalian spermatids during spermiogenesis is essentially filled with actin in its filamentous form (F-actin) (Vogl, 1989). In late spermatids and spermatozoa of many species, most or all of the F-actin is depolymerized to the globular form (G-actin), and appears to be redistributed in the cytoplasm in a species-specific pattern (Vogl, 1989). Therefore, although the role of F-actin and its interactions with other PT proteins are currently unclear, some of its possible roles include: 1) anchoring the acrosome to the underlying nuclear envelope and to the overlying plasma membrane, 2) shaping the acrosome or nucleus (spermatid head) by capping the nuclear envelope, and 3) organizing specialized spermatid membrane domains.

First, the PT-related proteins that are currently known to have actin-binding properties shall be discussed. These include calcinin (Lécuyer et al., 2000) and cyclin II (Rousseaux-Prevost et al., 2003). CPα3 (Tanaka et al., 1994; Hurst et al., 1998) and CPβ3 (von Bulow, 1997) are also strong candidates for serving as actin-binding proteins. Others have also been reported; Arp-T1 and Arp-T2 (Heid et al., 2002) and FSCN3 (Tubb et al., 2002).

Calcinin is a member of the kelch-family of actin-interacting proteins, and contains three kelch repeats and a BTB/POZ domain. The three consecutive elements, kelch elements, associate with actin-rich intracellular bridges (ring canals) linking nurse cells to the oocyte (Xue and Cooley, 1993). Calcin is initially superimposed on actin in the sub-acrosomal space and subsequently in the peri-acrosomal and sub-acrosomal space during spermiogenesis (Lécuyer et al., 2000). In addition, calcinin has the ability to form homomultimers which could contribute to the rigidity of the PT, based on its known characteristics. The affinity of calcinin to F-actin suggests the possibility that calcinin may be targeted at the sub-acrosomal space of round spermatids, and it has been proposed that its ability to form homomultimers contributes to the formation of a rigid PT
Fig. 6. Legend on the opposite page.
(Lécuyer et al., 2000). Thus, calcin is presumed to be involved in anchoring the nuclear envelope to surrounding structures.

Cyclin II has recently been reported to be both a PT and novel actin-binding protein (Rousseaux-Prevost et al., 2003). Cyclin II belongs to a cyclin multigene family, or a member of the multiple band polypeptide (MBP) group (Hess et al., 1993, 1995), one group of very basic proteins that were first characterized by Longo et al. (1987). Porcine clycin II is a basic 60 kDa protein that is homologous to bovine and human clycin II. Cyclin II is localized in the acrosomal region of round spermatids and in the postacrosomal region of late spermatids and spermatozoa. Interestingly, clycin II is reported to contain two potential bipartite nuclear localization signals (positions 431–448 and 461–478 of bovine clycin II). However, the issue of how clycin II plays a role in actin-related events that occur during early events of fertilization remains unclear.

The expression of CPA3 (Hurst et al., 1998) and CPβ3 (von Buolow, 1997) is also closely related to formation and nuclear elongation. FSCN3, expressed only in elongating spermatids, is localized at the dorsal and ventral head of a spermatid, where actin filaments are also densely localized (Tubb et al., 2002). Actin-related proteins, Arp-T1 and Arp-T2, have been recently reported to be present as calyx in the sub-acrosomal PT (Heid et al., 2002), but the details of this are currently unclear.

Other proteins that are likely to be involved in acrosome formation are PERF15, STAT4, and SubH2Bv.

PERF15 is related to the superfamilies of lipophilic transport proteins, and is the major protein of the sub-acrosomal PT (Okó and Morales, 1994; Poursertail et al., 1997; Breed et al., 2000). PERF15 is progressively concentrated as a sub-acrosomal substance over the anterior half of the spermatid nucleus during the development of round and early elongated spermatids. The possibility cannot be excluded that the PERF15 protein interacts with the acrosomal membrane, playing a role in the structural arrangement and stability of the acrosome during spermiogenesis.

STATs are essential transcription factors which mediate the biological effects of cytokines. Among them, STAT4 is present in the PT, and is capable of relocating to the nucleus to activate transcription (Herrada and Wolgemuth, 1997). STAT4 may be involved in the onset of zygotic transcription. However, how STAT4 is involved in acrosome docking and zygotic transcription is not clear.

It is likely that SubH2Bv is involved in acrosome assembly and acrosome-nuclear docking, since SubH2Bv is a sub-acrosomal substance and contains a nuclear localization signal (Aul and Okó, 2002). One possible mechanism for this is that SubH2Bv directs the nascent acrosome (acrosome vesicle)–to which PT-proteins are bound–to the nuclear envelope. Once the SubH2Bv-surrounded acrosome vesicle becomes attached to the nuclear envelope, the two membranes (nuclear envelope and acrosomal membrane) then bind to each other through the formation of dimeric complexes of SubH2Bv, thus stabilizing the acrosome.

Thus, the PT components are transported along with acrosome granules and assembled in the perinuclear region during early spermiogenesis. Later, during the elongating spermatid stage, the PT materials are further assembled through or via the use of the microtubule-machinery, the manchette, and are then organized into the postacrosomal region. PT components therefore play multifunctional, at least bifunctional, roles: initially, an instrumental role in formation (attachment and elongation) of the nucleus and acrosome during spermiogenesis, and later, a functional role in oocyte activation at fertilization.

Oocyte activation is briefly discussed below. The most important role of the PT is to provide oocyte activating factor(s), the sperm-borne oocyte-activating factor(s) (SOAF), to the oocyte (Kimura et al., 1998; Perry et al., 1999, 2000). Since the final organization and components of the PT are closely related to the expression process of the SOAF during sperm-egg fusion, this point is discussed further.

In mature spermatozoa, the SOAF is thought to be rather freely present as a soluble substance or one weakly associated with the PT component in the equatorial segment or proximal postacrosomal region or both. This is because...
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oocyte activation is rapidly induced soon after the sperm-egg fusion that first occurs at equatorial segment, and then at the proximal postacrosomal region, and because the SOAF is more rapidly dispersed into the ooplasm than the initiation of any degradation of PT structures (Toshimori et al., 1993; Manandhar and Toshimori, 2003; Sutovsky et al., 2003). All these facts, as well as the cytoskeletal nature and the specific localization of the PT, provoke an intriguing question, i.e., how the PT component is organized at the PT of the elongating sperm head. The SOAF is apparently organized as PT materials in the spermatid during spermiogenesis, presumably associated with some components of the PT. This point needs to be precisely clarified, since recent advances in assisted reproductive technology (ART) such as intracytoplasmic sperm injection (ICSI) or round spermatid injection (ROSI) are routinely performed on the clinical side to treat idiopathic infertility syndromes caused by the sperm factor. The SOAF is a yet-to-be-characterized substance (s) at this moment.

Finally, we present an overview of the failure of acrosome and nucleus elongation caused by gene knockout in order to understand better the sperm head formation.

3. Failure of acrosome and nucleus elongation in a gene knockout model mice; involvement of the manchette and the acroplaxome

An impairment in spermiogenesis induces pleiotropic head deformities in mammalian spermatozoa, i.e., various types of teratozoospermia. Molecules or genes involved in teratozoosperma with abnormal heads have been reported in the following gene knockout mice: Hrb (Kang-Decker et al., 2001), GOPC (Golgi associated PDZ-and coiled- and coil motif containing protein; Yao et al., 2002), and mGCL-1 (Kimura et al., 2003).

Hrb (also called Rab or hRip) contains the Asn-Pro-Phe (NPF) motif that interacts with protein Eps 15 homology domains. Eps 15 is a transport vesicle adaptor protein for endocytosis and vesicle sorting. Eps 15 homology domains interact with Hrb and several other NPF proteins. Thus, Hrb is regarded as a critical component in the docking and/or fusion machinery that forms the Golgi-driven proacrosomal vesicles. In the case of Hrb, deletion of the Hrb gene causes the proacrosomic vesicles to fail to fuse in very early round spermatids (at step 2), and unfused vesicles accumulate in the space between the juxta-Golgi apparatus and the nuclear surface. Eventually, the mutant male mouse generates a typical phenotype of a head spermatozoa “globozoospermia” that lacks acrosomes (Kang-Decker et al., 2001). Further details as to how the acrosome is detached await clarification.

In the case of GOPC, the acrosome is not completely formed, but becomes fragmented (Fig. 7; Yao et al., 2002). Eventually, all acrosomes of the GOPC null mouse are completely detached from the nulei of all the mature spermatozoa. Since acrosome fragmentation occurs during the round spermatid stage and since the detachment of fragmented acrosomes from the nuclear membrane start prior to nuclear elongation, it is thought that the manchette is not directly involved in acrosome elongation, or at least, the manchette does not provide sufficient force for anchoring the acrosome sac to the nucleus. Interestingly, the acroplaxome does not appear to be fully developed in GOPC null spermatids; the details of this issue are under investigation in our laboratory. If the acroplaxome is not well developed throughout spermiogenesis, then the acroplaxome would be expected to play a significant role in the attachment of the acrosome to the nuleus. It should also be noted that MN13 is not detectable in GOPC homo spermatozoa (unpublished observation, these data to be published separately).

In the case of the mGCL-1 (mouse homologue of Drosophila melanogaster germ cell less) null male mouse, even though both the manchette and the acrosome—including the acroplaxome—appear to be normally formed, the resulting acrosome and head are deformed into various shapes. This implies that other mechanisms for head formation/defomation may be viable; for example, nuclear lamin could be related to the condensation and elongation of the nucleus (Kimura et al., 2003).

In other cases of head anomalies such as gene knockout mice of Tnp2 (Adham et al., 2001), and Casein kinase II (CK2), a cyclic-AMP and calcium-independent serine-threonine kinase with two catalytic subunits (α and α’ ) and two regulatory β-subunits (Xu et al., 1999), the acrosomes

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Fig. 7. TEM images showing unfused acrosomic vesicles in a round spermatid (A), an abnormally formed round spermatid (B), and mature spermatozoa (C) from GOPC null (+−) male mice. A: acrosomic vesicles (●) are unable to fuse with each other and accumulate in small fragments adjacent to the nucleus (N). B: no acrosome, but a vacuole, is present at the apical side of the condensing nucleus ( ● ). C: round headed spermatozoa, which lack acrosomes, from cauda epididymis. Note a normal spermatozoan (arrow) from GOPC wild (+/+) male mice that was artificially mixed in the sample as a control.
of many spermatozoa are also detached from the nuclear envelope, but the details how this happens remain unclear.

**Conclusion**

The mechanism of sperm head formation—including elongation of the acrosome and nucleus—is complex, but two key structures appear to be involved in the mechanism: one is the "acroplaxome," the electron-dense plate-like complex at the distal end of the acrosome, and the other is the PT materials, including the PAS. The PT is a complex of multimers. The acroplaxome appears to play a role in the attachment and elongation of the acrosome in the round spermatid stage, while the PT is presumed to function in attaching the acrosome and the PAS to the underlying nuclear envelope and to the overlying plasma membrane throughout spermiogenesis. The PT materials are presumably assembled by the aid of the microtubules, the manchette, which are believed to serve as conveyors of the PT materials to the perinuclear region at the postacrosomal region, since this event actively occurs during the elongating spermatid stage.

In addition, the manchette also functions as a convoy of a variety of materials that are required for the tail assembly including neck components such as the centrosome, which is necessary for early embryonal development, e.g., for sperm aster formation in which microtubules are generated. In summary, the mechanisms of male gametogenesis and certain types of idiopathic infertility are better understood by gaining knowledge at the subcellular and molecular levels.

**References**


