MAP/microtubule affinity-regulating kinases, microtubule dynamics, and spermatogenesis

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Abstract

During spermatogenesis, spermatids derived from meiosis simultaneously undergo extensive morphological transformation, to become highly specialized and metabolically quiescent cells, and transport across the seminiferous epithelium. Spermatids are also transported back-and-forth across the seminiferous epithelium during the epithelial cycle until they line up at the luminal edge of the tubule to prepare for spermiation at stage VIII of the cycle. Spermatid transport thus requires the intricate coordination of the cytoskeletons in Sertoli cells (SCs) as spermatids are nonmotile cells lacking the ultrastructures of lamellipodia and filopodia, as well as the organized components of the cytoskeletons. In the course of preparing this brief review, we were surprised to see that, except for some earlier eminent morphological studies, little is known about the regulation of the microtubule (MT) cytoskeleton and the coordination of MT with the actin-based cytoskeleton to regulate spermatid transport during the epithelial cycle, illustrating that this is a largely neglected area of research in the field. Herein, we summarize recent findings in the field regarding the significance of actin- and tubulin-based cytoskeletons in SCs that support spermatid transport; we also highlight specific areas of research that deserve attention in future studies.

Introduction

Eukaryotic cells, including those in health (e.g. spermatogenesis) and in disease (e.g. cancer cells during tumorigenesis), are structurally supported by three extensive cytoskeletal networks, which are composed of the actin-, intermediate filament-, and microtubule (MT)-based cytoskeletons. It is conceivable that these three cytoskeletons must be precisely coordinated and tightly regulated in order to maintain cellular homeostasis at all levels, encompassing cell movement, metabolism, cell proliferation, synthesis, secretion, endocytic vesicle-mediated protein trafficking, and others (Mooren et al. 2012, Stehbens & Wittmann 2012, Vignaud et al. 2012, Matsuuchi & Naus 2013). Filamentous-actin (F-actin), vimentin, and tubulin proteins comprise the actin-, intermediate filament-, and MT-based cytoskeletons respectively. These three cytoskeletal proteins are essential for maintaining the integrity of a cell and performing a variety of functions in addition to providing structural support, such as cell movement, maintenance of cell junctions, and intracellular trafficking (Vogl et al. 2008, Lie et al. 2010, Su et al. 2013). MTs in particular, which are made up of α-tubulin and β-tubulins, are crucial for...
cellular processes such as mitosis, cell polarization, cell motility, neuronal differentiation, and organelle transport (Drewes et al. 1995, Etienne-Manneville 2010, Meunier & Vernos 2012, Su et al. 2012).

MTs are regulated by a variety of factors and proteins, including one class of proteins called the MT-associated proteins (MAPs). As MAPs found in the vertebrate brain have been widely studied, so too have their regulators. One regulator, MAP/MT-affinity regulating kinase (MARK), was first discovered in the brain for its role in phosphorylating tau, a type of MAP protein (Drewes et al. 1997). MARK was found to phosphorylate the LXGS motifs of tau, causing detachment of the MAP from MTs. A balance between attachment and detachment of tau from MTs is necessary for normal functioning of neurons. However, in Alzheimer’s disease, this balance is offset and tau becomes hyperphosphorylated (Timm et al. 2006). Hyperphosphorylation of tau is associated with its own abnormal protein aggregation, a hallmark of Alzheimer’s disease (Marx et al. 2010). The aggregation of tau leads to the development of neurofibrillary tangles in the nerve cells of the brain (Drewes 2004). Studies have shown that MARK co-localizes with these neurofibrillary tangles in the brain (Matena & Mandelkow 2009).

Though there is much interest in uncovering the role of MARKs in the brain, there is a dearth of information currently available on the function of MARKs in the testis, as virtually all the studies conducted in the last several decades on the role of cytoskeletons, in particular MTs in spermatogenesis, are nonfunctional but morphologically based (Russell 1993, Vogl et al. 2008). Studying the regulation of the three cytoskeletons in the testis is of great relevance for the development of novel male contraceptives and new approaches in dealing with the problem of male infertility, a global concern. Thus, it is pertinent to study the function of MARKs during spermatogenesis, as such the process of spermatid development during spermiogenesis and spermiation. Spermatogenesis takes place in the seminiferous epithelium of the seminiferous tubules in the mammalian testis (de Kretser & Kerr 1988, Hess & de Franca 2008, Cheng & Mruk 2009). The Sertoli cell (SC) in the seminiferous epithelium, a specialized microenvironment devoid of blood vessels and nerves, provides nourishment and structural support for the germ cells that will develop into spermatozoa. As mentioned, the seminiferous tubule is the functional unit where spermatozoa are produced, and in order for this highly regulated process to occur, the tubule must be protected from harmful substances that are found in the systemic circulation in the host body.

The blood–testis barrier (BTB), which is constituted by tight junctions (TJs) coexisting with basal ectoplasmic specialization (basal ES), as well as gap junctions and desmosomes between adjacent SCs near the basement membrane, thus segregates the events of meiosis I/II and post-meiotic spermatid development from systemic circulation (Wong & Cheng 2005, Pelletier 2011, Cheng & Mruk 2012, Franca et al. 2012). SCs have been deemed the ‘nurse cells,’ as they provide germ cells in the seminiferous epithelium with the proper environment for development. The SCs themselves comprise a very extensive network of the actin-, vimentin-, and tubulin-based cytoskeletons and thus serve a unique structural and supporting role during spermatogenesis (Vogl et al. 2008, Lie et al. 2010). In this brief review, we present an overview of MARK proteins and their possible function in regulation of the cytoskeleton dynamics in the testis.

**Overview of MTs and MAPs**

The dynamic nature of MTs gives rise to the multitude of functions they serve in any mammalian cell. MTs are polarized cylindrical structures comprising heterodimers of α-tubulin and β-tubulin, which polymerize to form protofilament strands (Mandelkow & Mandelkow 1995, Desai & Mitchison 1997). A single MT is usually formed by the association of 13 protofilaments (Tilney et al. 1973, Kueh & Mitchison 2009). A tubulin heterodimer is made up of an α-tubulin subunit and a β-tubulin subunit; tubulin dimers interact with each other in a head-to-tail manner where the β-subunit of one dimer contacts the α-subunit of another dimer. Thus, in a single protofilament, there will be one α-subunit exposed, designated as the plus-end, and one β-subunit, termed the minus-end (Etienne-Manneville 2010). MTs have an intrinsic polarity due to the nature of the ends of the MTs. The minus end of a MT undergoes slow growth and is commonly anchored to cellular structures such as the MT organizing center (Lie et al. 2010; Fig. 1). The plus, or growing, end of an MT undergoes phases of growth and shrinkage; this occurrence is regarded as dynamic instability (Mitchison & Kirschner 1984, Erickson & O’Brien 1992). Some of the biological functions of dynamic instability include rapid reorganization of cytoskeleton, mitosis, and cell motility (Erickson & O’Brien 1992, Etienne-Manneville 2010).

Due to the inherently unstable nature of MTs, there are a host of proteins that interact with MTs to regulate them. There are three classes of proteins that interact with MTs: MAPs, motor proteins (e.g. myosin VII, dynein, and kinesin in the testis (Lee & Cheng 2004, Vaid et al. 2007b)).
and non-MAP proteins that associate with MTs but are not considered MAPs such as glycolytic enzymes and kinases (Mandelkow & Mandelkow 1995). Such regulators of MT dynamic instability include destabilizing factors, severing proteins, plus- and minus-end capping proteins, and stabilizing factors like MAPs (de Forges et al. 2012; see Table 1 for a list of proteins that interact with MTs). MAPs play an important role in regulating the dynamic nature of MTs by attaching to the sides of MTs, which can slow down or even reverse the shrinkage of MTs (Burbank & Mitchison 2006). MAPs have been shown to control MT dynamics both in vitro and in vivo (Drechsel et al. 1992, Illenberger et al. 1996).

A number of different MAPs have been identified, with the most studied MAPs found in the vertebrate nervous system (Illenberger et al. 1996). Tau, MAP2, and MAP4 are some of the best-studied MAPs; tau is localized in axons, MAP2 in dendrites, and MAP4 is found in various cell and tissue types (Illenberger et al. 1996). MAPs are good substrates for many protein kinases and are regulated through phosphorylation. Phosphorylation of MAPs causes them to detach from MTs, resulting in MT destabilization (Illenberger et al. 1996). However, detachment of MAPs from MTs also allows for other classes of proteins to interact with MTs. Thus, a regulatory system must be in play in order to allow other MT proteins to

Figure 1
A schematic drawing illustrating the likely mechanism of spermatid transport across the seminiferous epithelium during spermatogenesis using the MT-based cytoskeleton and the intricate relationship between MT and F-actin-rich apical and basal ES. MTs are orientated with their plus (+) and minus (−) ends in the SC of the seminiferous epithelium and stabilized by MAPs, which are being used as the track for spermatids (cargoes) to transport across the epithelium, involving motor proteins (e.g. myosin VIIa), at different stages of the epithelial cycle, moving ‘up’ and ‘down’ the epithelium until fully developed elongated spermatids detach from the epithelium at spermiation. The precise mechanism of spermatid transport remains unknown as many of the crucial molecules involved in this event have yet to be identified and studied in the testis.
Table 1  Functional proteins that interact with microtubules

<table>
<thead>
<tr>
<th>Protein type</th>
<th>Function</th>
<th>Examples</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stabilizing MAPs</td>
<td>Stabilize MTs; known for regulatory role of MTs in axons and dendrites</td>
<td>MAP2, tau</td>
<td>Mandelkow et al. (1995) and Drewes (2004)</td>
</tr>
<tr>
<td>Plus-end tracking proteins (+ TIPS)</td>
<td>Functionally diverse: regulate MT dynamics, favor MT assembly, can link MT ends to actin</td>
<td>EB1, CLIP-170</td>
<td>Dixit et al. (2009), Wade (2009) and Kumar &amp; Wittmann (2012)</td>
</tr>
<tr>
<td>Assembly proteins</td>
<td>Promote growth of MTs</td>
<td>Dis1/XMAP215</td>
<td>Brouhard et al. (2008) and Al-Bassam &amp; Chang (2011)</td>
</tr>
<tr>
<td>Severing proteins</td>
<td>Cut MTs into fragments for reorganization of the MT cytoskeleton</td>
<td>Katanin, spastin</td>
<td>Wade (2009), Lumb et al. (2012) and Smith et al. (2012)</td>
</tr>
<tr>
<td>Disassembly proteins</td>
<td>MT disassembly, depolymerization</td>
<td>MCAK, stathmin</td>
<td>Nakamura et al. (2007), Wade (2009) and Belletti &amp; Baldassarre (2011)</td>
</tr>
<tr>
<td>Motor proteins</td>
<td>ATP-dependent movement along MTs</td>
<td>Dynein, kinesin</td>
<td>Endow (1995), Wu et al. (2006) and Su et al. (2012)</td>
</tr>
</tbody>
</table>

interact with MTs without compromising MT integrity (Matenia & Mandelkow 2009). For example, in neuronal axons, the binding of MAPs to MTs stabilizes MTs, which is necessary to ensure MT-dependent axonal transport of cargo, like organelles and vesicles, by motor proteins (Matenia & Mandelkow 2009). Though MT stability is required for axonal transport, it must be noted that MAPs are also competing with motor proteins for MT binding. The concept that MTs serve as the track for the translocation of developing spermatids as ‘cargoes’ across the seminiferous epithelium has been proposed for years following the discovery of several MT-based motor proteins (Guttman et al. 2000, Vaid et al. 2007a,b, Vogl et al. 2008); however, many other important players that are crucial to MT dynamics have yet to be identified and studied in the testis. In short, the model depicted in Fig. 1 is rather preliminary, yet it serves as a helpful guide for the design of functional experiments in future studies.

**MARK, a regulator of MTs via MAPs**

**Structure**

MARKs were first discovered in studies to uncover the pathogenesis of Alzheimer’s disease (Drewes et al. 1995, Matenia & Mandelkow 2009). There are four MARK isoforms (MARK1–4) and they belong to the AMPK subfamily of calcium/calmodulin-dependent protein kinases (CaMKs) (Kemphues 2000, Timm et al. 2008b, Marx et al. 2010). CaMKs are ubiquitous and multi-functional protein kinases that phosphorylate a host of substrates upon activation by calcium-bound calmodulin (Braun & Schulman 1995). In addition to MARK1–4, other isoforms are also found due to alternative splicing (Matenia & Mandelkow 2009). MARK1–4 exhibit the following conserved functional regions: N-terminal header (N), catalytic kinase domain (KD), common docking domain (CD), ubiquitin-associated domain (UBA), spacer region, and kinase-associated domain 1 (KA1) motif-containing tail domain (Fig. 2). The spacer region is the least conserved and the most variable region among the four MARK isoforms (Timm et al. 2008b, Matenia & Mandelkow 2009).

**Regulation of MARKs**

Like many kinases, MARKs are involved in multiple signaling pathways and are regulated by a variety of mechanisms. Phosphorylation of the KD by upstream kinases like MARK kinase (MARKK) can lead to activation of MARK. Activated MARK can phosphorylate tau protein and other related MAP proteins such as MAP2 and MAP4, which have affinities for stabilizing MTs, and also can phosphorylate other proteins involved in cell signaling and 14-3-3 (also known as Par 5, partitioning defective protein 5) binding (Drewes 2004, Timm et al. 2008b). However, phosphorylation of KD by a kinase such as glycogen synthase kinase 3β (GSK3β [GSKB]) can lead to MARK inhibition. MARKs can also be activated in a phosphorylation-independent manner through binding of regulatory protein, such as the activating protein-2 (AP-2) protein complex to the KD (Schmitt-Ulms et al. 2009). Likewise, MARKs can also be inhibited through...
other mechanisms. For example, a conformational change where the tail domain binds to the KD can lead to MARK inhibition (Elbert et al. 2005, Matenia & Mandelkow 2009). Table 2 illustrates some of the known regulators of MARK in mammalian cells.

### Function of MARKs

Sertoli cell (SC) MTs play an indispensable role during spermatogenesis. MTs are involved in maintenance of SC structure, vesicle transport, tubule fluid secretion, and transport of maturing spermatids (Redenbach & Vogl 1991, Vogl et al. 2008, Smith et al. 2012). These events are possible only because of the dynamic nature of MTs. Throughout spermatogenesis, MTs are never in a static state and must be regulated by a host of factors. For example, KATNAL1, a recently identified MT severing protein, is one such regulator of MTs in the SC (Smith et al. 2012). MAPs are another class of proteins that regulate MTs; however, much of the current information regarding the regulation of MAPs is not specific to the testis. Interestingly, the testis is an abundant source of MTs (Loveland et al. 1996). MTs are intimately associated with the cycle of events that take place in SCs with regard to the progression of spermatogenesis (Vogl et al. 2008). Thus, discovering new information regarding MT regulation in

### Table 2 Regulators of MARK in mammalian cells

<table>
<thead>
<tr>
<th>Name of regulator</th>
<th>Method of regulation</th>
<th>Effect on MARK</th>
<th>Additional information</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MARKK/TAO-1</td>
<td>Phosphorylation of Thr in KD (T208 in MARK2)</td>
<td>Activation</td>
<td>Upstream activating kinase TESK1 (LIM-motif containing kinase) can inactivate MARKK</td>
<td>Timm et al. (2006, 2008b), Johnne et al. (2008) and Matenia &amp; Mandelkow (2009)</td>
</tr>
<tr>
<td>LKB1</td>
<td>Phosphorylation of Thr in KD (T208 in MARK2)</td>
<td>Activation</td>
<td>Upstream activating kinase</td>
<td>Brajenovic et al. (2004), Kojima et al. (2007) and Tanwar et al. (2012)</td>
</tr>
<tr>
<td>CAMK1</td>
<td>Phosphorylation of KD</td>
<td>Activation</td>
<td>Phosphorylation sites differ from MARKK and LKB1 Can override activation by MARKK or LKB1 Also independently affects actin cytoskeleton by activating cofilin</td>
<td>Timm et al. (2008a)</td>
</tr>
<tr>
<td>GSK3β</td>
<td>Phosphorylation of Ser (S212 in MARK2)</td>
<td>Inhibition</td>
<td></td>
<td>Timm et al. (2006)</td>
</tr>
<tr>
<td>PAK5</td>
<td>Binds to KD</td>
<td>Inhibition</td>
<td></td>
<td>Schmitt-Ulms et al. (2009)</td>
</tr>
<tr>
<td>AP-2 protein complex</td>
<td>Binds to KD</td>
<td>Regulation of MT-dependent trafficking of CCVs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aPKC</td>
<td>Phosphorylation of spacer domain</td>
<td>Inhibition</td>
<td>Creates 14-3-3 binding motif Involved in conferring cell polarity</td>
<td>Hurov et al. (2004), Watkins et al. (2008) and Matenia &amp; Mandelkow (2009)</td>
</tr>
<tr>
<td>Adaptor protein 14-3-3 (Par5)</td>
<td>Binds to spacer domain (phosphorylation dependent) or interacts with KD (phosphorylation independent)</td>
<td>Inhibition</td>
<td>Causes conformational change</td>
<td>Elbert et al. (2005) and Matenia &amp; Mandelkow (2009)</td>
</tr>
<tr>
<td>Tail domain</td>
<td>Binds to KD or N-terminal head</td>
<td>Inhibition</td>
<td></td>
<td>Benton &amp; St Johnston (2003) and Matenia &amp; Mandelkow (2009)</td>
</tr>
<tr>
<td>Cofactors</td>
<td>Bind to CD site</td>
<td>Multiple interactions with upstream and downstream effectors</td>
<td></td>
<td>Timoue &amp; Nishida (2003) and Timm et al. (2008b)</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>Polyubiquitination of UBA domain</td>
<td>Intracellular signaling</td>
<td>This is only a proposed regulatory role based on similarity to UBA domain of MAP kinases</td>
<td>Panneerselvam et al. (2006)</td>
</tr>
</tbody>
</table>

CCVs, clathrin-coated vesicles; Ste20, sterile 20 protein; PAK5, p21-activated kinase 5; TAO-1, thousand and one amino acid protein kinase; TESK1, testis-specific protein kinase 1; T, Thr; S, Ser.
the testis may not only lead to a better understanding of processes such as spermatogenesis but may also contribute to what is known about MT regulation as a whole across all cell types. The following will highlight one type of MAP regulator, MARK protein, and its proposed role in the testis.

MARKs and their homologs are functionally diverse protein kinases possessing functional roles in cell polarity, MT stability, cell cycle control, and intracellular signaling (Tassan & Le Goff 2004). Par (partitioning defective) kinases are essential for cytoplasmic partitioning, asymmetric cell division, and establishment of cell polarity (Kemphues 2000, Marx et al. 2010). MARK is the mammalian homolog of Par1 (partition-defective kinase 1), which is a serine/threonine kinase first identified in Caenorhabditis elegans, and subsequently identified in Drosophila melanogaster, for its role in antero-posterior (A/P) axis development during embryogenesis (Kemphues et al. 1988, Tassan & Le Goff 2004, Matenia & Mandellkow 2009, Marx et al. 2010). The diverse roles of MARKs are likely due to the nature of the MTs in which they regulate. For instance, cell polarization is possible because the dynamic instability of MTs is regulated by an interplay between stabilizing and destabilizing events (Kaverina & Straube 2011).

**MARKs in the testis**

Given that MARKs are involved in a diverse array of cellular processes, it is conceivable that they also serve important roles in the testis. Among all the MARKs, MARK4 is the more prominent MARK isoform found in the testis (Trinczek et al. 2004, Tang et al. 2012). Thus, our recent study focused on the possible localization and role of MARK4 in the SC. MARK4 was detected at the apical ES, BTB, and basement membrane, suggesting that the protein has a functional role in the apical ES–BTB–hemidesmosome/basement membrane axis, which coordinates cellular events like degeneration of apical ES and restructuring of the BTB during spermatogenesis (Yan et al. 2008). Below are some proposed roles of MARK4 in facilitating cross talk and cell polarity in the testis based on current information from the literature and findings from our own study.

**MARK4 and cell polarity**

MARKs play a role in cell polarization by regulating the activity of MAPs through phosphorylation. The MAPs that MARKs regulate serve as a bridge between signal transduction cascades and MTs (Etienne-Manneville 2010). However, the exact functional role of MARK in cell polarization in the testis has not been fully elucidated. Cell polarity proteins have recently been identified in the testis, specifically in Sertoli and germ cells, most notably spermatids. As polarity protein complexes, such as the Par- and Scribble-based protein complexes, can each recruit its binding partners and these two protein complexes also display mutually exclusive distribution patterns, their presence in SCs and spermatids thus confer cell polarity (Iden & Collard 2008, Wong & Cheng 2009). For example, the partitioning-defective3/partitioning-defective6/atypical protein kinase C (Par3/Par6/aPKC) protein complex was shown to regulate spermatid adhesion and confer spermatid polarity during spermiogenesis (Wong et al. 2008). As summarized in Table 2, aPKC can inhibit MARK by phosphorylating the kinase domain. Studies on C. elegans have shown that aPKC of the Par3/Par6/aPKC complex, found at the anterior cortex, can phosphorylate Par1, which is located in the posterior cortex (Hurov et al. 2004). Studies on epithelial cells also reveal that the Par3/Par6/aPKC complex and Par1, a homolog of MARK, are located in different areas of the cell, at TJs and laterally beneath TJs respectively. These findings suggest that Par1 must be physically sequestered from the Par3/Par6/aPKC complex in order to establish cell polarity (Watkins et al. 2008). In the testis, the Par3/Par6/aPKC complex is found at the apical ES (Wong & Cheng 2009). Interestingly, MARK4 was also detected at the apical ES, but its spatiotemporal expression and localization at the apical ES (Tang et al. 2012) is not identical to the Par6-based polarity complex (Wong et al. 2008). For instance, Par6 is limited mostly to the convex side of the elongating/elongated spermatids in stage VII–VIII tubules until it is considerably diminished by stage VIII (Wong et al. 2008), whereas MARK4 is found surrounding the entire head of the developing spermatids in stage IV–VI tubules; however, MARK4 is limited almost exclusively to the concave side of the elongated spermatid heads in stage VII tubules and it becomes dispersed to the entire tip of the spermatid head until it is rapidly diminished by stage VIII of the epithelial cycle (Tang et al. 2012). This distinctive spatiotemporal expression and distribution pattern during the epithelial cycle between MARK4 and Par6 thus illustrates that these two groups of proteins may be working synergistically to confer spermatid polarity during spermatogenesis.

14-3-3 protein has been shown to regulate cell adhesion at the apical ES in the testis and at the BTB to facilitate preleptotene spermatocyte transit (Wong et al. 2009). Studies on C. elegans have shown that phosphorylation by aPKC on MARK can lead to the creation of a 14-3-3 protein binding motif to recruit other binding partners to a specific cellular domain. Thus, it is plausible...
that MARK in the testis may play a similar role in cell polarity through 14-3-3 protein.

In our study, we used the adjudin model to examine MARK4 expression and localization in the seminiferous epithelium. Adjudin is known to induce germ cell loss, most notably spermatids by disrupting the apical ES (Cheng et al. 2011). Treatment of adult rats with adjudin that induced premature loss of elongating/elongated spermatids from the epithelium was found to coincide with a downregulation of MARK4 expression and its mislocalization at the apical ES. In short, the premature release of spermatids from the epithelium is associated with a considerable loss of MARK4 at the apical ES (Tang et al. 2012), similar to the disappearance of Par6 and 14-3-3 at the apical ES in departing spermatids in adjudin-treated rats (Wong et al. 2008, 2009). It is likely that MARK4 exerts its effects, at least in part, by modulating the Par3/Par6/aPKC complex and 14-3-3 at the apical ES to induce premature spermatid loss, analogous to spermatiation that takes place at stage VIII of the epithelial cycle. This possibility must be carefully elucidated in future studies.

**MARK4 and cross talk between cytoskeletons** In eukaryotic cells, actin-, MT-, and IF-based cytoskeletons work in a concerted manner to maintain cell integrity. Though these cytoskeletons are unique and consist of different proteins, they are perpetually in a state of communication with each other. This cross talk has been observed in multiple epithelia, but what remains to be uncovered is the mechanism(s) of cross talk among the cytoskeletal elements in SCs during spermatogenesis.

There is evidence of cross talk between actin and MTs in SCs. During spermatogenesis, developing germ cells are transported from the basal to the apical compartment of the seminiferous epithelium as they mature into spermatids. This spermatid transport process is thought to be the result of a communicative effort among different SC elements. One such element is known as the ES, which is a tripartite complex found at both SC–SC and spermatid–SC interfaces in the seminiferous epithelium (Vogl et al. 2000). It comprises F-actin bundles sandwiched in between cisternae of endoplasmic reticulum and the cytoplasmic side of the SC (Vogl et al. 2000, 2008). The ES is an atypical, testis-specific, adherens junction (AJ) (Mruk & Cheng 2004a). AJs are ubiquitously found at cell junctions, but the ES, in contrast to other AJs, contains protein complexes comprising nectins, cadherins, connexins, JAM-C, and integrins (Mruk & Cheng 2004a, b), in which connexins, JAMs, and integrins are usually restricted to gap junctions, TJs, focal adhesion (FA) complex (or focal contact) at the cell–extracellular matrix interface, respectively. It has been proposed that as F-actin found at the apical ES is noncontractile, and spermatids are nonmotile cells *per se*, longitudinal transport of elongating spermatids is mediated by MT-based motors such as dynein and kinesin (Guttman et al. 2000, Vogl et al. 2000, Lie et al. 2010).

In addition to providing a transport mechanism for elongating/elongated spermatids in the SCs, MTs may also play a role in apical ES restructuring (Lie et al. 2010). A function of the apical ES is to serve as an anchor for developing spermatids. However, before spermatiation, the apical ES must be deconstructed in order for mature spermatids to be released into the lumen at spermatiation (O’Donnell et al. 2011). Disassembly of the apical ES occurs from late stage VII through to stage VIII of the epithelial cycle (Lie et al. 2010). As previously discussed, MTs have an intrinsic polarity. Unlike most motile cells (e.g. fibroblasts and macrophages), the MTs of SCs do not exhibit centrosomal organization in which MT polymerization is initiated at the centrosome (Dammermann et al. 2003). As a result, MTs are oriented longitudinally, rather than radially, with their minus ends pointing apically as in other polarized epithelial cells (Dammermann et al. 2003). Minus ends of MTs are normally sites of anchorage and disassembly (Akhmanova et al. 2009).

We have shown that localization of MARK4 in the seminiferous epithelium is stage specific during the epithelial cycle. When the apical ES begins to degenerate at stage VII or early stage VIII of the seminiferous epithelial cycle, MARK4 is strongly expressed at the concave side of the apical ES (Tang et al. 2012), which is also the site where extensive endocytic vesicle-mediated protein trafficking takes place. It is now known that as the apical ES begins to degrade at late stage VII, it degenerates into an ultrastructure formerly known as apical tubulobulbar complexes where extensive protein endocytosis takes place (Upadhyay et al. 2012), so that apical ES proteins can be transcytosed and recycled to assemble ‘newly’ developed step 8 spermatids in the seminiferous epithelium. Indeed, because ES has been shown to associate with motor proteins at the cytoplasmic face of the ER component of the ES (Guttman et al. 2000), the localization of MARK4 at this site suggests that MARK4 may be facilitating these protein trafficking events. MARK4 expression at the apical ES during the subsequent late stage VIII was greatly reduced. The almost non-detectable level of MARK4 at this stage, when spermatiation occurs, seems to be correlated with spermatid loss. Taken
collectively, these findings suggest that the actin filaments of the apical ES and MTs localized in close vicinity at the apical ES may be involved in cross talk regulated in part by MARK4, such that the reorganization of these two cytoskeletons can be coordinated to facilitate spermatid transport and spermiation.

There are ultrastructures of the SC that resemble components of motile cells. In motile cells, FAs relay regulatory signals and physically participate in cell migration. The dynamic nature of FAs can be attributed to their rapid growth and disassembly. However, FAs per se are not found in the seminiferous epithelium such as by electron microscopy, and MTs in SCs are not as dynamically unstable as in motile cells. Because minus ends do not polymerize in vivo, they require stabilization and can attach to cell junctions, such as AJ s (Dammermann et al. 2003, Lie et al. 2010). In a recent review (Lie et al. 2010), it was suggested that the MT minus ends may participate in the assembly of apical ES and/or may play a role in apical ES restructuring. As the ES is defined as an AJ, MT interaction with the ES may result in transporting the proper signals for restructuring and disassembly of the cell junction during spermatogenesis (Lie et al. 2010). In addition, while there are no FAs in the seminiferous epithelium, they are similar to apical ES in makeup. As both contain similar structural proteins, it is plausible that apical ES disassembly may follow a mechanism similar to FA disassembly.

Cross talk in the testis between MTs and IFs is another area that requires much research. It has already been shown that the interactions between MTs and IFs are important in maintaining the structure of SCs (Amlani & Vogl 1988). SC MTs are parallel in orientation with their minus ends apparently positioned apically and plus ends directed basally; IFs that are concentrated basally in the SC anchor the plus ends of MTs (Neely & Boekelheide 1988, Vogl et al. 1995). A previous co-immunoprecipitation experiment showed that actin-related adaptor proteins, such as zyxin and axin, and Wiskott–Aldrich syndrome protein (WASP) can interact with both vimentin and tubulin (Lee et al. 2004, Lie et al. 2010). This suggests that these adaptor proteins play a role in regulating MTs and desmosomes in the testis; they also support the notion of cross talk among all three cytoskeletons within the SC.

Our initial findings suggest that MARK4 plays a role in regulating desmosomes at the BTB (Tang et al. 2012). Desmosomes are intercellular junctions in which IFs are anchored to integral desmosomal cadherin proteins via cytolinker desmoplakin (Delva et al. 2009, Lie et al. 2011). Armadillo proteins, such as the plakophilins, reinforce cadherin–desmoplakin interactions (Cowin & Burke 1996). Using co-immunoprecipitation, MARK4 was shown to structurally associate with plakophilin-2 (PKP2; Tang et al. 2012). A previous study sought to examine the physiological significance of the desmosome at the BTB in the testis and reported that a protein complex comprised of connexin 43 and PKP2 regulates BTB dynamics (Li et al. 2009). The findings from this study suggest that the desmosome facilitates the transport of preleptotene spermatocytes at the BTB while maintaining the BTB integrity, and as MARK4 structurally associates PKP2, it is likely that MARK4 plays a functional role in BTB regulation and cross talk between MTs and IFs.

Concluding remarks and future perspectives

This review briefly summarizes current knowledge of MT regulation in the testis. Results from our recent study on MARK4 suggest that this MAP/MT regulating protein may play a critical role in the regulation of MTs during spermatogenesis. The expression of MARK4 in the seminiferous epithelium is highly spatiotemporal during the epithelial cycle, and MARK4 is found in close vicinity of actin filament bundles at the apical ES, coexisting with actin-regulatory proteins at the apical ES (e.g. ARP3 (ACTR3)), illustrating that this MT-regulatory protein may mediate cross talk between the actin- and the MT-based cytoskeletons. Further studies should examine the role of MARK4 and other actin-regulatory proteins (e.g. the Arp2/3-N-WASP protein complex, filamins) and polarity protein complexes (e.g. Scribble/Lgl/Dlg complex, Par-based complex) in coordinating changes in the organization of the actin filament bundles and the MTs at the apical ES to affect spermatid transport and movement during spermatogenesis. Furthermore, much work is needed to identify other players in regulating MT dynamics in the testis and many questions are open to be addressed. For instance, what are the molecules involved in directing the transport of spermatids? This likely involves small GTPases and ATPs, and perhaps other non-receptor proteins kinases, such as FAK, c-Src, and c-Yes.

Declaration of interest
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