The acrosome-acroplaxome-manchette complex and the shaping of the spermatid head

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Summary. A combination of exogenous contractile forces generated by a stack of F-actin-containing hoops embracing the apical region of the elongating spermatid nucleus and an endogenous modulating mechanism dependent on the spermatid-containing acrosome-acroplaxome-manchette complex may play a cooperative role in the shaping of the spermatid head. In addition, the manchette is a key element in the transport of vesicles and macromolecules to the centrosome and developing spermatid tails as well as in nucleocyttoplasmic transport. The proposed model of spermatid head shaping is based on: 1) currently known structural and molecular components of the F-actin hoops, the main cytoskeletal element of the Sertoli cell ectoplasmic specializations; 2) the molecular features of acrosome biogenesis; 3) the assembly of a subacrosomal cytoskeletal plate called the acroplaxome; and 4) the spatial relationship of the acrosome-acroplaxome complex with the manchette, a transient microtubular/actin-containing structure. During acrosome biogenesis, the acroplaxome becomes the nucleation site to which Golgi-derived proacrosomal vesicles tether and fuse. The acroplaxome has at least two functions: it anchors the developing acrosome to the elongating spermatid head. It may also provide a mechanical scaffolding plate during the shaping of the spermatid nucleus. The plate is stabilized by a marginal ring with junctional complex characteristics, adjusting to exogenous clutching forces generated by the stack of Sertoli cell F-actin-containing hoops applied to the elongating spermatid head. A tubulobulbar complex, formed by cytoplasmic processes protruding from the elongating spermatid head extending into the adjacent Sertoli cell, is located at the concave side of the spermatid head. The tubulobulbar complex might provide stabilizing conditions, together with the actin-afadin-nectin-2/nectin-3 adhesion unit, to enable sustained and balanced clutching exogenous forces applied during the elongation of the spermatid head.

Introduction

One of the major causes of male infertility is the abnormal development of the spermatid into a mature and motile sperm. Globozoospermia (round-headed sperm) and coiled sperm tails are two severe abnormalities that lead to infertility. Globozoospermia correlates with defective acrosome biogenesis and tail defects that have a disruption in the intramanchette (IMT, Kierszenbaum, 2002) and intragollicellar transport systems (IFT, Rosenbaum and Witman, 2002) of molecules to the centrosome and the developing tail. Recent research has provided molecular data concerning the impact of acrosome biogenesis on spermatid nuclear shaping, in particular on the relationship of deficient acrosome development and round-headed sperm. Two examples are the acrosome-deficient Hrb (Kang-Decker et al., 2001; Kierszenbaum et al., 2004) and the GOPC-deficient (Yao et al., 2002) mice mutants. Hrb is a protein associated with the cytosolic surface of proacrosomal transporting vesicles in round spermatids, and a lack of Hrb protein prevents the vesicles from fusing to form the acrosome. The GOPC protein is localized in the trans Golgi region of round spermatids, and a lack of this protein interferes with the transport of vesicles from the Golgi apparatus to the acrosomal sac and fusion to form the acrosome. Spermatids in both mutants share a common feature: Golgi-derived proacrosomal vesicles attach to and align along the...
acroplaxome, an actin-keratin-containing cytoskeletal plate anchoring the developing acrosome to the nuclear envelope (Kierszenbaum et al., 2003a). In the Hrb mutant, proacrosomal vesicles fuse forming a flat sac, called pseudacroplaxome, while the acroplaxome is deficient in the intermediate filament protein called Sak57 (for spermatogenic cell/sperm-associated keratin with a molecular mass of 57 kDa, Kierszenbaum et al., 1996), an ortholog of keratin 5 (K5) (Kierszenbaum et al., 2003a). Nucleopodes, protrusions of the spermatid nucleus with a bulb-shaped ending linked by a stalk to the main nuclear structure, develop at the Sak57/K5-deficient acroplaxome (Kierszenbaum et al., 2004). In the GOPC, proacrosomal vesicles are also seen attached to the acroplaxome while an acrosome fails to develop (Yao et al., 2002).

When acrosome biogenesis is in progress, the transient microtubule-containing manchette develops caudally to the acrosome. A perinuclear ring, the insertion site of manchette microtubules, assembles adjacent to the marginal ring of the actoplaxome from which it is separated by a narrow belt-like groove. A number of elongating spermatids from the mutant mouse azh (for abnormal sperm headshape, Meistrich, 1993) display an abnormally constricting manchette perinuclear ring as well as severe nuclear deformities at the acroplaxome site (Kierszenbaum et al., 2003a). In addition, spermatids and sperm from the azh mutant mouse have a lasso-like coiled tail with a high frequency of head dislocation and decapitation (Mochida et al., 1999; Kierszenbaum and Tres, 2002). A non-functional Hook-1 gene, predominantly expressed in azh spermatids, has been proposed as being responsible for the azh abnormal spermatid and sperm phenotype (Mendoza-Lujambio et al., 2002). The presence of the Hook-1 protein, which is truncated in the azh mutant, appears to establish a link between the membrane of vesicles and microtubules.

Spermatids develop a tubulobulbar complex at the concave side of the elongating head, which projects into matching infoldings of the adjacent Sertoli cells (Russell and Clermont, 1976). Each tubular element of the tubulobulbar complex is cuffed by F-actin bundles; each bulbar element is associated to a piece of the Sertoli cell endoplasmic reticulum (Guttman et al., 2004). The Sertoli cell region associated with the tubulobulbar complex can be regarded as a confined modification of Sertoli cell ectoplasmic specializations, consisting of the plasma membrane, harboring the afadin/nectin-2 complex (Mueller et al., 2003; Guttman et al., 2004), F-actin bundles and associated cisternae of the endoplasmic reticulum as predominant elements. A clear distinction is the planar arrangement of Sertoli cell ectoplasmic specializations at the convex side of the elongating spermatid head and with respect to the concave side, where the specializations adopt a folded arrangement in parallel to the shaft of each tubulobulbar extension. It was proposed that the tubulobulbar-Sertoli cell ectoplasmic specialization assembly may stabilize the spermatid heads undergoing elongation (Russell, 1979). More recently, it has been proposed that the tubulobulbar complex participates in both the disassembly of the ectoplasmic specializations and internalization of Sertoli cell-spermatid adhesion molecules during spermiation (Guttman et al., 2004).

From the foregoing account, it can be envisioned that an exogenous contractile component, represented by Sertoli cell ectoplasmic specialization, and an endogenous component, represented by the acroplaxome-actincomplex, may interact to modulate the shaping of the spermatid head. The interaction of exogenous and endogenous components may require a relatively stable head, a function presumably fulfilled by the tubulobulbar complex anchored in the adjacent Sertoli cell.

Details of the spermatid head shaping mechanism are not fully understood but pieces of the puzzle are beginning to emerge. In this review article, we focus on the mechanism of acrosome biogenesis and IMT involving the transport of Golgi-derived vesicles containing the Rab27a/b receptor and mobilized by the F-actin-dependent motor myosin Va (Kierszenbaum et al., 2003b). These two events, together with the acroplaxome and the function of the ectoplasmic specializations of the adjacent Sertoli cell, suggest a potential mechanism of spermatid head shaping based on observations in mouse mutants displaying globozoospermiata. It should be noted that in addition to vesicle transport, cytosol-derived protein cargos bound to protein rafts can be mobilized by molecular motors by IMT (see Kierszenbaum, 2002). Therefore, both vesicle and protein cargo transport might take place simultaneously within the manchette and along the axoneme.

The Rab27a/b-MyRIP-myosin Va complex and organelle transport

Acrosome biogenesis consists of the transport of Golgi-derived vesicles containing acrosomal hydrolytic proteins to the acrosomal sac, which is associated to one of the poles of the spermatid nucleus. The opposite pole of the nucleus harbors the centrosome, which gives rise to the axoneme, one of the components of the developing spermatid tail. The transport of proacrosomal vesicles requires microtubule-based and actin-based motor proteins (kinesin/dynein and myosin Va, respectively). Vesicles transported by the myosin Va motor require three elements: a vesicle receptor (Rab27a/b), a molecular motor (myosin Va), and a molecular motor recruiter (MyRIP; for myosin Va-Rab interacting protein). Figure 1 illustrates the localization of myosin Va...
and Rab27a/b in the Golgi and acroplaxome region (in particular in the marginal ring) as well as myosin Va in the manchette (Fig. 1E–G). In contrast, proteins not enclosed in vesicles and synthesized in the cytosol can be bound to a protein raft and transported by molecular motors to the centrosome and the developing sperm tail along microtubules of the manchette (see Kierszenbaum, 2002 and references therein). One of the components of the raft protein complex is the polaris, found in the manchette (Taulman et al., 2001). A mutation in Tg737, the gene encoding polaris, is associated with immotile cilia and abortive sperm axoneme. Therefore, a defective protein raft complex may hamper the timely delivery of molecules to the centrosome and developing spermatid tail.

Rab27a/b are members of a large family of Ras-related small GTPases. Rabs have distinct subcellular localizations and are believed to regulate specific steps of intracellular trafficking. Rab27a is unique at present because it is the

Fig. 1. Localization of myosin Va in the Golgi-acrosome-acroplaxome region and chromatoid body region and in the manchette of rat spermatids (A–G). The localization of Rab27a/b is shown in the Golgi and acrosome-acroplaxome region (H–K).
only known Rab associated with disease in humans (Seabra et al., 2002). Loss-of-function mutations in the Rab27a gene result in Griscelli syndrome, an autosomal disorder characterized by partial cutaneous albinism and immunodeficiency due to the failure of cytotoxic T cells to release the contents of their lytic granules. A natural mouse mutant, ashen (Rab27a¬), has a mutation in Rab27a and exhibits the same phenotypic features of Griscelli syndrome patients such as partial albinism and immunodeficiency (Wilson et al., 2000). Rab27a is associated with melanosomes in pigmented cells of skin and retina and regulates melanosome transport via its interaction with actin-based cellular motors such as myosin Va and myosin VIIa (Seabra et al., 2002; Wu et al., 2002; Tolmachova et al., 2004).

Myosins share a common structure and are composed of three modules: the head domain comprising the motor domain, which hydrolyzes ATP to power movement along F-actin; the neck domain, which acts as a lever arm and binds regulatory light chains such as calmodulin; and the tail domain, which is highly divergent among the different classes of myosin, serves to bind a specific cargo. Some myosins are processive (i.e., can perform many consecutive steps along F-actin without dissociating). Other myosins can exert tensions between actin filaments, or actin filaments and membrane domains.

The divergent tail domain of myosins contains the necessary information for targeting cargos to specific cell compartments. Until recently, little was known concerning the link between myosin tails and their cognate organelle. Genetic, morphologic, and biochemical analysis of three mouse coat color mutants, dilute, ashen, and leaden, gave some clues regarding the mechanism regulating the binding of one class of myosin V to melanosomes, the pigment containing granules of skin melanocytes. The dilute locus in mice encodes the murine ortholog of myosin Va. There are two dilute mutants: dilute-lethal, with a severe neurological condition leading to neonatal death due to loss of function, and dilute-viral, that exhibits only pigment dilution and expresses an alternative spliced form of myosin Va.

Myosin Va and myosin VIIa do not interact directly with Rab27a but require linker proteins, melanophilin and MyRIP, respectively, that bind GTP-bound Rab27a through a conserved N-terminal domain, and myosins through a medial domain (El-Amraoui et al., 2002; Fukuda et al., 2002; Wu et al., 2002). Melanophilin and MyRIP are products of the leaden gene. Recently, we reported the expression of MyRIP (previously designated Slac2-c) in mouse testis (Kierszenbaum et al., 2003b). MyRIP (or Slac2-c, a homologue of Slac2-a, Kuroda et al., 2002a, b) is widely expressed in most tissues and has the following characteristics: 1) it interacts specifically with Rab27a/Rab27b and myosin Va/myosin VIIa; 2) it displays an an binding site at the C-terminal region; and 3) it is highly expressed in brain, lung, and testis (Fukuda and Kuroda, 2002). Collectively, the ashen, dilute, and leaden gene products may be responsible for the recruitment of

![Fig. 2. Gene expression determined by RT-PCR of the molecular motors myosin Va and Myosin VIIa, the vesicle receptors Rab27a and Rab 27b and the molecular motor linkers MyRIP and melanophilin in mouse testis. The numbers at the left indicate the molecular marker size in kilobases (kb). The base pair (bp) size of each transcript is indicated.](image-url)
melanosomes to actin filaments. The absence of any of these gene products leads to a striking clustering of melanosomes around the nucleus of melanocytes. However, in spermatids, the switch cytoskeletal track model anticipates the coexistence of kinesin and myosin Va/myosin VIIa motors in the same vesicle; one motor becomes inactive when its companion motor binds to a filament. Therefore, a molecular motor compensatory mechanism based on redundancy may ensure undisrupted vesicular transport and a tug-of-war between motors does not occur. Figure 2 shows that mouse testis expresses the molecular motors myosin Va and myosin VIIa, the molecular motor recruiters MyRIP and melanophilin, and the vesicle receptors Rab27a and Rab 27b. Therefore, the possibility of a compensatory vesicle microtubule-based transport mechanism in ashen spermatids can be readily explored. Figure 3 provides a summary of the sorting pathways of Golgi-derived proacrosomal and non-acrosomal vesicles mobilized along manchette microtubules and manchette-associated F-actin as well as the molecular components of microtubule- and F-actin-dependent vesicle transporting machinery. There are two interesting aspects illustrated in the diagram to be emphasized. First, vesicles may remain linked to microtubules in a "holding pattern" until microtubule- and F-actin-based motors recruit them for transport. A candidate vesicle-microtubule linker is Hook1, a protein found in the manchette and encoded by the Hook1 gene. Hook1 is defective in the ashen mouse mutant (Mendoza-Lujambio et al., 2002), which produces sperm with abnormal head shape, head dislocation, and "lasso-like" coiled tails (Mochida et al., 1999). It is likely that in addition to Hook1, other vesicle-microtubule protein linkers may be present in the manchette. Second, the chromaoid body, a mass associated to nuclei of developing spermatids, is surrounded by myosin Va-decorated vesicles (see Fig. 1A–D). This observation suggests a possible function of the vesicles in the disposal of nuclear material generated during spermiogenesis.

A "switch cytoskeletal track model" has been proposed (Kierszenbaum et al., 2003b) to account for the coexistence in the manchette of F-actin with microtubules and the corresponding vesicle motors (myosin Va/myosin VIIa and kinesin/dynein, respectively) for the transport of cargos (Fig. 4). The model suggests that Golgi-derived vesicles, required for acrosome biogenesis and spermatid tail development, can switch from an F-actin to a microtubule track and vice versa. The competition between molecular motors may be determined by engaging effector molecules, such as MyRIP and melanophilin, which upon binding to Rab27a/b, recruit myosin Va or myosin VIIa. If MyRIP, or melanophilin, fail to bind to Rab27a/b on Golgi-derived vesicles, myosin Va and myosin VIIa will not be recruited. In a default pathway, the already present microtubule-based motor kinesin variants mobilize the vesicles in an anterograde direction. The model is based on recently reported data from our laboratory (Kierszenbaum et al., 2003a,b, 2004) and on additional data demonstrating a coexisting kinesin/myosin V heteromotor complex on Golgi-derived vesicles (Brown et al., 2004).

Rab27a is also required for secretion of lytic granules in cytotoxic T cells (Menasche et al., 2000). In ashen mice, lytic granules polarize correctly but are unable to kill target cells due to impaired secretion of the lytic granules (Stinchcombe et al., 2001). Melanocytes and cytotoxic T cells are only a subset of cell types expressing Rab27a. The Rab27a protein is expressed in the hematopoietic lineage, spleen, lung, eye, pancreas and the gastrointestinal tract (Seabra et al., 1995; Barral et al., 2002). In addition, recent studies suggest a role of Rab27a in exocytosis of insulin and chromaffin granules in endocrine cells (Fukuda et al., 2002; Yi et al., 2002). Rab27a is present in cortical granules aligned under the zona pellucida of mature mouse follicles (Tolmachova et al., 2004; Seabra M, personal communication). The presence of immunoreactive Rab27a/b and myosin Va during the early steps of acrosome biogenesis has been reported (Kierszenbaum et al., 2003b, 2004; see Fig. 1) and myosin Va can be found bound to Golgi-derived vesicles copurified with manchettes (Kierszenbaum et al., 2003b). Collectively, these data indicate that the Rab27a/b-myosin Va complex plays a role in immunity, pigmentation and reproductive function as well as other physiological activities (Tolmachova et al., 2004) with specific characteristics varying from one cell to another.

The temporal development of the acrosome-acroplaxome unit and the manchette

Golgi-derived proacrosomal vesicles initiate acrosome biogenesis by fusing into a single acrosomal sac bound to the nuclear envelope. After acrosome biogenesis starts, the microtubule-containing manchette assembles as the spermatid nucleus initiates its elongation. The manchette disassembles upon completion of nuclear elongation and condensation (reviewed by Clermont et al., 1993). The role of the manchette in spermatid nuclear shaping has been analyzed for many years (Cole et al., 1988; reviewed by Meistrich, 1993). Recent biochemical and structural evidences support an IMT mechanism for the delivery of molecules to the centrosome and developing spermatid tail (Rivkin et al., 1997; Kierszenbaum et al., 2002; Kierszenbaum, 2002). However, direct evidence of IMT of specific cargos needs to be experimentally shown.

Because of the tight acrosome-nuclear relationship, we have looked into a nuclear-acrosome anchoring
mechanism. We have identified a cytoskeletal plate, the acroplaxome, linking the developing acrosome to the spermatid nucleus (Kierszenbaum et al., 2003a). The acroplaxome consists of F-actin, Sak57, an ortholog of keratin 5 [K5] (Kierszenbaum et al., 2003a), and myosin Va (Kierszenbaum et al., 2003b). The marginal ring of the acroplaxome displays a desmosome-like structure consisting of a bundle of Sak57 [K5]-containing intermediate filaments connecting a plaque associated with the descending edge of the inner acrosomal membrane and an opposite plaque bound to the spermatid nuclear envelope (Fig. 5). The persistence in the acroplaxome marginal ring of myosin Va, presumably associated to Rab27a/b via the effector MyRIP, together with the report that another Rab27 effector, Munc13-14, is thought to regulate SNARE in vesicle fusion (Shirakawa et al., 2004), raises the interest-

Fig. 3. Diagrammatic representation of the two major intramanchette vesicle pathways and the components of the microtubule- and F-actin-based vesicle-motor complexes (diagram not to scale). 1: The acrosome biogenesis vesicle pathway includes transporting vesicles from the endoplasmic reticulum to the Golgi. The Golgi generates two types of vesicles: proacrosomal and non-acrosomal vesicles. Proacrosomal vesicles are transported to the acroplaxome by actin-based and microtubule-based molecular motors, where they fuse and organize the acrosome. 2: Non-acrosomal vesicles associate with microtubules of the manchette and are transported by microtubule-based molecular motors to the centrosome region and the developing spermatid tail (not shown). 3: Non-acrosomal vesicles can also associate with F-actin present in the manchette and transported by F-actin-based molecular motors. Non-acrosomal vesicles can remain in a “holding” pattern associated to manchette microtubules by vesicle-microtubule linkers until recruited by microtubule- or F-actin-based molecular motors for further intramanchette transport. The chromatoid body becomes surrounded by non-acrosomal vesicles probably mobilized by intramanchette transport.
ing possibility that Rabs may recruit hierarchically a set of effectors and thereby coordinate proacrosomal vesicle transport with tethering to the F-actin component of the acroplaxome and fusion to form the acrosome. Therefore, it is possible that the Rab27a/b-MyRIP-myosin Va complex participates in both acrosome biogenesis and acrosome reaction at fertilization. However, details of how this hierarchy could be achieved remain to be determined.

The lack of an acrosome in Hrb mutant spermatids does not prevent the development of the acroplaxome (Kierszenbaum et al., 2004). Yet, the acroplaxome in the Hrb mutant contains F-actin and myosin Va but is deficient in Sak57
In wild-type and Hrb mutant spermatids, myosin Va is prominent in the Golgi region where proacrosome vesicles are clustered (Fig. 1H–K). In the Hrb mutant, myosin Va-bound proacrosome vesicles coalesce along the acroplaxome and form a flat sac, the pseudoacrosome (Kierszenbaum et al., 2004). As spermiogenesis advances, round-shaped spermatid nuclei of the mutant display at the acroplaxome location several nuclear protrusions, designated nucleopodes (Kierszenbaum et al., 2004). The acroplaxome appears to provide a focal point for myosin Va-driven proacrosome vesicles to congregate and form an acrosome in wild-type spermatids or a pseudoacrosome in Hrb mutant spermatids.
Intramanchette transport of cargos by actin- and microtubule-based molecular motors

In addition to the acroplaxome, F-actin is also present in the manchette (Mochida et al., 1998; 1999; Kierszenbaum et al., 2003b). The finding of discrete β-actin immunoreactivity along interconnected tube-like assemblies of manchette microtubules (Fig. 4), together with myosin Va-decorated vesicles associated with the manchette (Fig. 1E–G), suggest that a dual microtubule- and actin-based motor protein mechanism may operate to mobilize vesicle cargos by IMT. Why might two transport systems be necessary in developing spermatids? Models have been proposed to explain how the microtubule- and actin-based transport systems may interact with each other. One model postulates a fast long-range transport of cargos mediated by microtubules, in contrast with an actin-based short-range local transport (Goode et al., 2000). By contrast, another model assumes that different types of motors operate simultaneously and that the resulting motion of a vesicle cargo results from a balance of the forces acting upon the cargo (Gross et al., 2002). An alternate mechanism is that intramanchette cargos may “switch” from a microtubule to an actin track and vice versa by exchanging a microtubule-based motor (kinesin- or dynein-like) for an actin-based motor (myosin Va/VIIa). Such a mechanism may involve Rab27a/b-specific motor recruiting proteins (such as MyRIP/melanophilin) to dictate the final destination of the cargo. Essentially, Rab27a/b may determine whether vesicle membrane domains are assembled for vesicle fusion or transport. Our finding that manchette-associated vesicles decorated with anti-myosin Va can be fractionated (Kierszenbaum et al., 2003b), opens new experimental possibilities for determining how manchette microtubules (and presumably F-actin) may regulate the mobility of vesicle cargos.

The microtubule-based motor kinesin KIFC1 and the actin-based motor myosin Va have been proposed as participating in the transport of Golgi-derived vesicles and acrosome biogenesis (Yang and Sperry, 2003; Kierszenbaum et al., 2003b, respectively). It remains to be determined how these two motors interact with distinct cytoskeletal systems during acrosome biogenesis.

The tubulobulbar complex, a mechanical stabilizer during spermatid head shaping

Tubulobulbar complexes consist of several projections from the spermatid plasma membrane and cytoplasm into corresponding invaginations of the adjacent Sertoli cell (Russell and Clermont, 1976; see Fig. 6). Although the precise function of the tubulobulbar complex has not been experimentally determined, several possibilities have been considered. One of the possibilities is an attachment device between Sertoli cells and spermatids, which disappears upon spermiation (Russell and Clermont, 1976). Another possibility is that tubulobulbar complexes represent a dynamic modification of the preexisting Sertoli cell ectoplasmic specializations tailored to facilitate mechanical events within the seminiferous epithelium during spermatogenesis, but not necessarily restricted to spermiogenesis (Russell, 1979). It has also been proposed that tubulobulbar complexes are involved in a dual function: the disassembly of Sertoli cell ectoplasmic specializations as well as in the uptake of cell adhesion molecules to enable the release of mature spermatids into the seminiferous tubular lumen (Guttman et al., 2004).

The tubulobulbar complexes may also play a role in spermatid head shaping. The elongating spermatid head displays a convex curvature in contact with Sertoli cell ectoplasmic specializations, and a concave curvature facing the tubulobulbar complex (Fig. 6). The Sertoli cell surface facing the convex side of the spermatid head displays significant characteristics: 1) It contains F-actin bundles stabilized by the actin-bundling protein espin (Bartles et al., 1996). 2) F-actin is the binding site of the afadin, a protein that links the cytoplasmic domain of nectin-2, forming an actin-afadin-nectin-2 adhesion unit (Mueller et al., 2003 and references therein). 3) The extracellular domain of nectin-2 (on Sertoli cell surfaces) and nectin-3 (on spermatid cell surfaces) establish a heterophilic binding partnership, thereby stabilizing adjacent Sertoli cell-spermatid surfaces (Ozaki-Kuroda et al., 2002). 4) The nectin-2/nectin-3 association appear critical to spermiogenesis as demonstrated by a disrupted Sertoli-spermatid association, abnormal spermatid head shape morphogenesis and infertility following inactivation of nectin-2 gene expression (Bouchard et al., 2000; Ozaki-Kuroda et al., 2002; Mueller et al., 2003). 5) The α6β1 integrin complex has been identified at the Sertoli cell ectoplasmic specialization (Palombi et al., 1992); yet, the binding partner on adjacent spermatogenic cells has not been determined. 6) The afadin-nectin-2 complex undergoes a gradient redistribution during the elongation of the spermatid head; the concentration of the afadin-nectin-2 complex at the Sertoli cell convex curvature declines in correspondence to an increase in concentration along the tubulobulbar complexes (Guttman et al., 2004). From these data, it can be concluded that a complex and dynamic cell-cell adhesion and mechanical mechanism stabilizes the positioning of the spermatids within the apical domain of the seminiferous epithelium as head elongation is in progress.
The acrosome-acroplaxome-manchette complex and sperm head shaping: a hypothesis

Components of the developing spermatid, such as the manchette and centrosomes, have been fractionated (manchette, Mochida et al., 1999; centrosomes, Kierszenbaum et al., 2002) and approaches are now being developed to fractionate the intact acroplaxome (our unpublished data) for molecular and biochemical analysis. Yet, mouse mutants provide at present a convenient approach to determine how the acrosome-acroplaxome-manchette complex and Sertoli cell ectoplasmic specializations might contribute to the shaping of the sperm head.

Here, we present a model for spermatid head shaping based on structural data derived from normal rats and wild-type and mutant mice. The model relies on two elements. The first element, the exogenous clutch, is represented by the spatial arrangement of stacked F-actin-containing hoops embracing the apical one-third of the spermatid head (Fig. 7). The hoops may be kept in place by the F-actin-linked afadin/nectin-2 and the nectin-2/nectin-2 adhesion complexes. How do hoops apply an exogenous constriction force? Conventional myosin motors, capable of interacting with F-actin and exerting contractile forces, have not been found along the Sertoli cell ectoplasmic specializations (Vogl and Soucy, 1985). However, unconventional myosins may be present. Dynein-type and kinesin-type molecules have been found in association with microtubules adjacent to the inner face of Sertoli cell endoplasmic reticulum components of the ectoplasmic specializations (Guttman et al., 2000). It has been proposed that molecular motors linked to microtubules adjacent to the endoplasmic reticulum at the ectoplasmic specialization site could mobilize the F-actin-containing plaques in an up direction (toward the seminiferous tubular lumen) and
down direction (toward the seminiferous tubular wall) to facilitate Sertoli cell displacement (Guttman et al., 2000). However, a bidirectional up-down movement of F-actin bundles is unlikely to generate constriction forces capable of participating in spermatid head shaping. An attractive alternative to be explored is the depolymerization-polymerization of F-actin, which could threadmill F-actin-containing hoops. Two regulatory molecules of F-actin assembly/disassembly are gelsolin and profilin. Gelsolin, a protein that both caps and severs F-actin, is found in Sertoli

Fig. 7. Role of exogenous and endogenous clutching forces and aspects of intramanchette and intraflagellar transport operating during the elongation of the spermatid head. A: The head of an elongating spermatid is enveloped by F-actin-containing parallel hoops and associated proteins exerting an exogenous clutching effect on the spermatid nucleus by gradual reduction of the hoop length (indicated by facing arrows). The marginal ring of the acroplaxome and the adjacent perinuclear ring of the manchette adjust their diameter in compliance to the elongation of the spermatid nucleus (endogenous clutch effect). A groove belt denotes the boundary between the acroplaxome marginal ring and the manchette perinuclear ring. B: In the elongated spermatid, the head is surrounded by F-actin hoops adjacent to endoplasmic reticulum cisternae on the Sertoli cell side. The shrinking marginal ring of the acroplaxome, anchored to the descending caudal edge of the acrosome and to the nuclear lamina as well as the narrowing perinuclear ring of the manchette, reach the caudal region of the nucleus. The manchette extends into the cytoplasm and is engaged in the intramanchette transport of vesicles and proteins to the centrosome and the developing tail. The manchette then disassembles. The progressive development of the axoneme and associated outer dense fibers and fibrous sheath (not shown) is facilitated by intraflagellar transport of the corresponding molecular building blocks.
cell ectoplasmic specializations (Guttman et al., 2002). A testis-specific isoform of profilin, a protein which blocks the incorporation of G-actin into filamentous actin and also facilitates actin polymerization by facilitating the exchange of bound ADP for ATP in G-actin, has been reported (profilin-3, Braun et al., 2002). Given the existence of a number of testis-specific actin-binding proteins (Kierszenbaum et al., 2003a and references therein), it is likely that an understanding of the nucleation and disassembly of F-actin in the Sertoli cell ectoplasmic specializations and the acroplaxome can shed light on the mechanistic functions of these two structures during spermatid head shaping.

The second element in the hypothesis of spermatid head shaping, the endogenous clutch, is the acrosome-acroplaxome-manchette complex (Fig. 7). The acrosome is tightly bound to the acroplaxome plate, which in turn is anchored to the nuclear envelope of the elongating spermatid nucleus. The acroplaxome seems to play two important roles. First, the acroplaxome is the site where proacrosomal vesicles tether to the F-actin component after being mobilized by the Rab27a/b-myosin Va complex along F-actin tracks (Kierszenbaum et al., 2004) and microtubule tracks by kinesin molecular motors (Yang and Sperry, 2003). Second, a specific feature of the acroplaxome is its marginal ring lodged in a shallow circumferential indentation of the spermatid nucleus. The indentation displays a narrow dense plaque spanning across the indentation and linked to the nuclear lamina. The subacroplaxome position of the nuclear plaque coincides with a distinct chromatin imprint denoted by propidium iodide staining (see Fig. 1D). On the opposite site, a bundle of Sak57/K5-containing intermediate filaments is anchored to a dense acrosomal plaque (Fig. 5). The marginal ring and associated dense plaques are reminiscent of a junctional belt linking membranes of adjacent epithelial cells. Subjacent to the junctional belt is the perinuclear ring of the manchette. Both the marginal ring of the acroplaxome and the perinuclear ring of the manchette reduce their diameter as they gradually descend along the elongating spermatid nucleus. Therefore, the dense plaques and associated cytoskeletal components appear to stabilize the tight association of the acrosome to the nuclear envelope as the caudal acrosome edge descends during the elongation of the spermatid head. In fact, hypotonic and strong detergent treatment can remove spermatid membranes but fail to dislodge the acroplaxome from its nuclear envelope attachment site (Fig. 1A).

How can the acroplaxome contribute to spermatid nuclear shaping? The presence of F-actin and Sak57/K5 in the acroplaxome plate suggests that this structure is stress-resistant and may transmit to the spermatid nucleus gentle and steady clutching forces generated by the Sertoli cell F-actin-containing hoops (Fig. 7). Supporting this assumption is the finding in the acrosome-deficient Hrb mutant of a number of nucleopodes developed at the Sak57/K5-defective acroplaxome site (Kierszenbaum et al., 2004). Both the marginal ring of the acroplaxome and the perinuclear ring of the manchette may act as endogenous constrictors in a sleeve-like manner, steering the elongation of the spermatid nucleus as both rings descend from the caudal two-thirds of the elongating spermatid head. The effective action of exogenous contractile forces requires the steady positioning of the spermatid head. In this regard, steady positioning may be provided by the tubulobulbar anchoring complex (Fig. 7) acting as a lever in concert with the interacting nectin-2/nectin-3 heterotypic complex and β1 integrin. The various elements of the proposed model might provide a framework for further studies leading to the complete characterization of the molecular components of the acroplaxome and the manchette in a number of mutant mice where the development of the acrosome and the integrity of the acroplaxome and the manchette are defective.

References


Spermatid head shaping


