Published in final edited form as:

Biol Cell. 2012 May ; 104(5): . doi:10.1111/boc.201100102.

The dynamic cytoskeleton of the developing male germ cell

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Abstract

Mammalian spermatogenesis is characterized by dramatic cellular change to transform the non-polar spermatogonium into the highly polarized, functional spermatozoon. The acquisition of cell polarity is a requisite step for formation of viable sperm. The polarity of the spermatozoon is clearly demonstrated by the acrosome at the apical pole of the cell and the flagellum at the opposite end. Spermatogenesis consists of three basic phases: mitosis, meiosis, and spermiogenesis. The final phase represents the period of greatest cellular change where cell-type specific organelles such as the acrosome and the flagellum form, the nucleus migrates to the plasma membrane and elongates, chromatin condenses, and residual cytoplasm is removed. An important feature of spermatogenesis is the change in the cytoskeleton that occurs throughout this pathway. In this review, the author will provide an overview of these transformations and provide insight into possible modes of regulation of these rearrangements during spermatogenesis. Although primary focus will be given to the microtubule cytoskeleton, the importance of actin filaments to the cellular transformation of the male germ cell will also be discussed.

Keywords

cytoskeleton; microtubule; cellular motors; spermatogenesis; manchette

Introduction

Correct cell polarity is essential for proper cellular function in many cell types including epithelial cells, neurons, and sperm. The intrinsic polarity of microtubules confers polarity on cells by determining the direction of movement of vesicles and other cargoes based on the preferential motility of microtubule-based motor proteins. In many cell types the plus-end, or faster growing end, of microtubules is located at the periphery of the cell in close proximity to the plasma membrane. This arrangement is consistent with the polarized transport of vesicles to the plasma membrane for purposes of cell growth, delivery of membrane proteins, and release of neurotransmitters in neurons, among other functions. During development, microtubules rearrange to accommodate the cell-specific functions of the differentiated state. For example, epithelial cells have highly asymmetric membrane domains separated by tight junctions. In order to accommodate delivery of specific cargo to these distinct domains, the microtubule cytoskeleton of epithelial cells rearranges during cell specification. During differentiation, the centrosome-based radial microtubule array transforms into parallel bundles lacking discernable centrosomes with minus-ends, or slower growing ends, oriented toward the apical membrane and plus-ends toward the basal membrane. Such an arrangement is well suited for the various exocytic and endocytic pathways essential for proper function of this cell type (Musch, 2004).

Spermatogenesis is a splendid example of the dramatic microtubule rearrangements necessary for production of a functional differentiated cell (Figure 1). During this developmental pathway, spindle structures are formed during two successive rounds of cell division to ultimately produce spermatids each with a haploid DNA content. Spermatids then undergo striking changes in cellular architecture including formation of the acrosome and flagellum, elongation and condensation of the nucleus, and removal of excess cytoplasm to produce a spermatozoon that can successfully deliver genetic material to the oocyte during fertilization. Precise execution and regulation of this complex pathway is essential as disruption at any step can render the organism infertile or result in abnormal progeny.

Cell division

Mitosis

Spindle formation and regulation in spermatogonia, the sperm cell precursor, are similar to that occurring during mitosis in somatic cells which has been reviewed extensively elsewhere (Roof et al., 1992; Barton and Goldstein, 1996; Endow, 1999; Sharp et al., 2000; Walczak and Heald, 2008; Gatlin and Bloom, 2010); therefore, only an overview will be provided in this review. Spindle assembly and function is driven by the action of numerous molecular motors, both minus-end directed and plus-end directed. The minus-end motor dynein/dynactin is localized to the kinetochore where it aids in the movement of chromosomes towards the poles at telophase and to the centromere where it helps link spindle and astral microtubules (Echeverri et al., 1996; Quintyne et al., 1999). Dynein/dynactin is also required for removal of checkpoint proteins from the kinetochore at the onset of anaphase (Ozaki et al., 2011). Microtubule dynamics, regulated by depolymerizing kinesins, also participates in chromosome movement toward the poles (reviewed in Ems-McClung and Walczak, 2010).

The kinesin-14 class of motor proteins mediates microtubule focusing at the centrosome through their combined ability to move toward microtubule minus-ends and to crosslink microtubules (Endow et al., 1990; McDonald and Goldstein, 1990). The motor activity of the kinesin-5 subfamily balances the inward force produced by kinesin-14 subfamily motors through their capacity to crosslink antiparallel microtubules and move toward microtubule plus-ends (Sawin et al., 1992; Heck et al., 1993). Members of the kinesin-7 subfamily of motors are located at the kinetochore and are important for proper alignment of chromosomes at the metaphase plate (Yen et al., 1991). The depolymerizing kinesins, members of the kinesin-13 subfamily, are also located at the kinetochore where their activity drives movement of chromosomes to poles during telophase and is important for detection of chromosome-microtubule attachment errors (Wordeman and Mitchison, 1995). Chromokinesins, members of either the kinesin-4 and kinesin-10 subfamily, associate with chromosome arms and have multiple functions in mitosis including chromosome condensation, spindle organization, chromosome alignment, and cytokinesis (reviewed in Mazumdar and Misteli, 2005). Members of the kinesin-6 subfamily are required for assembly of the central spindle and cytokinesis (Zhu et al., 2005).

The actin-based molecular motor myosin II plays several roles in cytokinesis including controlling cell rounding during prophase and providing essential motor activity for constriction of actin filaments during telophase. Recent data demonstrates that in addition to these important functions, myosin II participates in spindle positioning and karyokinesis (reviewed in Matsumura et al., 2011). Myosin II activity in cytokinesis, as in other examples of cell motility, is regulated by reversible protein phosphorylation of its associated light chains and has been the subject of a recent review (Matsumura, 2005).

Reversible protein phosphorylation is also a major mode of regulation of microtubule-based motor activity in mitosis. Aurora B kinase phosphorylates kinesin-13 subfamily members thereby decreasing their polymerization activity and is important for error correction of microtubule-kinetochore attachment (reviewed in Moore and Wordeman, 2004; Walczak and Heald, 2008). PLK1, polo-like kinase-1, also phosphorylates MCAK, a kinesin-13 subfamily member, but the result is the inverse of Aurora phosphorylation with activation of MCAK's depolymerization activity rather than inactivation (Zhang et al., 2011). Members of the kinesin-5 subfamily of bipolar plus-end motors can be phosphorylated at both their head and tail domains by the Wee1 homolog and p34(cdc2) kinases, respectively, in order to regulate association of these motors with dynactin or microtubules (Blangy et al., 1997; Garcia et al., 2009).

Dynein/dynactin is also regulated by reversible phosphorylation, primarily through phosphorylation of dynein's associated intermediate chains (Huang et al., 1999; Whyte et al., 2008). Phosphorylation of dynein intermediate chain regulates targeting of dynein to kinetochores and the phosphorylation state of this motor reflects the attachment status of spindle microtubules (Whyte et al., 2008).

The small GTP binding protein Ran participates in another possible method of regulating mitotic motor proteins (reviewed in Walczak and Heald, 2008). This type of regulation relies on a Ran-GTP gradient established by the chromatin linked Ran-GEF (guanine nucleotide exchange factor) RCC1 that loads the small GTPase Ran with GTP leading to a high concentration of Ran-GTP near chromosomes. In addition to its effects on other proteins including spindle assembly factors, the high concentration of Ran-GTP near chromosomes binds to MCAK thereby stimulating spindle formation *in vitro* (Ems-McClung et al., 2004). Recent data reveals an additional and unique role for Ran and importins in delivering proteins into vertebrate primary cilia (Fan et al., 2011).

Meiosis

Although mitotic and meiotic spindles are functionally similar, they can differ in one distinct respect: some subclasses of meiotic spindles self organize without the need for centrosomes and therefore have distinct requirements for molecular motor activity (reviewed in Compton, 1998). Formation and stability of acentriolar spindles depend upon the capture of microtubules by kinetochores, bundling of antiparallel microtubules by kinesin-5 subfamily members, and microtubule-focusing of parallel microtubules by cytoplasmic dynein and the kinesin-14 subfamily. Such spindles are also more dependent on the Ran pathway for their assembly and maintenance than their centrosome-driven counterparts (Kalab et al., 2006). In mouse oocytes, the spindle is formed from multiple microtubule organizing centers (MTOCs) present in the oocyte cytoplasm whereas in rodent spermatocytes a miniature spindle with paired centrosomes forms outside the nuclear membrane (Schatten et al., 1986; Kallio et al., 1998). Upon nuclear envelope breakdown, the spermatocyte spindle assembles chromosomes and lengthens followed by chromosome separation. Regulation of meiotic spindle function with respect to microtubule motor activity and microtubule dynamics is similar to that described for mitosis.

Spermiogenesis

Once haploid germ cells are produced by meiosis, they begin the long and intricate process of cellular transformation necessary to form a mature spermatozoon capable of transferring genetic material to the oocyte upon fertilization. Microtubules participate in several important events in this process including nuclear elongation, cytoplasmic redistribution and reduction, and development of the flagellum.

Nuclear elongation

Shortly after formation of round spermatids, the acrosome forms and flattens along one pole of the nucleus accompanied by movement of the nucleus to contact the plasma membrane. In round spermatids, microtubules are initially randomly arranged in the cytoplasm without a visible centrosome (Moreno and Schatten, 2000). An increase in microtubules around the nucleus occurs and presages the formation of the manchette, a unique microtubule-based structure that eventually surrounds the nucleus just distal to the acrosome (Wolosewick and Bryan, 1977). The manchette is a transient assembly, forming in early spermatids and completely dissolving by the time mature sperm are formed. Because the manchette forms during the time that the nucleus compacts and reshapes, it has been proposed to aid in streamlining of the nucleus (Cole et al., 1988; Meistrich et al., 1990). In addition to the manchette, an actin-based structure termed the acroplaxome is proposed to underlie the acrosome and to transfer force produced by actin cables in the Sertoli cell to the nearby elongating spermatid nucleus (Kierszenbaum et al., 2003).

Like microtubules of the spindle, molecular motor proteins are associated with microtubules of the manchette; however, because of the lack of a suitable culture system, the underlying molecular mechanisms involving motor proteins in manchette assembly, function, and disassembly are not well understood. Cytoplasmic dynein is associated with microtubules of the manchette and with the spermatid nuclear membrane (Hall et al., 1992; Yoshida et al., 1994). Kinesin motors have also been detected on the manchette (Hall et al., 1992; Yang and Sperry, 2003; Saade et al., 2007; Wang et al., 2010). The orientation of manchette microtubules is unclear; however, the presence of the plus-end binding protein CLIP-170 and its subfragment CLIP-50 at the perinuclear ring suggests that microtubule plus-ends reside in this structure (Tarsounas et al., 2001; Akhmanova et al., 2005). Supporting this idea is the finding that γ -tubulin, a marker of the centrosome where the minus-ends of microtubules are found, is absent from the perinuclear ring of the spermatid manchette (Fouquet et al., 1998).

Cytoplasmic redistribution

The placement of the manchette along the nucleus between the apical and distal aspects of the spermatid is suggestive of a role for this structure in redistribution of cytoplasmic contents necessary for their removal prior to spermiation. The term intramanchette transport (IMT) has been coined to reflect similarities between this type of transport and intraflagellar transport (IFT), which is also mediated by microtubule-based molecular motor proteins (reviewed in Kierszenbaum, 2002). A distinct difference between these two forms of transport is that IFT is conducted almost exclusively on microtubules whereas both microtubules and actin filaments of the manchette support IMT (Figure 1). Along with the microtubule-based motors kinesin and dynein that are resident on the manchette and are proposed to power vesicular movement, the actin-based molecular motor myosin Va is also found in the acroplaxome and the manchette of developing spermatids (Kierszenbaum et al., 2003; Hayasaka et al., 2008). The manchette, through its support of IMT proteins, provides a route for proteins destined for the centrosome and the developing sperm flagellum (Kierszenbaum, 2002). These proteins include elements of the proteasome (Rivkin et al., 1997; Rivkin et al., 2009; Bao et al., 2010); Ran GTPase and its binding partners (Kierszenbaum et al., 2002; Bao et al., 2011); flagella components including Sak57, Spag4, and ODF1 (Tres and Kierszenbaum, 1996; Shao et al., 1999); and IFT proteins (Taulman et al., 2001; Sironen et al., 2010; Kierszenbaum et al., 2011).

Flagellar formation

The biogenesis, structure, and regulation of flagella and cilia in sperm and in other cell types has been the subject of numerous reviews and therefore will not be recapitulated in this

article (Goodenough and Heuser, 1985; Goodenough, 1989; Blair and Dutcher, 1992; Walczak and Nelson, 1994; Silflow and Lefebvre, 2001). However, significant advances in the past several years have linked disruption of the growth of cilia and flagella via IFT with an ever-widening array of human genetic disorders due primarily to interruption of the hedgehog-signaling pathway facilitated by primary cilia (reviewed in D'Angelo and Franco, 2009; Tobin and Beales, 2009; Waters and Beales, 2011). The diversity of symptoms experienced by affected individuals includes reversal of left-right asymmetry, defects in bone and brain development, polydactyly, polycystic kidney disease, retinal degeneration, and infertility.

The precise role of the hedgehog-signaling pathway in mammalian spermatogenesis is incompletely understood; however, ablation of desert hedgehog, the isoform expressed in Sertoli cells, in all tissues results in male-specific sterility (Bitgood et al., 1996). Constituents of the hedgehog-signaling pathway found in spermatogenic cells include Gli family members, patched-1 (PTCH1), smoothened (SMO), fused (STK36, aka *Drosophila Fu*), and suppressor of fused (SUFU) (Persengiev et al., 1997; Kroft et al., 2001; Szczepny et al., 2006; Morales et al., 2009).

Recently, a previously uncharacterized protein termed testis leucine rich repeat (TLRR, aka *lrrc67*) was found associated with the centrosome of developing male germ cells in the mouse (Wang and Sperry, 2008; Wang et al., 2010; Wang and Sperry, 2011). Interestingly, TLRR has been identified as a putative member of the ciliome and is enriched in tissues displaying cilia and/or flagella (Li et al., 2004; McClintock et al., 2008). The localization of TLRR in spermatids, as well as its expression in retina and olfactory epithelium, is suggestive of a role for this protein in biogenesis of cilia and flagella (Wang and Sperry, 2011). TLRR has a protein phosphatase-1 (PP1) binding site and is therefore a putative PP1 regulatory protein. Recently, phosphoproteomic studies using mice null for the testis-specific PP1 isoform, PP1 γ 2, identified tubulin as a potential target of PP1 γ 2 in the testis providing a possible link between TLRR and the microtubule rearrangements of spermiogenesis (Henderson et al., 2010).

Post-translationally modified tubulin in spermatid structures

Tubulin is subject to numerous types of post-translational modification including phosphorylation, glutamylation, detyrosination, and glycylation that may alter its function in the microtubule structures where they are found (reviewed in Luduena, 1998). Modified tubulin is differentially expressed in male germ cells reflecting the diverse functions of the microtubule structures in these cells (Fouquet et al., 1994; Kann et al., 2003). Axonemal tubulin is heavily modified through acylation, polyglutamylation, and detyrosination (Mary et al., 1997; Plessmann and Weber, 1997; Mochida et al., 1999; Kann et al., 2003); reviewed in Kierszenbaum, 2002). Monoglycylated tubulin is distributed in a gradient in the axoneme with the highest concentration at the base while polyglycylated tubulin is also distributed in a gradient but instead the highest concentration is found at the tip of the flagellum (Kann et al., 1998). Polyglutamylated tubulin is also differentially distributed in the axoneme and is important for flagellar motility (Gagnon et al., 1996; Huitorel et al., 2002; Kann et al., 2003). The importance of polyglutamylation to sperm function was recently underscored by the report of a null mutant in the mouse tubulin tyrosine ligase-like 1 gene that results in foreshortened flagella with a thickened midpiece (Vogel et al., 2010).

Manchette microtubules are acetylated to a greater extent than observed in microtubules of the axoneme (Mochida et al., 1999). Glutamylated and detyrosinated tubulin are found in both the manchette as well as the axoneme (Mochida et al., 1999). Another difference in tubulin modification between the manchette and the axoneme is glycylation, which is not found in the manchette (Kann et al., 1998). In addition to tubulin modification, a unique

isoform of tubulin, δ -tubulin, is found in the perinuclear ring of the manchette and in the intracellular bridges connecting spermatocyte and spermatid cohorts (Smrzka et al., 2000; Kato et al., 2004).

Because modification is associated with more stable microtubules, these changes may provide additional stability to microtubule-based arrays in spermatids (Luduena, 1998). In addition, the activity of molecular motors can be influenced by the modification state of the substrate microtubule lattice (Rosenbaum, 2000; Dunn et al., 2008; Peris et al., 2009). Recently, the transition from detyrosinated to acetylated tubulin has been shown to coincide with the acquisition of polarity in cultured epithelial cells (Quinones et al., 2011). This result provides additional evidence for the important role of tubulin modification in the establishment of cell polarity.

Summary

Spermatogenesis is an excellent model system for study of the acquisition of polarity during differentiation. As in many developmental pathways, microtubule rearrangement plays a central role in this process. Molecular motors power changes in cellular structure observed during mitosis and meiosis and are regulated in much the same manner as in somatic cells. Particularly striking, however, are the changes in cell architecture that accompany spermiogenesis, the last phase of spermatogenesis. Briefly, the acrosome forms and flattens along the spermatid nucleus, the nucleus moves to contact the plasma membrane, the sperm flagellum forms, the microtubule manchette surrounds the nucleus, chromatin compacts, the nucleus elongates, and cellular contents are reorganized and finally discarded prior to sperm release. The precise purpose and molecular mechanism of manchette function are not known; however, this structure contains both microtubule- and actin-based molecular motor proteins. As in other developmental pathways, tubulin modification accompanies cell polarization during spermatogenesis including phosphorylation, glutamylation, detyrosination, and glycylation. Such tubulin modifications may stabilize spermatid microtubule structures and regulate the binding and therefore the activity of molecular motor proteins. Further understanding of cytoskeletal reorganization, particularly focused on the proteins associated with the manchette and their role in flagellar biogenesis, will provide insight into the common and testis-specific molecular mechanisms underlying cell polarization during development.

Acknowledgments

The author wishes to thank Ms. Nicole DeVaul for her careful reading of this manuscript and Ms. Rong Wang for her technical expertise in some of the work summarized here.

Funding: This work is supported by grant NIH HD058027.

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Figure 1. Schematic of cytoskeletal rearrangements during spermatogenesis

A highly stylized schematic of the reorganization of the male germ cell cytoskeleton is presented. Elements of the microtubule cytoskeleton are indicated in red while the actin component of the manchette is shown in yellow. The manchette and associated structures are enlarged to highlight some of the motors that participate in IMT (kinesin, cytoplasmic dynein, and myosin Va). The motors and mechanisms involved in the mitotic and meiotic spindles are the subject of excellent reviews (Barton and Goldstein, 1996; Endow, 1999; Sharp et al., 2000; Walczak and Heald, 2008; Gatlin and Bloom, 2010) as are those that participate in IFT (Rosenbaum and Witman, 2002; Scholey, 2003; Pedersen and Rosenbaum, 2008; Silverman and Leroux, 2009); therefore, they are not depicted here. The cargo for all motors is symbolized by a yellow circle for simplicity; however, the transported cargo is obviously motor-specific, variable, and complex. A, acrosome; PR, perinuclear ring; Sg, spermatogonium, 1°Sc, primary spermatocyte; 2°Sc; secondary spermatocyte; St, spermatid; Sz, spermatozoon.