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Microtubule-associated proteins (MAPs) in microtubule cytoskeletal dynamics and spermatogenesis

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Summary. The microtubule (MT) cytoskeleton in Sertoli cells, a crucial cellular structure in the seminiferous epithelium of adult mammalian testes that supports spermatogenesis, was studied morphologically decades ago. However, its biology, in particular the involving regulatory biomolecules and the underlying mechanism(s) in modulating MT dynamics, are only beginning to be revealed in recent years. This lack of studies in delineating the biology of MT cytoskeletal dynamics undermines other studies in the field, in particular the plausible therapeutic treatment and management of male infertility and fertility since studies have shown that the MT cytoskeleton is one of the prime targets of toxicants. Interestingly, much of the information regarding the function of actin-, MT- and intermediate filament-based cytoskeletons come from studies using toxicant models including some genetic models. During the past several years, there have been some advances in studying the biology of MT cytoskeleton in the testis, and many of these studies were based on the use of pharmaceutical/toxicant models. In this review, we summarize the results of these findings, illustrating the importance of toxicant/pharmaceutical models in unravelling the biology of MT dynamics, in particular the role of microtubule-associated proteins (MAPs), a family of regulatory proteins that modulate MT dynamics but also actin- and intermediate filament-based cytoskeletons. We also provide a timely hypothetical model which can serve as a guide to design functional experiments to study how the MT cytoskeleton is regulated during spermatogenesis through the use of toxicants and/or pharmaceutical agents.

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Introduction

MAPs (microtubule-associated proteins) are comprised of multiple families of proteins known to regulate microtubule (MT) dynamics, namely MT assembly, nucleation or polymerization, disassembly or depolymerization and shrinkage (also called catastrophe), rescue, and stabilization. MAPs are present in virtually all mammalian cells, most notably Sertoli cells in the testis, to support spermatogenesis by conferring changes of MT cytoskeletal organization in the seminiferous epithelium during different stages of the epithelial cycle. The dynamic changes of MTs across the seminiferous epithelium are necessary to support extensive cellular changes during self-renewal of undifferentiated spermatogonia via mitosis, and meiosis to produce haploid spermatids, to be followed by changes in cell shape, packaging of genetic materials, and development of the acrosome and spermatid head, as well as elongation of the tail (Hermo et al., 2010a-e). However, few in-depth reports are found in the literature that probe the role of MAPs in modulating MT cytoskeletal function in the testis to support spermatogenesis until recent years (O'Donnell and O'Bryan, 2014; Tang et al., 2016a). Based on their different actions, MAPs can be categorized into: (i) structural MAPs (Figs. 1-3; Table 1) which stabilize MTs (Bodakuntla et al., 2019; Melková et al., 2019) and are involved in modulating MT dynamics (Fig. 4); (ii) motor proteins that drive the transport of germ cells and cargoes (e.g., phagosomes, residual bodies) along the MT-based tracks (Fig. 1) (Hirokawa and Noda, 2008; Hirokawa et al., 2009; Bhabha et al., 2016) such as dynein 1 as noted in Fig. 5; (iii) end-binding proteins

that associate with plus (+) or minus (-) ends of MTs to stabilize MTs (Akhmanova and Steinmetz, 2015; Mao et al., 2020) (Fig. 1); (iv) proteins that depolymerize or cleave/severe MTs (e.g., katanin, spastin, fidgetin) (Goodson and Jonasson, 2018; McNally and Roll-Mecak, 2018) (Figs. 1, 4); and (v) MT nucleators (Roostalu and Surrey, 2017; Tovey and Conduit, 2018) (Fig. 1). These various functions of different categories of MAPs thus illustrate the physiological significance of MAPs in modulating MT cytoskeleton organization and MT dynamics in the testis to support spermatogenesis. Nonetheless, the precise roles of these proteins in supporting spermatogenesis in the testis remain largely unexplored (O'Donnell and O'Bryan, 2014; Tang et al., 2016a). Herein, we place emphasis of our discussion on structural MAPs, MT-dependent motor proteins and the end-binding proteins since abundant emerging evidence in these several research areas should spark interest

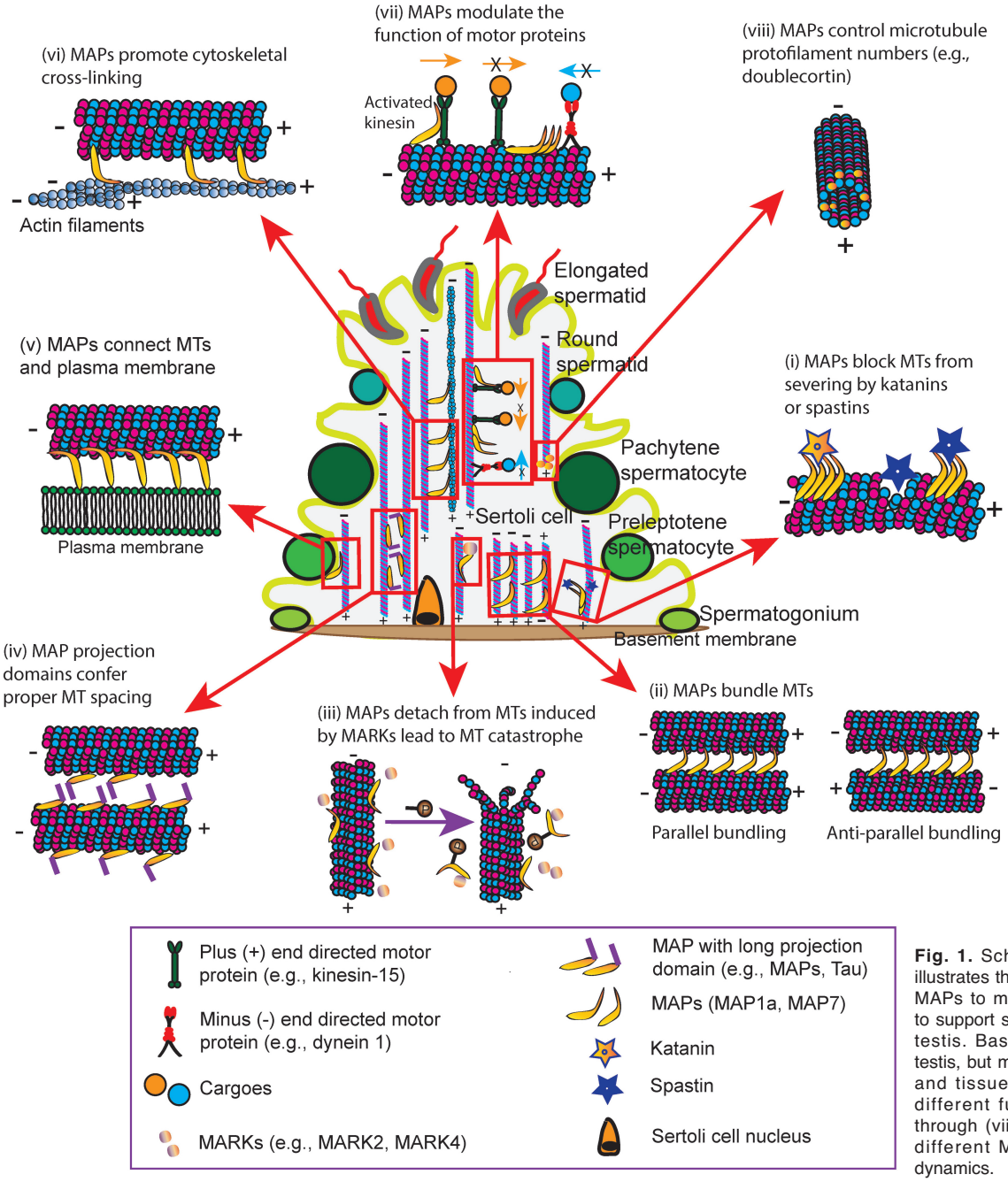

Fig. 1. Schematic drawing that illustrates the different functions of MAPs to modulate MT dynamics to support spermatogenesis in the testis. Based on studies in the testis, but mostly in other epithelia and tissues/organs, at least 8 different functions, namely (i) through (viii) can be ascribed to different MAPs to support MT dynamics.

among reproductive biologists. Interestingly, some of the observations discussed here were unintended discoveries and obtained based on the use of toxicant and/or pharmaceutical models originally aimed at other purposes. As such, studies based on the use of these models should be of great help to reproductive biologists

Table 1. Functions of various structural MAPs in mammalian cells and tissues.

Function	Related proteins and references
1. Structural MAPs bind to MTs to block MTs from cleavage/severing by katanins, spastins, and related MT severing proteins	MAP4 from Xenopus laevis can inhibit katanin-mediated microtubule severing *in vitro* (McNally et al., 2002); Tau, MAP2, and MAP4 can protect microtubules against severing by overexpressed katanin in mammalian cells (Qiang et al., 2006); overexpression of tau protects against spastin-induced MT-severing in cells (Yu et al., 2008).
2. Structural MAPs bind to MTs to promote MT parallel or anti-parallel bundling	PRC1, the human MAP65, crosslinks antiparallel microtubules (Bieling et al., 2010; Subramanian et al., 2010); tripartite motif containing (TRIM) protein TRIM46 forms closely spaced parallel microtubule bundles (van Beuningen et al., 2015); AtMAP65-5 and AtMAP65-1, both proteins promote the formation of a planar network of antiparallel microtubules.(Gaillard et al., 2008).
3. Structural MAPs bind to MTs to stabilize microtubules, preventing MTs from undergoing shrinkage and catastrophe	MARKs, such as MARK2 and MARK4, can phosphorylate MAPs, causing their detachment from microtubules, leading to MT shrinkage and catastrophe (Ramkumar et al., 2018).
4. Structural MAPs bind to MTs to confer property MT spacing	MAP2 (Chen et al., 1992), MAP4 (Nguyen et al., 1997) and Tau (Chen et al., 1992) with projection domain.
5. Structural MAPs bind to MTs to connect to plasma membrane	Tau localizes to the inner side of the plasma membrane (Brandt et al., 1995), by connecting microtubules to the plasma membrane; MACF1 also associates with the membrane of Golgi vesicles, mediating their transport from the trans-Golgi network to the cell periphery (Kakinuma et al., 2004); MAP1B regulates the degradation of Rab35 (Villarroel-Campos et al., 2016), suggesting a role in membrane trafficking in neurons.
6. Structural MAPs bind to MTs to promote cytoskeletal cross-linking, such as with actin filaments or intermediate filaments	Microtubule-actin crosslinking factors 1(MACF1) was initially discovered as actin crosslinking factor 7 (ACF7) (Leung et al., 1999a,b; Karakesisoglou et al., 2000); all members of the MAP1 family (Halpain and Dehmelt, 2006) including MAP1A (Pedrotti et al., 1994), MAP1B (Tögel et al., 1998), and MAP1S interact with actin filaments through their corresponding actin binding domain.
7. Structural MAPs bind to MTs to modulate the function of motor proteins either by activating motor proteins or acting as road blocks to inactivate motor proteins dynein and kinesin	MAP7 family proteins regulate kinesin-1 through recruitment and activation (Barlan et al., 2013; Hooikaas et al., 2019); MAP7 is a positive regulator of kinesin-1, and kinesin-3 (KIF1A) is inhibited by MAP7 but MAP7 has no effect on dynein 1 (Monroy et al., 2018); doublecortin (DCX) and doublecortin-like kinase 1 (DCLK1) promote the motility of kinesin-3 motors in neurons (Liu et al., 2012; Lipka et al., 2016); neuronal MAP tau reduces kinesin-1 activity. (Vershinin et al., 2007; Dixit et al., 2008).
8. Structural MAPs bind to MTs to control the 13 protofilaments in MTs	Doublecortin (DCX) (Fourniol et al., 2010).

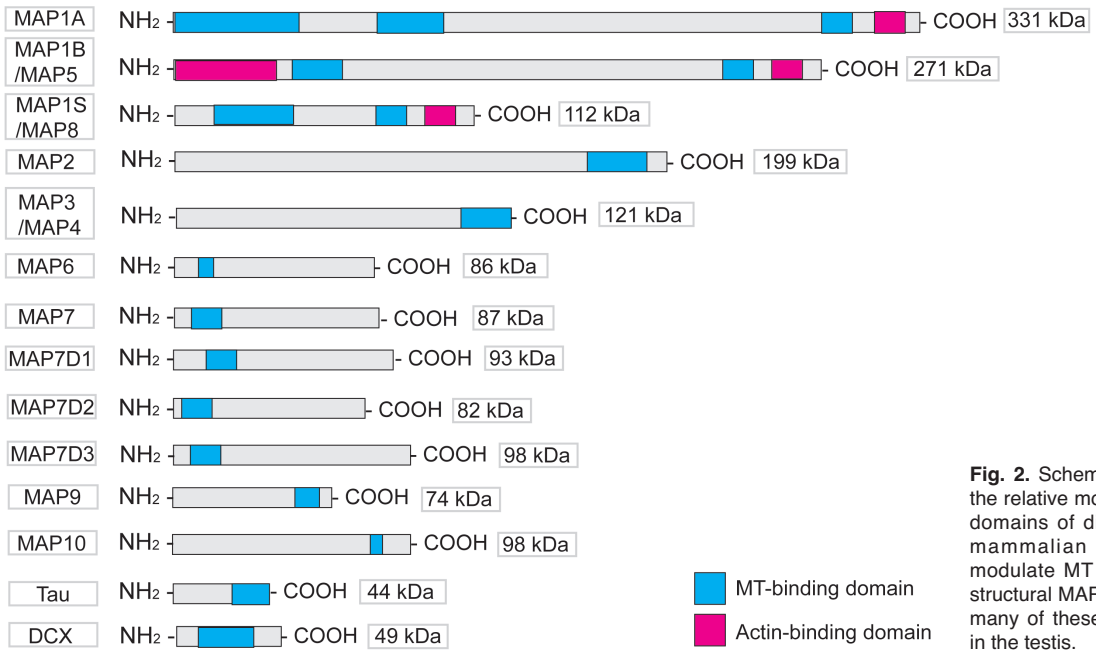

Fig. 2. Schematic drawing that illustrates the relative molecular sizes and functional domains of different structural MAPs in mammalian cells and tissues that modulate MT dynamics. Representative structural MAPs are shown here, although many of these remain to be investigated in the testis.

in the years to come besides reproductive toxicologists.

Structural MAPs

Introduction

Studies have shown that structural MAPs are comprised of more than a dozen proteins (Bodakuntla et al., 2019). Some representative MAPs are shown in Figs. 1-3, including the best studied Tau protein in the brain which is crucial to promote MT stabilization, bundling and polymerization based on studies *in vitro* (Murphy and Borisy, 1975; Weingarten et al., 1975; Sloboda et al., 1976; Kanai et al., 1989). Furthermore, studies on MAP8, initially called VCY2-interacting protein 1 (VCY2IP-1) which interacts with a testis-specific protein VCY2, have shown that MAP8 is crucial to support spermatogenesis and male fertility (Wong et al., 2004). Since the amino acid sequence of VCY2IP-1 (i.e., MAP8) shares 41.9% and 59.3% homology to MAP1A and MAP1B in humans (Wong et al., 2004), it is now referred to as MAP1S (Orbán-Németh et al., 2005) (Fig. 2). To date, structural MAPs have been shown to bind to MTs, which in turn affect MT structure and function by promoting MT stability through their effects (Figs. 2-4; Table 1) (Barlan and Gelfand, 2017; Bodakuntla et al., 2019) that: (i) block MT from severing by katanin or related MT severing proteins (e.g., katanin-like protein, spastin and fidgetin) (Sharp and Ross, 2012; McNally and Roll-Mecak, 2018); (ii) promote MT parallel bundling (e.g., TRIM46) or anti-parallel bundling (e.g., PRC1) (Walczak and Shaw, 2010; Royle, 2013; Harterink et al., 2019); (iii) promote cytoskeletal cross-linking, such as with actin filaments and also vimentin-based intermediate filaments (Cammarata et al., 2016; Miao et al., 2017); (iv) connect to plasma membrane (Royle, 2013), (v) confer proper MT spacing (such as MAP2 through its projection domains) (Chen et al.,

Table 2. Phenotypes in the testis following KO of structural MAPs in mice.

Gene name	Study model	Phenotypes
Map1a	Map1a-/- mice	Mice had learning and memory disturbances, due to defects in neuron synapse function (Takei et al., 2015).
	Spontaneous mutation of nm2719 that disrupts Map1a gene in mice	Tremors, ataxia, and loss of cerebellar Purkinje neurons in aged homozygous mice (Liu et al., 2015a).
Map1b	Map1b-/- mice	Map1b KO mice had defects in synapse formation in the brain (Bodaleo et al., 2016).
	MAP1BΔ93, an allele carrying a deletion of most of the Map1b gene	Mice heterozygous for Map1b deletion had no detectable defects. Homozygous mutants were viable but displayed developmental defects in the brain such as considerable reduction of myelinated axons that led to reduced nerve conduction velocity (Meixner et al., 2000).
	Map1b mutants (R21 mutant mice)	Heterozygotes of Map1b disruption exhibited no overt abnormalities in development and behavior, homozygotes displayed a slightly reduced brain weight and delayed nervous system development (Takei et al., 1997).
	Map2-/-Map1b-/- mice	Map2-/-Map1b-/- mice died perinatally due to retarded neuronal migration, suppression of axonal and dendritic elongation and defects in microtubule bundling in neurons (Teng et al., 2001).
Map1s	Map1s-/- mice	Map1s KO mice had shortened lifespan due to defects in liver function marked with sinusoidal dilation and increased oxidative stress (Li et al., 2016); no obvious defects were found in development, reproduction, or behavior, but these mice had defects in nutritive stress-induced autophagy for nutrient recycling (Xie et al., 2011); renal fibrosis found in aged >12 month-old mice (Xu et al., 2016).
Map2	Map2-/- mice	Map2-/- mice were viable, fertile, and apparently normal (Teng et al., 2001; Harada et al., 2002); but expression of kinase-associated phosphatase (KAP) was reduced in cortical dendrites and the amount of KAP bound to microtubules in the brain of Map2-/- mice was considerably reduced (Iriuchijima et al., 2005); considerable reduction of PKA in dendrites in hippocampal tissue and neurons (Harada et al., 2002); while Map2-/- mice were fertile and no evidence of premature mortality, but their body weights were 10-20% less vs. the littermate controls (Harada et al., 2002).
Map6 (also known as STOP, stable tubule-only polypeptide)	Map6-/- mice	Map6-/- mice had defects in neurotransmission of signals due to reduced synaptic function which led to behavioral disorders such as schizophrenia (Fournet et al., 2012; Daoust et al., 2014); Map6 deletion in mice also led to defects in brain and skeletal muscle function that contributed to schizophrenia-like phenotype (Sebastien et al., 2018).
	MAP6+/- mice	Reduced expression of MAP6/STOP in mice led to cognitive defects (Volle et al., 2013).
Map7(Mtap7)	Map7-/- mice	Map7-/- male mice were sterile (Magnan et al., 2009).
Mapt (Map tau)	Mapt-/- mice	Aged (19-20 month-old), but not mid-aged (8-9 month-old), tau KO mice developed Morris Water Maze (MWM) deficits and loss of hippocampal acetylated α-tubulin and excitatory synaptic proteins, and hippocampal deficits. Tau deletion also led to increased levels of Map1a, Map1b and Map2 (Ma et al., 2014); homozygous Mapt-/- mice developed normally and did not display any overt histological abnormalities, but these mice had muscle weakness and memory disturbance (Ikegamia et al., 2000).
	Mapt-/-Map1b-/- double mutant mice	MT disorganization in growth cones; delayed neuronal migration that led to perturbed neuronal layer formation; considerably high incidence of postnatal death in about ~80% of the double mutant mice (Takei et al., 2000).

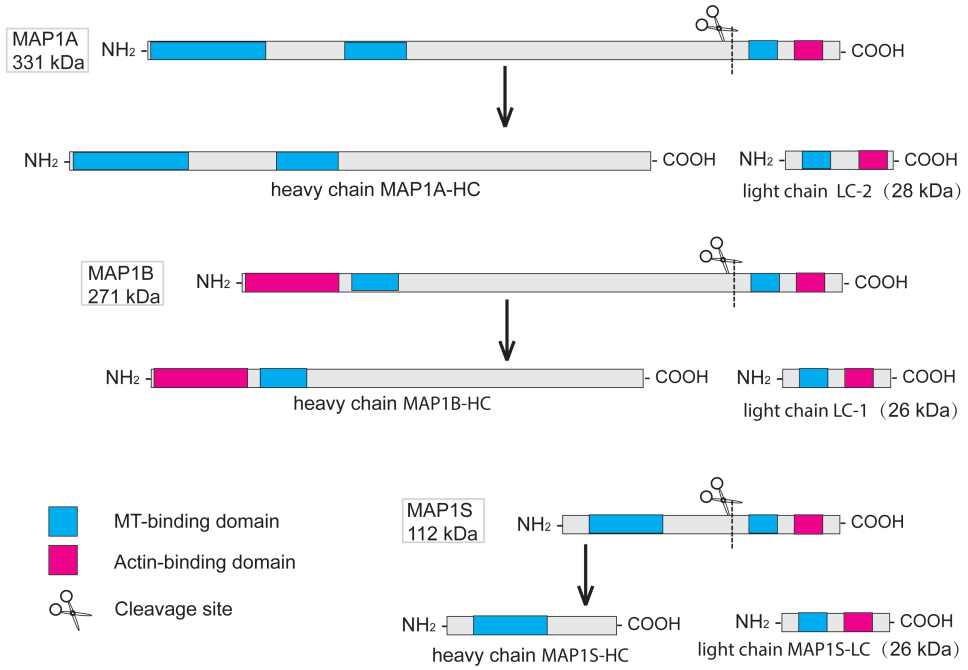

Fig. 3. Schematic drawing that illustrates the vertebrate MAP1 family of MAPs of MAP1A, MAP1B and MAP1S. All three members of the MAP1 family of MAPs are highly expressed in the central and peripheral nervous system and testes (Schoenfeld and Obar, 1994), but MAP1S is ubiquitously expressed (Orbán-Németh et al., 2005). These three proteins are synthesized as precursor polypeptides, which are then cleaved into a heavy chain (HC) and a light chain (LC), which in turn interact with each other to create the MAP1 complex. The HC and LC of MAP1 proteins contain structurally and functionally conversed domains to mediate heavy chain-light chain interactions, microtubule binding and the potential to interact with F-actin and also intermediate filament (see also Fig. 1).

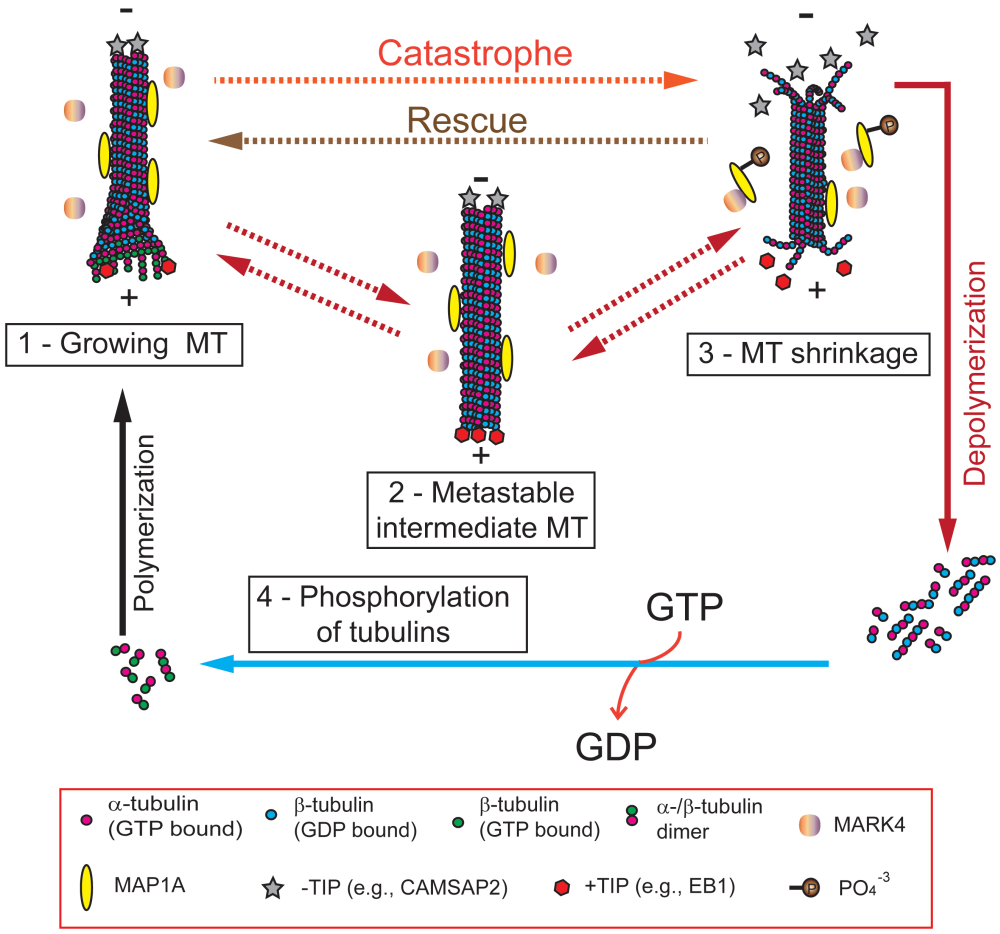

Fig. 4. Schematic drawing that illustrates the different states of the MTs in the testis to confer MT dynamics to support different cellular events pertinent to spermatogenesis in the testis. The growing MTs can undergo rapid shrinkage that leads to catastrophe due to phosphorylation of MAPs, such as MAP1A, which in turn detach from microtubules, thereby destabilizing MTs to facilitate catastrophe. Growing MTs can also assume a metastable state of intermediate MTs. However, when more MAPs are available that bind to MTs to stabilize microtubules, an MT shrinkage state can be rescued. Also, MT catastrophe can lead to depolymerization, and the α- and ß-subunits can be phosphorylated to undergo polymerization to generate MTs to support subsequent MT growth in a specific cellular domain across the seminiferous epithelium to support spermiogenesis, spermatid transport and other pertinent events of spermatogenesis.

1992; Mukhopadhyay and Hoh, 2001); (vi) regulate numbers of microtubule protofilaments to be assembled as an MT-based track (e.g., Tau) (Safinya et al., 2016); (vii) modulate the function of motor proteins either by activating motor proteins (e.g., activation of kinesin by MAP7) or acting as a road block to inactivate motor proteins dynein and kinesin (Royle, 2013; Alfaro-Aco and Petry, 2015); and (viii) promote MT stabilization unless it is phosphorylated by MARKs to induce detachment from MTs (Ramkumar et al., 2018) (Fig. 1,

Table 3. Genetic variations and mutations of selected structural MAPs that lead to pathological conditions in humans.

Gene name	Phenotypes
MAP1A	MAP1A protein-truncating variants (PTVs) was associated with autism spectrum disorder (ASD) and/or attention deficit hyperactivity disorder (ADHD) (Satterstrom et al., 2019).
MAP1B	A MAP1B mutation due to a 966 kb deletion that led to intellectual disability, borderline microcephaly, seizures, and normal brain MRI (Liu et al., 2015b); patients (n=5) with predicted loss of function mutations in MAP1B led to periventricular nodular heterotopia (PVNH), with seizures, cognitive impairment, and dysmorphic features (Heinzen et al., 2018); MAP1B mutations led to intellectual disability and extensive white matter deficit (Walters et al., 2018); a patient with a de novo MAP1B mutation led to a phenotype of PVNH, dysgenesis of corpus callosum, global developmental delay, microcephaly, short stature, mild conductive hearing loss, focal epilepsy, and dysmorphic features (Julca et al., 2019).
MAP2	Lack of MAP2 expression is associated with mantle cell lymphoma (MCL) pathogenesis (Vater et al., 2009).
MAP6 (STOP)	STOP mutation is associated with prostate cancer (Barbieri et al., 2012; Hieronymus and Sawyers, 2012).
MAPT	Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17 (Frontotemporal Dementia with Parkinsonism linked to chromosome 17) (Wszolek et al., 2006); a number of sporadic tauopathies, in particular PSP (progressive supranuclear palsy) and CBD (corticobasal degeneration), have been linked to haplotype-specific sequence variations in MAPT (Baker et al., 1999; Di Maria et al., 2000).

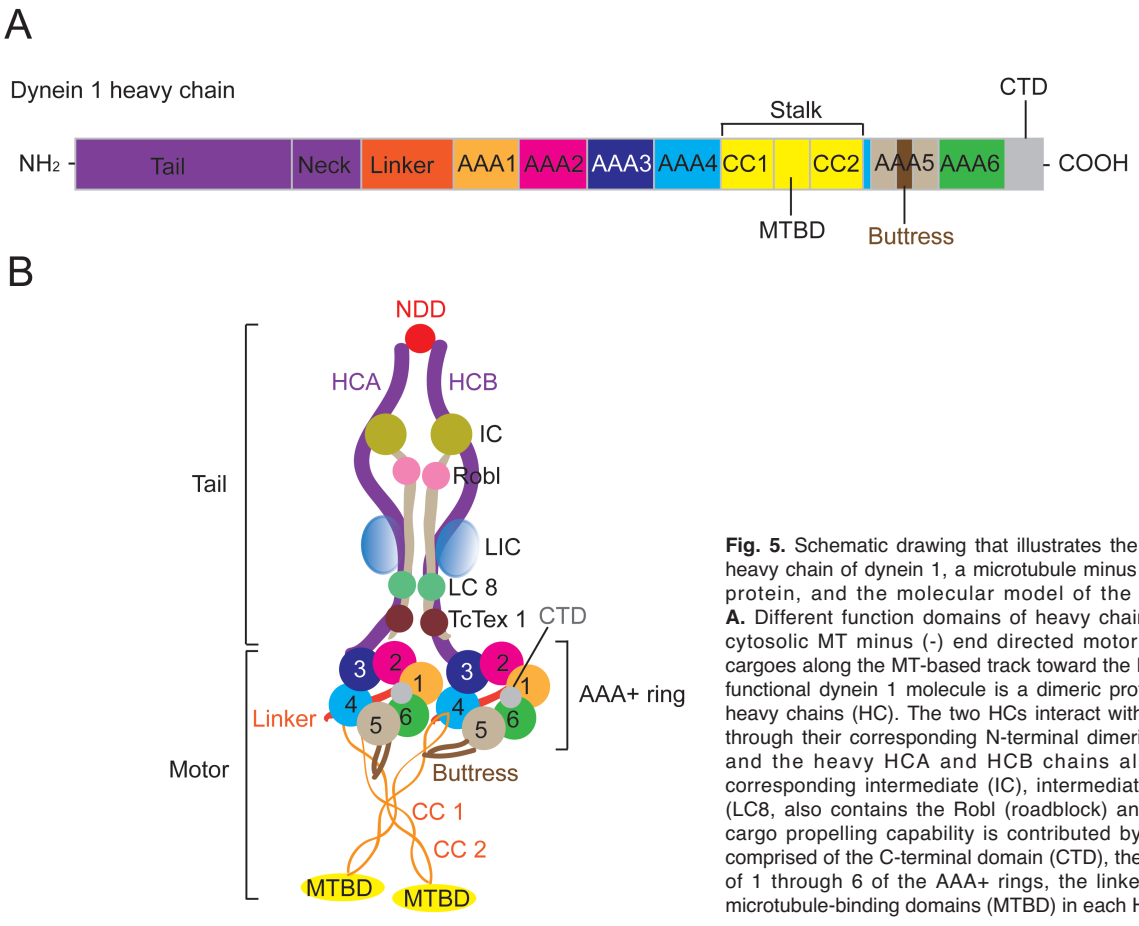

Fig. 5. Schematic drawing that illustrates the functional domains of heavy chain of dynein 1, a microtubule minus (-) end directed motor protein, and the molecular model of the functional dynein 1. **A.** Different function domains of heavy chain (HC) of dynein 1, a cytosolic MT minus (-) end directed motor protein that delivers cargoes along the MT-based track toward the MT minus (-) end. **B.** A functional dynein 1 molecule is a dimeric protein, comprised of two heavy chains (HC). The two HCs interact with each other at the tail through their corresponding N-terminal dimerization domain (NDD), and the heavy HCA and HCB chains also interact with the corresponding intermediate (IC), intermediate light (LIC) and light (LC8, also contains the Robl (roadblock) and TcTex) chains. The cargo propelling capability is contributed by the "motor" which is comprised of the C-terminal domain (CTD), the six ATPases domains of 1 through 6 of the AAA+ rings, the linker, the stalks, and the microtubule-binding domains (MTBD) in each HC.

Table 1). In brief, MAPs, besides regulating MT dynamics, also play a crucial role in modulating actin- and intermediate filament-based cytoskeletons. On the other hand, MAPs can also affect the function of each other, such as by competing with each other to bind onto the same sites in MTs, illustrating the physiological significance of these proteins. For instance, MAP7 has been shown to displace the binding of Tau protein onto MTs *in vitro* (Monroy et al., 2018) and both proteins have been shown to have antagonistic effects *in vivo*, and they both strongly inhibit kinesin-3 motor function (Seitz et al., 2002; Vershinin et al., 2007; Stern et al., 2017; Tymanskyj et al., 2017; Monroy et al., 2018). While the functions of many of the MAPs in the testis

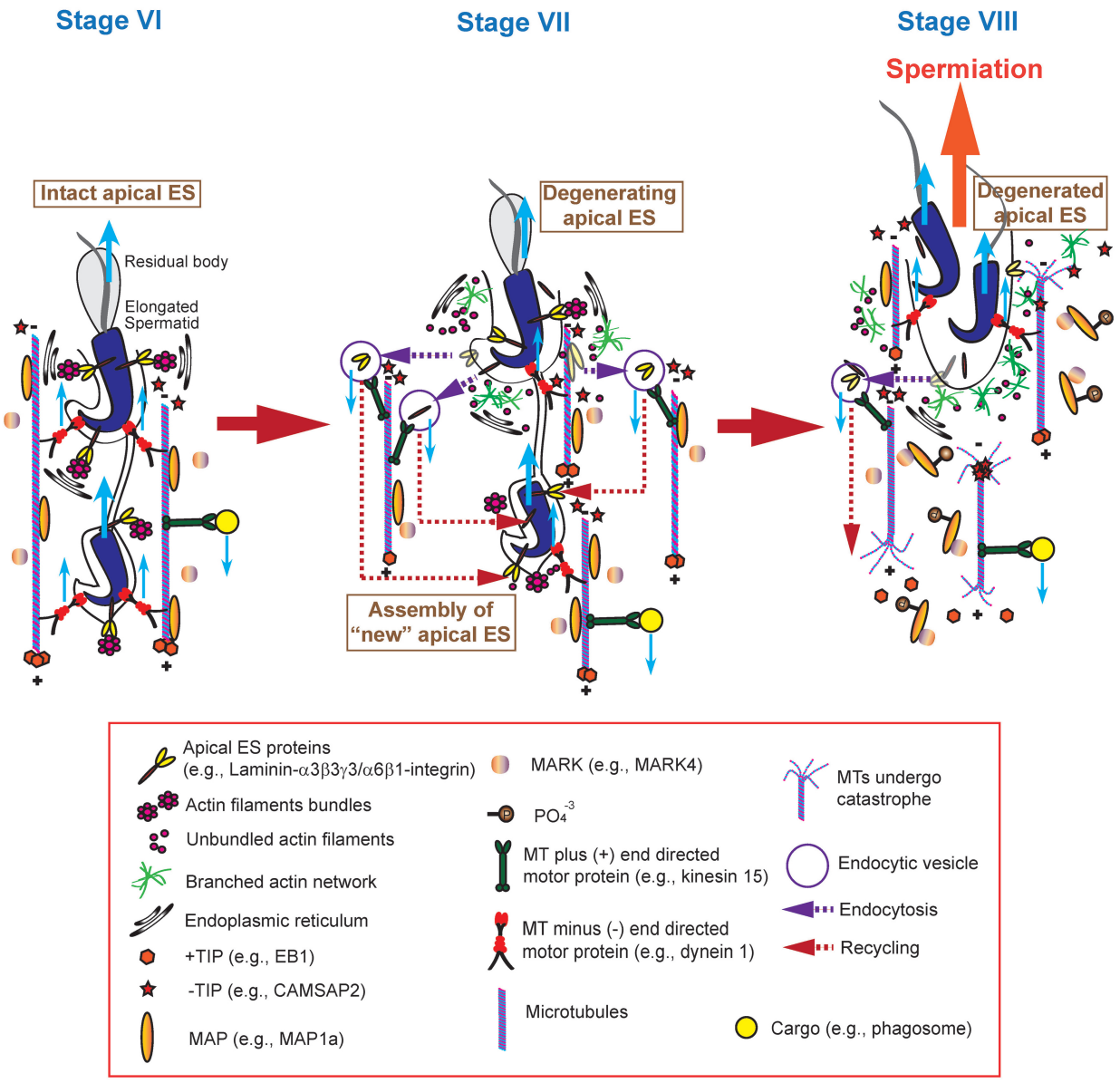

Fig. 6. A hypothetical model on the role of MAPs and the interactions of MAPs with MARKs to modulate MT dynamics to support spermatid transport across the seminiferous epithelium during spermiogenesis. Developing spermatids and other cargoes (e.g., residual bodies, phagosomes) are to be transported to the microtubule minus (-) or (+) end of the MTs during the epithelial cycle. This can be efficiently done via the use of minus (-) end (e.g., dynein 1) or plus (+) end (e.g., kinesins) directed motor proteins using the corresponding MT-based tracks. On the other hand, the integrity of MT-based tracks are maintained by the MAPs (e.g., MAP1A), and +TIPs (e.g., EB1), whereas, MT catastrophe is also necessary to shrink MTs to confer MT plasticity through the action of MARKs (e.g., MARK4) that phosphorylate MAP1A to induce its detachment from MTs and/or the spatial expression of -TIPs (e.g., CAMSP2), thereby facilitating MT shrinkage.

remain to be investigated and better understood regarding their role in supporting spermatogenesis through their effects on MT cytoskeletal organization, the findings summarized in Fig. 4 and Table 1 are important to provide the necessary information to design future functional studies in the testis. Furthermore, findings noted in studies using genetic models to probe the functions of different MAPs to support cell and tissue homeostasis are summarized in Table 2. Table 3 also summarizes results noted in genetic studies in humans when genes encoding for some the structural MAPs are mutated that lead to different variants, which in turn cause different pathological conditions. These findings further support the physiological significance of

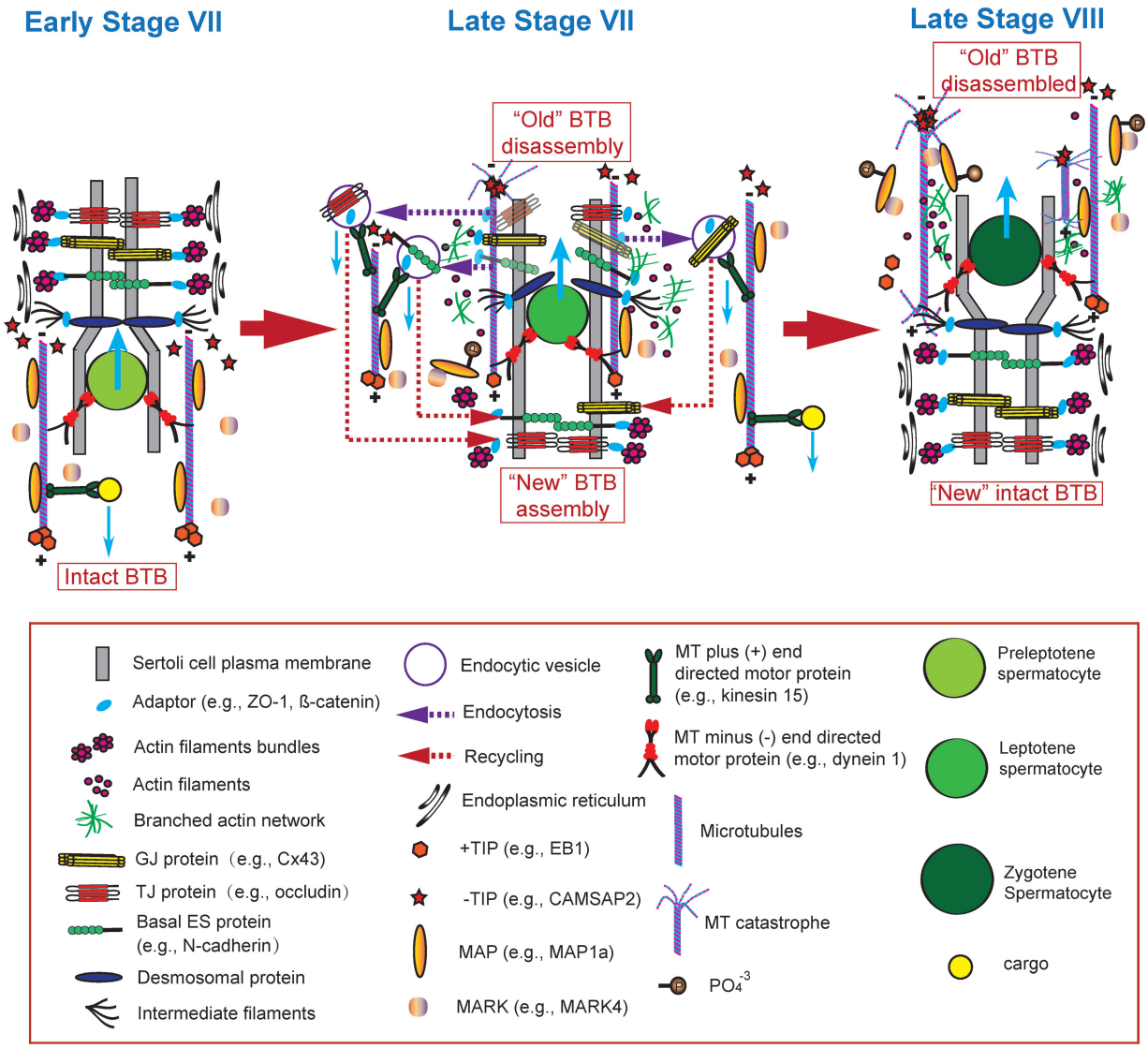

Fig. 7. A hypothetical model on the role of MAPs and the interactions of MAPs with MARKs to modulate MT dynamics at the BTB to support the transport of preleptotene spermatocytes across the immunological barrier during spermatogenesis. The transport of preleptotene spermatocytes across the immunological barrier are analogous to the transport of developing elongating/elongated spermatids across the seminiferous epithelium, involving the microtubule (-) end motor protein dynein 1 that propels preleptotene spermatocytes to pass through the immunological barrier, while differentiating into leptotene spermatocytes using the MTs as the tracks. At the same time, the "old" BTB undergoes remodeling through an increase in protein trafficking events including endocytosis and recycling, wherein the "old" TJ (e.g., occludin, ZO-1), and basal ES (e.g., N-cadherin, ß-catenin) proteins are being transported to the site behind the preleptotene spermatocytes for the assembly of a "new" BTB. The integrity of the MT-based tracks is maintained by MAPs (e.g., MAP1A) and also the binding of +TIP (e.g., EB1) to the MT plus (+) ends. However, phosphorylation of MAPs (e.g., MAP1A) by MARKs (e.g., MARK4) leads to destabilization of MTs, or an enhanced binding of -TIP (e.g., CAMSAP2) to the microtubule minus (-) end also induces MT shrinkage that lead to MT catastrophe, which in turn support the degeneration of the "old" BTB to facilitate the transport of preleptotene spermatocytes across the immunological barrier.

MAPs in both rodents and humans.

Adjudin model

Reports based on the use of a pharmaceutical/toxicant model of probing the role of MAPs (e.g., MAP1a, dynein 1, EB1, MARK4) in modulating MT dynamics to support spermatogenesis in the testis are through the use of an animal model wherein adult rats were treated with adjudin [1-(2,4-dichlorobenzyl)-1H-indazole-3-carbohydrazide] via oral gavage (Mao et al., 2019a; Wu et al., 2019; Yan et al., 2019). Studies have shown that adjudin is a non-hormonal male contraceptive that exerts its contraceptive effects by perturbing Sertoli-germ cell adhesion, which effectively induces germ cell exfoliation, without compromising the population of spermatogonial stem cells and also Sertoli cells in the testis. As such, its effects are highly reversible once it is metabolically cleared from the animals (Mruk et al., 2008; Mok et al., 2012a; Cheng, 2014). Studies have shown that the effective dose of adjudin to induce reversible male contraception in adult rats was at 37.5-50 mg/kg b.w. (via oral gavage), administered weekly with a total of 3 doses (Cheng et al., 2005). In brief, all the seminiferous tubules became virtually devoid of all germ cells (with the exception of undifferentiated spermatogonia) following the first dose of adjudin between 37.5-50 mg/kg b.w. (oral gavage) within ~7-days but spermatozoa found in the epididymis were largely unaffected. Thereafter, germ cells of different classes gradually repopulated the seminiferous epithelium in about 8-10 weeks (Mruk et al., 2008; Mok et al., 2012a; Cheng, 2014). Using this model, it was found that adjudin at a dose as low as 5 mg/kg b.w. (oral gavage) when notable phenotypes of germ cell loss across the seminiferous epithelium were not detected, this low-dose treatment was still capable of down-regulating MAP1a expression in the testis considerably when examined by immunoblot analysis (Yan et al., 2019). At a dose of 50 mg/kg b.w. (via oral gavage), adjudin was able to induce extensive seminiferous epithelial damage, most notably germ cell exfoliation from the testis (Cheng, 2014) by 14 days post-treatment, and >95% of the tubules were devoid of most germ cells except for spermatogonial stem cells and undifferentiated spermatogonia (Mok et al., 2012a; Yan et al., 2019). Concomitant with these changes, the expression of MAP1a in the testis was reduced by as much as 90% when examined by immunoblotting (Yan et al., 2019). When examined by immunofluorescence microscopy using corresponding specific antibodies, MAP1a was robustly expressed across the seminiferous epithelium in normal (control) adult rat testes, appearing as track-like structures. This pattern of staining was analogous to MTs (visualized by staining with α-tubulin, which together with ß-tubulin create the α-/ß-tubulin dimers, serving as the building blocks of MTs) that lie perpendicular to the basement membrane of the seminiferous tubule (Tang et al., 2015, 2016b; Yan et al., 2019). However, few MAP1a track-like structures were detected across the epithelium following adjudin treatment, and for the remaining MAP1a that were detected, they no longer appeared as tracks but defragmented, truncated and considerably shortened as "broken" tracks (Yan et al., 2019). These findings are important, because they illustrate that the adjudin-mediated disruption on MT cytoskeletal organization may relate to a considerable decline in MAP1a expression across the seminiferous epithelium, thereby failing to promote MT integrity to support spermatogenesis. This possibility is further supported by the findings that exposure of rats to adjudin, where its permeation into the adluminal compartment of the seminiferous epithelium in adult rat testes was facilitated by perturbing the BTB transiently via the use of an endogenously produced BTB modifier, the F5-peptide (Mao et al., 2019b). In this study, the expression of EB1 (end binding protein 1, a microtubule plus (+) end tracking protein, +TIP) across the seminiferous epithelium was also considerably down-regulated by at least 80% (Mao et al., 2019b). More important, a similar pattern of expression across the epithelium for CAMSAP2 (calmodulin-regulated spectrin-associated protein, a microtubule minus (-) end targeting protein (-TIP)) was noted (Mao et al., 2019b). Studies based on other epithelia have shown that these +TIP and -TIP proteins, which are members of MAPs, are crucial to support MT dynamics via their differential effects on MTs (Akhmanova and Steinmetz, 2008, 2015). Furthermore, adjudin treatment in adult rats also considerably perturbed the distribution and expression of MARK4 (Mao et al., 2019b), a non-receptor Ser/Thr protein kinase expressed in the testis (Tang et al., 2012), which is known to phosphorylate MAPs to induce their detachment from MTs, thereby facilitating MT catastrophe (Tang et al., 2013; Ramkumar et al., 2018) (Figs. 1, 4). In fact, the pattern of MARK4 distribution following adjudin treatment (Tang et al., 2012) was remarkably similar to EB1, CAMSAP2 and MAP1a (Mao et al., 2019b; Yan et al., 2019). Collectively, the use of the adjudin-based pharmaceutical model thus illustrates that structural MAPs (e.g., MAP1a), and other MAPs (e.g., EB1, a +TIP; CAMSAP2, a -TIP; and MARK4) are functionally involved in maintaining the integrity and functionality of MT-based tracks that laid across the seminiferous epithelium through their effects to confer the MT plasticity. This, in turn, is necessary to support the transport of developing haploid spermatids and other cellular organelles (e.g., residual bodies, phagosomes) across the epithelium to sustain spermatogenesis (Tang et al., 2016b; Li et al., 2017). In fact, it is based on the use of this pharmaceutical/toxicant model, it was able to reconstruct the timely cellular events pertinent to the transport of developing

haploid spermatids across the seminiferous epithelium during spermiogenesis. This concept is shown in the hypothetic model in Fig. 6. The hypothetical concept regarding the transport of preleptotene spermatocytes across the BTB in stage VIII-IX tubules is also summarized in Fig. 7. Other studies have shown that adjudin is a remarkable activator of rpS6, the downstream signaling protein of mTORC1 *in vitro* (Mok et al., 2012b) and *in vivo* (Li et al., 2018). Subsequent studies have confirmed the earlier observations that the mTORC1/p-rpS6/p-Akt1/2 signaling complex is a putative BTB regulatory protein complex, which is capable of perturbing the immunological barrier when p-rpS6 was overexpressed in the Sertoli cell BTB model *in vitro* (Mok et al., 2014, 2015) or in the testis *in vivo* (Li et al., 2018). Indeed, overexpression of a constitutively active mutant of rpS6 designated p-rpS6-MT, wherein the four phosphorylation sites of S235/S236 and S240/S244 were mutated to S235E/S236E and S240E/S244E in p-rpS6 (i.e., a quadruple phosphomimetic mutant), in the testis *in vivo* was able to considerably perturb the Sertoli cell BTB integrity *in vivo*. This change was also associated with extensive germ cell loss from the seminiferous epithelium (Mao et al., 2019a; Wu et al., 2019; Yan et al., 2019). These findings are also consistent with the use of a genetic model when mTOR in Sertoli cells was deleted, which led to male infertility wherein the tubules were devoid of haploid spermatids, and a concomitant surge in p-rpS6-S240/S244 (Boyer et al., 2016). In brief, the initial observation that adjudin exerts its effects to down-regulate the expression of MAP1a (Yan et al., 2019) and other MAPs (e.g., dynein 1, EB1 and CAMSAP2) (Mao et al., 2019a; Mao et al., 2019c; Yan et al., 2019) (see sections below), and a concomitant surge in p-rpS6 expression to activate the mTORC1/p-rpS6 signaling complex (Mok et al., 2012b; Yan et al., 2019) thus provide us with the information to prepare the hypothetical models depicted in Figs. 6, 7.

PFOS model

PFOS (perfluorooctanesulfonate) is a synthetic chemical that acts as a stain repellent used in many consumer products such as carpets, drapery, and clothing, but it is also a known environmental toxicant with a relative long half-life of about 5.4 years (Wan et al., 2013; Tsuda, 2016). Exposure of this toxicant was found to be associated with health risks in humans and animals including reduced fetal growth and endocrine disruption, reproductive dysfunction, and neonatal mortality (Lau et al., 2004; Olsen et al., 2009; Taxvig et al., 2014; Bach et al., 2015). Thus, the use of PFOS in consumable products was banned in Europe, Canada and the U.S. in the 2000s, and eventually China in 2020, except for industrial use in many nations including the United States, such as hydraulic fluids for aviation, photolithography and others. Due to its long half-life, a considerable amount of PFOS could still build up in humans, illustrating that a potential health risk remains following prolonged exposure. Studies using a primary Sertoli cell model *in vitro* in rats (Wan et al., 2014; Gao et al., 2017) and humans (Chen et al., 2017) have shown that the use of PFOS at 20 μM to treat Sertoli cell epithelium with an established functional TJ-permeability barrier that mimics the *in vivo* Sertoli cell BTB for 24 hrs led to transient BTB disruption. Using this PFOS-based toxicant model, it was shown that PFOS exerted its disruptive effects through a down-regulation on the expression of p-Akt1-S473 and p-Akt2-S474, but not total Akts (Gao et al., 2017). This, in turn, perturbed the organization of MTs across the Sertoli cell cytosol since the MT-based cytoskeleton was grossly truncated and failed to stretch across the cell cytosol when compared to control rat Sertoli cells (Gao et al., 2017). This PFOS-mediated MT cytoskeletal disorganization through p-Akt1/2 down-regulation was confirmed via the use of SC79 (2-amino-6-chloro-α-cyano-3-(ethoxycarbonyl)-4H-1-benzopyran-4-acetic acid ethyl ester, Mr 364.78), a specific activator of p-Akt1/2 (Jo et al., 2012), which was found to block the PFOS-mediated Sertoli cell TJ-barrier disruption, but was also capable of blocking the PFOS-mediated defects in Sertoli cell MT cytoskeleton (Gao et al., 2017). For instance, the use of SC79 coupled with PFOS (20 μM) exposure rendered the Sertoli cell epithelium to have proper distribution of MTs across the Sertoli cell cytosol, making them indistinguishable from control cells but in sharp contrast to cells treated with PFOS (20 μM) without SC79 (Gao et al., 2017). More importantly, human Sertoli cells treated with PFOS (20 μM) also displayed similar phenotypes as of the rat Sertoli cells wherein MTs in the human Sertoli cell epithelium were grossly disrupted with truncated and considerably shortened MTs (Chen et al., 2017). However, overexpression of a human p-FAK-Y407E mutant [a constitutively active mutant of p-FAK-Y407, a known BTB promoting signaling protein (Lie et al., 2012), by converting amino acid residue Tyr 407 to Glu, namely p-FAK-Y407E by site directed mutagenesis (Chen et al., 2017) in human Sertoli cell epithelium was capable of blocking the PFOS-mediated Sertoli cell TJ-permeability barrier disruption (Chen et al., 2017). Interestingly, these PFOS-mediated defects in Sertoli cell MT cytoskeleton organization were caused by a defect in spatial expression of EB1 (a +TIP protein in the MAP protein family, see Fig. 1, Table 1) wherein EB1 no longer properly distributed along the MTs as noted in control cells (Chen et al., 2017). However, overexpression of the p-FAK-Y407E mutant was capable of blocking the PFOS-mediated mis-distribution of EB1 across the human Sertoli cell cytosol, making these cells have similar phenotypes of EB1 distribution and MT organization as noted in control cells (Chen et al., 2017). These findings are important since the use of this PFOS-based toxicant model has demonstrated that PFOS exerts its toxic effects in perturbing the MT cytoskeleton

through disruptive expression of p-Akt1/2 and p-FAK-Y407, and by targeting these signaling proteins, it is possible to manage the PFOS-mediated Sertoli cell injury, providing helpful clues to manage toxicant-mediated male reproductive dysfunction.

MT-dependent motor proteins

Introduction

In the testis, one of the best studied MT-dependent motor proteins, also a MAP protein, is dynein 1 (Wen et al., 2018). Cytoplasmic dynein 1 is a MT-dependent minus (-) end directed motor protein which moves cargoes including proteins, food vacuoles, mRNA complexes, endosomes, mitochondria and cell nuclei (Xiang et al., 2015; Carter et al., 2016; Schmidt and Carter, 2016) using the MT-based tracks across the cell cytosol. A functional dynein 1 consists of two identical dynein heavy chains of 500 kDa each and several other subunits (Wen et al., 2016). Each dynein heavy chain (HC), in turn, binds to a light intermediate chain (LIC, 60 kDa), an intermediate chain (IC, 70 kDa), and three light chains (LCs) of LC7, LC8 and Tctex 1 (also known as DYNLT, dynein light chain Tctex-type 1, which forms a tripartite complex with dynein intermediate chain and RagA by linking the small RagA GTPase to the dynein motor protein to modulate dynein 1 function) (Xiang et al., 2015; Carter et al., 2016; Schmidt and Carter, 2016; Wen et al., 2016) (Fig. 5). As such, a functional dynein 1 motor protein is a large multi-protein complex, which utilizes energy generated via ATP hydrolysis to propel cargo (e.g., spermatid) transport across the seminiferous epithelium using MTs as the track (Fig. 5). In this context, it is of interest to note that the dynein 1 motor protein is located in the Sertoli cell cytosol but adjacent to the testis-specific Sertoli cell-elongate spermatid adhesion site known as ectoplasmic specialization (apical ES) (Vogl et al., 2008; Wong et al., 2008) whereas the elongate spermatid under transport is located outside the Sertoli cell yet adjacent to the apical ES. Due to the unique ultrastructural features of the apical ES and the unusual adherence of elongated spermatid onto the Sertoli cell cytoskeleton at the site, the force generated by the dynein 1 motor protein is capable of propelling the elongated spermatid along the MT-based tracks, moving spermatids across the seminiferous epithelium. In fact, this concept is supported by the localization of dynein 1 in the testis, using a specific antibody against the heavy chain of dynein 1, which was found to co-localize with the MT-based tracks across the seminiferous epithelium (Wen et al., 2018). This finding is also consistent with its role to serve as a MT-dependent minus (-) end directed motor protein to transport cargoes (e.g., spermatids) from the base to the tubule lumen across the seminiferous epithelium (Wen et al., 2016) due to the polarized nature of the MTs across the epithelium (Figs. 6, 7).

Dynein 1 knockdown model

The notion regarding the role of dynein 1 in supporting spermatid transport across the seminiferous epithelium is also illustrated by studies wherein dynein 1 was silenced by RNAi in the testis *in vivo*. For instance, following dynein 1 knockdown in the testis, the transport of elongated spermatids across the epithelium was considerably perturbed since step 19 spermatids remained trapped deep inside the seminiferous epithelium in stages IX, X, XI, XII and XIII tubules when they should have been released into the tubule lumen at late stage VIII of the epithelial cycle (Wen et al., 2018). Furthermore, the transport of phagosomes across the epithelium was also perturbed. For instance, phagosomes which should have been transported to the base of the epithelium to facilitate their eventual degradation by Sertoli cells in late stage III-stage IX (Clermont et al., 1987) were found to remain localized in the epithelium near the tubule lumen in these stages (Wen et al., 2018). More important, dynein 1 knockdown in the testis *in vivo* and also Sertoli cells cultured *in vitro* impeded MT polymerization based on biochemical assays using lysates of testes or Sertoli cells (Wen et al., 2018) since intracellular trafficking necessary to coordinate the cellular events of MT dynamics as noted in Figs. 1, 4 to support spermatogenesis in the testis was disrupted. Equally important, dynein 1 knockdown in the testis *in vivo* or primary Sertoli cells cultured *in vitro* also impeded actin polymerization and bundling activity using biochemical assays with lysates of either testes or Sertoli cells (Wen et al., 2018). This latter finding is physiologically important since defects in MT-dependent cellular transport would perturb actin-based cytoskeletal function as these two networks are tightly associated to function to maintain cellular homeostasis. For instance, some MAPs serve as cytoskeletal crosslinkers that bring the actin- and MT-based cytoskeletons together (Fig. 1) to allow these two cytoskeletons to work in concert to support cargo transport and other essential cellular functions, such as directional cell migration (Palazzo and Gundersen, 2002). In fact, actin filament bundles most notably detected at the ES are localized in the testis in close proximity with the MT cytoskeleton to support cell adhesion and transport function (Vogl et al., 2008; Tang et al., 2016a). The two best studied MAPs that function as bona fide crosslinkers of the microtubule and actin cytoskeletons are the microtubule-actin crosslinking factors 1 and 2 [MACF1, also known as actin crosslinking factor 7 (Bernier et al., 1996), and MACF2, also called dystonin (Bernier et al., 1995; Leung et al., 1999b; Karakesisoglou et al., 2000)]. Interestingly, MACF1 and MACF2 also cross-link microtubules and intermediate filaments (Yang et al., 1996; Leung et al., 1999a), which are likely crucial to support ES and BTB function in the testis (Lie et al., 2011). In brief, these findings are important since they illustrate the significance of this class of MAPs, namely MT-dependent motor proteins, to support spermatogenesis

through their role in providing the "engines" to propel cargo (e.g., spermatids) transport using the MT tracks as the "freeways", consistent with the function of this class of MAPs (Fig. 1, Table 1). More important, a knockdown of the MT-dependent motor protein dynein 1 in the testis by RNAi was found not just affected spermatid and cargo transport across the seminiferous epithelium, its knockdown also caused considerable damage to testicular sperm maturation, most likely due to defects in intracellular protein trafficking function (Wen et al., 2018). For instance, dynein 1 silencing in the testis also affected epididymal sperm morphology as multiple sperm defects were noted in the epididymis (Wen et al., 2018). Defects noted in epididymal spermatozoa in the report include the following (Wen et al., 2018). First, sperm heads failed to attach to the sperm tails due to defective mid-piece. Second, the persistent presence of a residual body which engulfed the entire epididymal sperm head. Third, grossly malformed sperm heads such as epididymal sperm with missing tails but only sperm heads. It is of interest to note that structural MAPs also work closely with MT-dependent motor proteins (e.g., dynein 1, kinesins) to modulate cellular functions. As noted in Fig. 1, virtually all MAPs can act as road blocks to disrupt the motor path (Bodakuntla et al., 2019) used by dynein 1 and kinesins (which are corresponding MT minus (-) end and plus (+) end directed motor proteins) (Wen et al., 2016), effectively blocking the motor transport function to the minus (-) or plus (+) end of MTs in mammalian cells. On the other hand, MAPs, most notably MAP7 (also known as ensconsin, and members of the MAP7 family), have been shown to regulate the motor activity of kinesin-1 directly by recruiting kinesin-1 and its activation in HeLa cells (Hooikaas et al., 2019), and also in *Drosophila* S2 cells (Barlan et al., 2013), but it has no effects on dynein 1 (Monroy et al., 2018; Chaudhary et al., 2019).

Adjudin model

Studies using the adjudin model have shown that this pharmaceutical agent that effectively induces germ cell exfoliation by disrupting the actin and MT cytoskeletons also considerably perturbs and down-regulates the expression of dynein 1 across the seminiferous epithelium, wherein dynein 1 no longer expresses and co-localizes with MTs in the epithelium following adjudin treatment (Mao et al., 2019a; Yan et al., 2019). Instead, dynein 1 expression was reduced by almost 90% across the epithelium and the remaining cytoplasmic dynein 1 no longer associated with the MT-based tracks, which were truncated into shorter pieces that laid randomly across the epithelium (Yan et al., 2019). These findings are in agreement with earlier reports that cytoskeletons are the cellular target of many toxicants (e.g., 2,5-hexanedione, carbendazim, cadmium, glycerol) and pharmaceutical agents (e.g., adjudin, vinblastine, Taxol, colchicine) (Russell et al., 1981; Richburg and Boekelheide, 1996; Boekelheide et al., 2003; Cheng, 2014; Johnson, 2014). More important, it appears that these toxicants/pharmaceutical agents exert their effects through the disruptive spatial expression and/or distribution of cytoskeletal regulatory proteins across the seminiferous epithelium. These findings thus illustrate the plausible therapeutic application by targeting motor proteins to manage unexplained male infertility by promoting MT-dependent motor protein function in men, in particular if defects in motor protein function, such as dynein 1 or kinesins, are identified.

Concluding remarks and future perspectives

The use of pharmaceutical/toxicant models *in vitro* and *in vivo*, coupled with studies using gene knockout models by RNAi, have yielded new information regarding the role of MAPs in modifying Sertoli cell MT cytoskeletal organization, which in turn modulates Sertoli cell functions and spermatogenesis. Importantly, findings obtained by using these approaches in studies *in vitro* are also consistent with *in vitro* data. This thus demonstrates that the use of toxicant/pharmaceutical models are helpful tools to study the complex cellular events of spermatogenesis, in particular how cytoskeletal functions are regulated to support testis function. Based on these findings, we propose a hypothetical model regarding the transport of maturing elongate spermatids across the seminiferous epithelium during spermiogenesis (Fig. 6), and the transport of preleptotene spermatocytes across the BTB (Fig. 7). As noted in Figs. 6, 7, MTs serve as the tracks, analogous to the "freeways" on which the MT-based motor proteins, such as dynein 1 (Fig. 5), a MT minus (-) end directed motor protein, coupled with other proteins (e.g., kinesins, many of which are MT plus (+) end directed motor proteins), serve as "the motors in vehicles" to propel spermatids (Fig. 6) and spermatocytes (Fig. 7) across the seminiferous epithelium and the BTB during the epithelial cycle. Based on the model illustrated in Figs. 6, 7, several obvious questions must be addressed in future studies. For instance, how MAPs coordinate with other cytoskeletons, such as actin filaments and vimentin-based intermediate filaments, via the cross-linking MAPs (Fig. 1). What are the upstream signaling biomolecules and cascade that govern the timely spatiotemporal expression of these MAPs briefly summarized here to modulate the organization of MTs as noted in Fig. 4, namely the growth phase, the metastable phase, the shrinkage/catastrophe phase, and the phosphorylation and polymerization phase of MTs? More importantly, what are the commanding regulatory biomolecules that regulate the activation of the signaling biomolecules and the subsequent cascade? Do these involve cytokines and chemokines? Once this information is available, we will have enough knowledge to bring these studies a step closer to the clinic to tackle male infertility or to provide a novel approach to manage (or treat) male fertility including a non-hormonal male pill for men.

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Conflicts of Interest. Authors have nothing to declare.

References

Akhmanova A. and Steinmetz M.O. (2008). Tracking the ends: A dynamic protein network controls the fate of microtubule tips. Nat. Rev. Mol. Cell Biol. 9, 309-322.

Akhmanova A. and Steinmetz M.O. (2015). Control of microtubule organization and dynamics: Two ends in the limelight. Nat. Rev. Mol. Cell Biol. 16, 711-726.

Alfaro-Aco R. and Petry S. (2015). Building the microtubule cytoskeleton piece by piece. J. Biol. Chem. 290, 17154-17162.

Bach C.C., Bech B.H., Brix N., Nohr E.A., Bonde J.P. and Henriksen T.B. (2015). Perfluoroalkyl and polyfluoroalkly substances and human fetal growth: A systematic review. Crit. Rev. Toxicol. 45, 53-67.

Baker M., Litvan I., Houlden H., Adamson J., Dickson D., Perez-Tur J., Hardy J., Lynch T., Bigio E. and Hutton M. (1999). Association of an extended haplotype in the tau gene with progressive supranuclear palsy. Hum. Mol. Genet. 8, 711-715.

Barbieri C.E., Baca S.C., Lawrence M.S., Demichelis F., Blattner M., Theurillat J.P., White T.A., Stojanov P., Van Allen E., Stransky N., Nickerson E., Chae S.S., Boysen G., Auclair D., Onofrio R.C., Park K., Kitabayashi N., MacDonald T.Y., Sheikh K., Vuong T., Guiducci C., Cibulskis K., Sivachenko A., Carter S.L., Saksena G., Voet D., Hussain W.M., Ramos A.H., Winckler W., Redman M.C., Ardlie K., Tewari A.K., Mosquera J.M., Rupp N., Wild P.J., Moch H., Morrissey C., Nelson P.S., Kantoff P.W., Gabriel S.B., Golub T.R., Meyerson M., Lander E.S., Getz G., Rubin M.A. and Garraway L.A. (2012). Exome sequencing identifies recurrent spop, foxa1 and med12 mutations in prostate cancer. Nat. Genet. 44, 685-689.

Barlan K. and Gelfand V.I. (2017). Microtubule-based transport and the distribution, tethering, and organization of organelles. Cold Spring Harb. Perspect. Biol. 9, a025817.

Barlan K., Lu W. and Gelfand V.I. (2013). The microtubule-binding protein ensconsin is an essential cofactor of kinesin-1. Curr. Biol. 23, 317-322.

Bernier G., Mathieu M., De Repentigny Y., Vidal S.M. and Kothary R. (1996). Cloning and characterization of mouse ACF7, a novel member of the dystonin subfamily of actin binding proteins. Genomics 38, 19-29.

Bernier G., Brown A., Dalpé G., De Repentigny Y., Mathieu M. and Kothary R. (1995). Dystonin expression in the developing nervous system predominates in the neurons that degenerate in dystonia musculorum mutant mice. Mol. Cell. Neurosci. 6, 509-520.

Bhabha G., Johnson G.T., Schroeder C.M. and Vale R.D. (2016). How dynein moves along microtubules. Trends Biochem. Sci. 41, 94-105.

Bieling P., Telley I.A. and Surrey T. (2010). A minimal midzone protein module controls formation and length of antiparallel microtubule overlaps. Cell 142, 420-432.

Bodakuntla S., Jijumon A.S., Villablanca C., Gonzalez-Billault C. and Janke C. (2019). Microtubule-associated proteins: Structuring the cytoskeleton. Trends Cell. Biol. 29, 804-819.

Bodaleo F.J., Montenegro-Venegas C., Henriquez D.R., Court F.A. and Gonzalez-Billault C. (2016). Microtubule-associated protein 1B (map1B)-deficient neurons show structural presynaptic deficiencies *in vitro* and altered presynaptic physiology. Sci. Rep. 6, 30069.

Boekelheide K., Fleming S.L., Allio T., Embree-Ku M.E., Hall S.J., Johnson K.J., Kwon E.J., Patel S.R., Rasoulpour R.J., Schoenfeld H.A. and Thompson S. (2003). 2,5-hexanedione-induced testicular injury. Annu. Rev. Pharmacol. Toxciol. 43, 125-147.

Boyer A., Girard M., Thimmanahalli D.S., Levasseur A., Celeste C., Paquet M., Duggavathi R. and Boerboom D. (2016). MTOR regulates gap junction alpha-1 protein trafficking in Sertoli cells and is required for the maintenance of spermatogenesis in mice. Biol. Reprod. 95, 13.

Brandt R., Léger J. and Lee G. (1995). Interaction of tau with the neural plasma membrane mediated by tau's amino-terminal projection domain. J. Cell Biol. 131, 1327-1340.

Cammarata G.M., Bearce E.A. and Lowery L.A. (2016). Cytoskeletal social networking in the growth cone: How +tips mediate mcirotubule-pactin cross-linking to drive axon outgrowth and guidance. Cytoskeleton 73, 461-476.

Carter A.P., Diamant A.G. and Urnavicius L. (2016). How dynein and dynactin transport cargos: A structural perspective. Curr. Opin. Struct. Biol. 37, 62-70.

Chaudhary A.R., Lu H., Krementsova E.B., Bookwalter C.S., Trybus K.M. and Hendricks A.G. (2019). MAP7 regulates organelle transport by recruiting kinesin-1 to microtubules. J. Biol. Chem. 294, 10160-10171.

Chen J., Kanai Y., Cowan N.J. and Hirokawa N. (1992). Projection domains of MAP2 and tau determine spacings between microtubules in dendrites and axons. Nature 360, 674-677.

Chen H., Gao Y., Mruk D.D., Xiao X., John C.M., Turek P.J., Lui W.Y., Lee W.M., Silvestrini B. and Cheng C.Y. (2017). Rescue of PFOS-induced human sertoli cell injury by overexpressing a p-FAK-Y407E phosphomimetic mutant. Sci. Rep. 7, 15810.

Cheng C.Y. (2014). Toxicants target cell junctions in the testis - insights from the indazole-carboxylic acid model. Spermatogenesis 4, e981485.

Cheng C.Y., Mruk D.D., Silvestrini B., Bonanomi M., Wong C.H., Siu M.K.Y., Lee N.P.Y. and Mo M.Y. (2005). AF-2364 [1-(2,4-dichlorobenzyl)-1H-indazole-3-carbohydrazide] is a potential male contraceptive: A review of recent data. Contraception 72, 251-261.

Clermont Y., Morales C. and Hermo L. (1987). Endocytic activities of Sertoli cells in the rat. Ann. NY Acad. Sci. 513, 1-15.

Daoust A., Bohic S., Saoudi Y., Debacker C., Gory-Faure S., Andrieux A., Barbier E.L. and Deloulme J.C. (2014). Neuronal transport defects of the MAP6 ko mouse - a model of schizophrenia - and alleviation by epothilone D treatment, as observed using memri. Neuroimage 96, 133-142.

Di Maria E., Tabaton M., Vigo T., Abbruzzese G., Bellone E., Donati C., Frasson E., Marchese R., Montagna P., Munoz D.G., Pramstaller P.P., Zanusso G., Ajmar F. and Mandich P. (2000). Corticobasal degeneration shares a common genetic background with progressive supranuclear palsy. Ann. Neurol. 47, 374-377.

Dixit R., Ross J.L., Goldman Y.E. and Holzbaur E.L.F. (2008). Differential regulation of dynein and kinesin motor proteins by tau.

Science 319, 1086-1089.

Fournet V., Schweitzer A., Chevarin C., Deloulme J.C., Hamon M., Giros B., Andrieux A. and Martres M.P. (2012). The deletion of STOP/MAP6 protein in mice triggers highly altered mood and impaired cognitive performances. J. Neurochem. 121, 99-114.

Fourniol F.J., Sindelar C.V., Amigues B., Clare D.K., Thomas G., Perderiset M., Francis F., Houdusse A. and Moores C.A. (2010). Template-free 13-protofilament microtubule-map assembly visualized at 8 a resolution. J. Cell Biol. 191, 463-470.

Gaillard J., Neumann E., Van Damme D., Stoppin-Mellet V., Ebel C., Barbier E., Geelen D. and Vantard M. (2008). Two microtubule-associated proteins of arabidopsis map65s promote antiparallel microtubule bundling. Mol. Biol. Cell 19, 4534-4544.

Gao Y., Chen H., Xiao X., Lui W.Y., Lee W.M., Mruk D.D. and Cheng C.Y. (2017). Perfluorooctanesulfonate (PFOS)-induced Sertoli cell injury through a disruption of F-actin and microtubule organization is mediated by Akt1/2. Sci. Rep. 7, 1110.

Goodson H.V. and Jonasson E.M. (2018). Microtubules and microtubule-associated proteins. Cold Spring Harb. Perspect Biol. 10, a022608.

Halpain S. and Dehmelt L. (2006). The MAP1 family of microtubule-associated proteins. Genome Biol. 7, 224.

Harada A., Teng J., Takei Y., Oguchi K. and Hirokawa N. (2002). MAP2 is required for dendrite elongation, PKA anchoring in dendrites, and proper PKA signal transduction. J. Cell Biol. 158, 541-549.

Harterink M., Vocking K., Pan X., Soriano Jerez E.M., Slenders L., Fréal A., Tas R.P., van de Wetering W.J., Timmer K., Motshagen J., van Beuningen S.F.B., Kapitein L.C., Geerts W.J.C., Post J.A. and Hoogenraad C.C. (2019). TRIM46 organizes microtubule fasciculation in the axon initial segment. J. Neurosci. 39, 4864-4873.

Heinzen E.L., O'Neill A.C., Zhu X., Allen A.S., Bahlo M., Chelly J., Chen M.H., Dobyns W.B., Freytag S., Guerrini R., Leventer R.J., Poduri A., Robertson S.P., Walsh C.A., Zhang M., Epi K.C. and Epilepsy Phenome/Genome P. (2018). De novo and inherited private variants in map1b in periventricular nodular heterotopia. PLoS Genet. 14, e1007281.

Hermo L., Pelletier R.M., Cyr D.G. and Smith C.E. (2010a). Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 2: Changes in spermatid organelles associated with development of spermatozoa. Microsc. Res. Tech. 73, 279-319.

Hermo L., Pelletier R.M., Cyr D.G. and Smith C.E. (2010b). Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 4: Intercellular bridges, mitochondria, nuclear envelope, apoptosis, ubiquitination, membrane/voltage-gated channels, methylation/acetylation, and transcription factors. Microsc. Res. Tech. 73, 364-408.

Hermo L., Pelletier R.M., Cyr D.G. and Smith C.E. (2010c). Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 5: Intercellular junctions and contacts between germ cells and sertoli cells and their regulatory interactions, testicular cholesterol, and genes/proteins associated with more than one germ cell generation. Microsc. Res. Tech. 73, 409-494.

Hermo L., Pelletier R.-M., Cyr D.G. and Smith C.E. (2010d). Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 3: Developmental changes in spermatid flagellum and cytoplasmic droplet and interaction of sperm with the zona pellucida and egg plasma membrane. Microsc. Res. Tech. 73, 320-363.

Hermo L., Pelletier R.M., Cyr D.G. and Smith C.E. (2010e). Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 1: Background to spermatogenesis, spermatogonia, and spermatocytes. Microsc. Res. Tech. 73, 241-278.

Hieronymus H. and Sawyers C.L. (2012). Traversing the genomic landscape of prostate cancer from diagnosis to death. Nat. Genet. 44, 613-614.

Hirokawa N. and Noda Y. (2008). Intracellular transport and kinesin superfamily proteins, KIFS: Structure, function, and dynamics. Phyisol. Rev. 88, 1089-1118.

Hirokawa N., Noda Y., Tanaka Y. and Niwa S. (2009). Kinesin superfamily motor proteins and intracellular transport. Nat. Rev. Mol. Cell Biol. 10, 682-696.

Hooikaas P.J., Martin M., Mühlethaler T., Kuijntjes G.J., Peeters C.A.E., Katrukha E.A., Ferrari L., Stucchi R., Verhagen D.G.F., van Riel W.E., Grigoriev I., Altelaar A.F.M., Hoogenraad C.C., Rüdiger S.G.D., Steinmetz M.O., Kapitein L.C. and Akhmanova A. (2019). MAP7 family proteins regulate kinesin-1 recruitment and activation. J. Cell Biol. 218, 1298-1318.

Ikegami S., Harada A. and Hirokawa N. (2000). Muscle weakness, hyperactivity, and impairment in fear conditioning in tau-deficient mice. Neurosci. Lett. 279, 129-132.

Iriuchijima N., Sato-Harada R., Takano M., Fujio K., Sato T., Goto F. and Harada A. (2005). Reduced expression of kinase-associated phosphatase in cortical dendrites of MAP2-deficient mice. Biochem. Biophys. Res. Commun. 338, 1216-1221.

Jo H., Mondal S., Tan D., Nagata E., Takizawa S., Sharma A.K., Hou Q., Shanmugasundaram K., Prasad A., Tung J.K., Tejeda A.O., Man H., Rigby A.C. and Luo H.R. (2012). Small molecule-induced cytosolic activation of protein kinase akt rescues ischemia-elicited neuronal death. Proc. Natl. Acad. Sci. USA 109, 10581-10586.

Johnson K.J. (2014). Testicular histopathology associated with disruption of the sertoli cell cytoskeleton. Spermatogenesis 4, e979106.

Julca D.M., Diaz J., Berger S. and Leon E. (2019). MAP1B related syndrome: Case presentation and review of literature. Am. J. Med. Genet. A 179, 1703-1708.

Kakinuma T., Ichikawa H., Tsukada Y., Nakamura T. and Toh B.H. (2004). Interaction between p230 and MACF1 is associated with transport of a glycosyl phosphatidyl inositol-anchored protein from the Golgi to the cell periphery. Exp. Cell Res. 298, 388-398.

Kanai Y., Takemura R., Oshima T., Mori H., Ihara Y., Yanagisawa M., Masaki T. and Hirokawa N. (1989). Expression of multiple tau isoforms and microtubule bundle formation in fibroblasts transfected with a single tau CDNA. J. Cell Biol. 109, 1173-1184.

Karakesisoglou I., Yang Y. and Fuchs E. (2000). An epidermal plakin that integrates actin and microtubule networks at cellular junctions. J. Cell Biol. 149, 195-208.

Lau C., Butenhoff J.L. and Rogers J.M. (2004). The developmental toxicity of perfluoroalkyl acids and their derivatives. Toxicol. Appl. Pharmacol. 198, 231-241.

Leung C.L., Sun D. and Liem R.K. (1999a). The intermediate filament protein peripherin is the specific interaction partner of mouse bpag1-n (dystonin) in neurons. J. Cell Biol. 144, 435-446.

Leung C.L., Sun. D., Zheng. M., Knowles. D.R. and Liem. R.K.H. (1999b). Microtubule actin cross-linking factor (MACF): A hybrid of dystonin and dystrophin that can interact with the actin and microtubule cytoskeletons. J. Cell Biol. 147, 1275-1286.

Li W., Zou J., Yue F., Song K., Chen Q., McKeehan W.L., Wang F., Xu G., Huang H., Yi J. and Liu L. (2016). Defects in MAP1s-mediated

autophagy cause reduction in mouse lifespans especially when fibronectin is overexpressed. Aging Cell 15, 370-379.

Li L., Tang E.I., Chen H., Lian Q., Ge R., Silvestrini B. and Cheng C.Y. (2017). Sperm release at spermiation is regulated by changes in the organization of actin- and microtubule-based cytoskeletons at the apical ectoplasmic specialization - a study using the adjudin model. Endocrinology 158, 4300-4316.

Li S.Y.T., Yan M., Chen H., Jesus T.T., Lee W.M., Xiao X. and Cheng C.Y. (2018). mTORC1/rpS6 regulates blood-testis barrier (BTB) dynamics and spermatogenetic function in the testis *in vivo*. Am. J. Physiol. Endocrinol. Metab. 314, E174-E190.

Lie P.P.Y., Cheng C.Y. and Mruk D.D. (2011). The biology of the desmosome-like junction: A versatile anchoring junction and signal transducer in the seminiferous epithelium. Int. Rev. Cell Mol. Biol. 286, 223-269.

Lie P.P.Y., Mruk D.D., Mok K.W., Su L., Lee W.M. and Cheng C.Y. (2012). Focal adhesion kinase-tyr407 and -tyr397 exhibit antagonistic effects on blood-testis barrier dynamics in the rat. Proc. Natl. Acad. Sci. USA 109, 12562-12567.

Lipka J., Kapitein L.C., Jaworski J. and Hoogenraad C.C. (2016). Microtubule-binding protein doublecortin-like kinase 1 (DCLK1) guides kinesin-3-mediated cargo transport to dendrites. EMBO J. 35, 302-318.

Liu J.S., Schubert C.R., Fu X., Fourniol F.J., Jaiswal J.K., Houdusse A., Stultz C.M., Moores C.A. and Walsh C.A. (2012). Molecular basis for specific regulation of neuronal kinesin-3 motors by doublecortin family proteins. Mol. Cell 47, 707-721.

Liu Y., Lee J.W. and Ackerman S.L. (2015a). Mutations in the microtubule-associated protein 1a (MAP1a) gene cause purkinje cell degeneration. J. Neurosci. 35, 4587-4598.

Liu Y.F., Sowell S.M., Luo Y., Chaubey A., Cameron R.S., Kim H.G. and Srivastava A.K. (2015b). Autism and intellectual disability-associated kirrel3 interacts with neuronal proteins MAP1b and myo16 with potential roles in neurodevelopment. PLoS One 10, e0123106.

Ma Q.L., Zuo X., Yang F., Ubeda O.J., Gant D.J., Alaverdyan M., Kiosea N.C., Nazari S., Chen P.P., Nothias F., Chan P., Teng E., Frautschy S.A. and Cole G.M. (2014). Loss of MAP function leads to hippocampal synapse loss and deficits in the morris water maze with aging. J. Neurosci. 34, 7124-7136.

Magnan D.R., Spacek D.V., Ye N., Lu Y.C. and King T.R. (2009). The male sterility and histoincompatibility (mshi) mutation in mice is a natural variant of microtubule-associated protein 7 (MAP7). Mol. Genet. Metab. 97, 155-162.

Mao B., Li L., Yan M., Wong C.K.C., Silvestrini B., Li C., Ge R., Lian Q. and Cheng C.Y. (2019a). F5-peptide and mTORC1/rpS6 effectively enhance BTB transport function in the testis-lesson from the adjudin model. Endocrinology 160, 1832-1853.

Mao B.P., Ge R. and Cheng C.Y. (2020). Role of microtubule +TIPs and -TIPs in spermatogenesis - insights from studies of toxicant models. Reprod. Toxicol. 91, 43-52.

Mao B.P., Li L., Yan M., Ge R., Lian Q. and Cheng C.Y. (2019b). Regulation of BTB dynamics in spermatogenesis - insights from the adjudin toxicant model. Toxicol. Sci. 172, 75-88.

Mao B.P., Li L., Ge R.S., Li C., Wong C.K.C., Silvestrini B., Lian Q. and Cheng C.Y. (2019c). CAMSAP2 is a microtubule minus-end targeting protein that regulates BTB dynamics through cytoskeletal organization. Endocrinology 160, 1448-1467.

McNally F.J. and Roll-Mecak A. (2018). Microtubule-severing enzymes: From cellular functions to molecular mechanism. J. Cell Biol. 217, 4057-4069.

McNally K.P., Buster D. and McNally F.J. (2002). Katanin-mediated microtubule severing can be regulated by multiple mechanisms. Cell Moti Cytoskeleton 53, 337-349.

Meixner A., Haverkamp S., Wässle H., Führer S., Thalhammer J., Kropf N., Bittner R.E., Lassmann H., Wiche G. and Propst F. (2000). MAP1b is required for axon guidance and is involved in the development of the central and peripheral nervous system. J. Cell Biol. 151, 1169-1178.

Melková K., Zapletal V., Narasimhan S., Jansen S., Hritz J., Škrabana R., Zweckstetter M., Ringkjøbing Jensen M., Blackledge M. and Žídek L. (2019). Structure and functions of microtubule associated proteins tau and MAP2c: Similarities and differences. Biomolecules 9, 105.

Miao Z., Ali A., Hu L., Zhao F., Yin C., Chen C., Yang T. and Qian A. (2017). Microtubule actin cross-linking factor 1, a novel potential target in cancer. Cancer Sci. 108, 1953-1958.

Mok K.W., Mruk D.D., Lee W.M. and Cheng C.Y. (2012a). Spermatogonial stem cells alone are not sufficient to re-initiate spermatogenesis in the rat testis following adjudin-induced infertility. Int. J. Androl 35, 86-101.

Mok K.W., Mruk D.D., Silvestrini B. and Cheng C.Y. (2012b). RpS6 regulates blood-testis barrier dynamics by affecting F-actin organization and protein recruitment. Endocrinology 153, 5036-5048.

Mok K.W., Mruk D.D. and Cheng C.Y. (2014). RpS6 regulates blood-testis barrier dynamics through Akt-mediated effects on MMP-9. J. Cell Sci. 127, 4870-4882.

Mok K.W., Chen H., Lee W.M. and Cheng C.Y. (2015). RpS6 regulates blood-testis barrier dynamics through Arp3-mediated actin microfilament organization in rat Sertoli cells. An *in vitro* study. Endocrinology 156, 1900-1913.

Monroy B.Y., Sawyer D.L., Ackermann B.E., Borden M.M., Tan T.C. and Ori-McKenney K.M. (2018). Competition between microtubule-associated proteins directs motor transport. Nat. Commun. 9, 1487.

Mruk D.D., Silvestrini B. and Cheng C.Y. (2008). Anchoring junctions as drug targets: Role in contraceptive development. Pharmacol. Rev. 60, 146-180.

Mukhopadhyay R. and Hoh J.H. (2001). AFM force measurements on microtubule-associated proteins: The projection domain exerts a long-range repulsive force. FEBS Letters 505, 374-378.

Murphy D.B. and Borisy G.G. (1975). Assocation of high-molecular weight proteins with microtubules and their role in microtubule assembly *in vitro*. Proc. Natl. Acad. Sci. USA 72, 2696-2700.

Nguyen. H.-L., Chari. S., Gruber. D., Lue. C.-M., Chapin. S.J. and Bulinski. J.C. (1997). Overexpression of full- or partial-length MAP4 stabilizes microtubules and alters cell growth. J. Cell Sci. 110, 281-294.

O'Donnell L. and O'Bryan M.K. (2014). Microtubules and spermatogenesis. Semin. Cell Dev. Biol. 30, 45-54.

Olsen G.W., Butenhoff J.L. and Zobel L.R. (2009). Perfluoroalkyl chemicals and human fetal development: An epidemiologic review with clinical and toxicological perspectives. Reprod. Toxicol. 27, 212-230.

Orbán-Németh Z., Simader H., Badurek S., Tranciková A. and Propst F. (2005). Microtubule-associated protein 1S, a short and ubiquitously expressed member of the microtubule-associated protein 1 family. J. Biol. Chem. 280, 2257-2265.

Palazzo A.F. and Gundersen G.G. (2002). Microtubule-actin cross-talk

at focal adhesions. Sci. STKE 2002, pe31.

Pedrotti. B., Colombo. R. and Islam. K. (1994). Microtubule associated protein MAP1a is an actin-binding and crosslinking protein. Cell Motil. Cytoskeleton 29, 110-116.

Qiang L., Yu W., Andreadis A., Luo M. and Baas P.W. (2006). Tau protects microtubules in the axon from severing by katanin. J. Neurosci. 26, 3120-3129.

Ramkumar A., Jong B.Y. and Ori-McKenney K.M. (2018). Remapping the microtubule landscape: How phosphorylation dictates the activities of microtubule-associated proteins. Dev. Dyn. 247, 138-155.

Richburg J.H. and Boekelheide K. (1996). Mono-(2-ethylhexyl) phthalate rapidly alters both sertoli cell vimentin filaments and germ cell apoptosis in young rat testes. Toxicol. Appl. Pharmacol. 137, 42-50.

Roostalu J. and Surrey T. (2017). Microtubule nucleation: Beyond the template. Nat. Rev. Mol. Cell Biol. 18, 702-710.

Royle S.J. (2013). Protein adaptation: Mitotic functions for membrane trafficking proteins. Nat. Rev. Mol. Cell Biol. 14, 592-599.

Russell L.D., Malone J.P. and MacCurdy D.S. (1981). Effect of the microtubule disrupting agents, colchicine and vinblastine, on seminiferous tubule structure in the rat. Tissue Cell 13, 349-367.

Safinya C.R., Chung P.J., Song C., Li Y., Ewert K.K. and Choi M.C. (2016). The effect of multivalent cations and tau on paclitaxel-stabilized microtubule assembly, disassembly, and structure. Adv. Colloid. Interface Sci. 232, 9-16.

Satterstrom F.K., Walters R.K., Singh T., Wigdor E.M., Lescai F., Demontis D., Kosmicki J.A., Grove J., Stevens C., Bybjerg-Grauholm J., Baekvad-Hansen M., Palmer D.S., Maller J.B., iPSYCH-Broad Consortium, Nordentoft M., Mors O., Robinson E.B., Hougaard D.M., Werge T.M., Bo Mortensen P., Neale B.M., Borglum A.D. and Daly M.J. (2019). Autism spectrum disorder and attention deficit hyperactivity disorder have a similar burden of rare protein-truncating variants. Nat. Neurosci. 22, 1961-1965.

Schmidt H. and Carter A.P. (2016). Review: Structure and mechanism of the dynein motor atpase. Biopolymers 105, 557-567.

Schoenfeld T.A. and Obar R.A. (1994). Diverse distribution and function of fibrous microtubule-associated proteins in the nervous system. Int. Rev. Cytol. 151, 67-137.

Sebastien M., Giannesini B., Aubin P., Brocard J., Chivet M., Pietrangelo L., Boncompagni S., Bosc C., Brocard J., Rendu J., Gory-Faure S., Andrieux A., Fourest-Lieuvin A., Faure J. and Marty I. (2018). Deletion of the microtubule-associated protein 6 (MAP6) results in skeletal muscle dysfunction. Skelet. Muscle 8, 30.

Seitz A., Kojima H., Oiwa K., Mandelkow E.M., Song Y.H. and Mandelkow E. (2002). Single-molecule investigation of the interference between kinesin, tau and MAP2c. EMBO J. 21, 4896-4905.

Sharp D.J. and Ross J.L. (2012). Microtubule-severing enzymes at the cutting edge. J. Cell Sci. 125, 2561-2569.

Sloboda R.D., Dentler W.L. and Rosenbaum J.L. (1976). Microtubule-associated proteins and the stimulation of tubulin assembly *in vitro*. Biochemistry 15, 4497-4505.

Stern J.L., Lessard D.V., Hoeprich G.J., Morfini G.A. and Berger C.L. (2017). Phosphoregulation of tau modulates inhibition of kinesin-1 motility. Mol. Biol. Cell 28, 1079-1087.

Subramanian R., Wilson-Kubalek E.M., Arthur C.P., Bick M.J., Campbell E.A., Darst S.A., Milligan R.A. and Kapoor T.M. (2010). Insights into antiparallel microtubule crosslinking by PRC1, a conserved nonmotor microtubule binding protein. Cell 142, 433-443.

Takei Y., Kondo. S., Harada. A., Inomata. S., Noda. T. and Hirokawa. N. (1997). Delayed development of nervous system in mice homozygous for disrupted microtubule-associated protein 1b (MAP1b) gene. J. Cell Biol. 137, 1615-1626.

Takei Y., Teng. J., Harada. A. and Hirokawa. N. (2000). Defects in axonal elongation and neuronal migration in mice with disrupted tau and MAP1b genes. J. Cell Biol. 150:989-1000.

Takei Y., Kikkawa Y.S., Atapour N., Hensch T.K. and Hirokawa N. (2015). Defects in synaptic plasticity, reduced NMDA-receptor transport, and instability of postsynaptic density proteins in mice lacking microtubule-associated protein 1a. J. Neurosci. 35, 15539-15554.

Tang E.I., Xiao X., Mruk D.D., Qian X.J., Mok K.W., Jenardhanan P., Lee W.M., Mathur P.P. and Cheng C.Y. (2012). Microtubule affinity-regulating kinase 4 (MARK4) is a component of the ectoplasmic specialization in the rat testis. Spermatogenesis 2, 117-126.

Tang E.I., Mruk D.D. and Cheng C.Y. (2013). MAP/microtubule affinity-regulating kinases, microtubule dynamics, and spermatogenesis. J. Endocrinol. 217, R13-R23.

Tang E.I., Mok K.W., Lee W.M. and Cheng C.Y. (2015). EB1 regulates tubulin and actin cytoskeletal networks at the sertoli cell blood-testis barrier in male rats - an *in vitro* study. Endocrinology 156, 680-693.

Tang E.I., Mruk D.D. and Cheng C.Y. (2016a). Regulation of microtubule (MT)-based cytoskeleton in the seminiferous epithelium during spermatogenesis. Semin. Cell Dev. Biol. 59, 35-45.

Tang E.I., Lee W.M. and Cheng C.Y. (2016b). Coordination of actin- and microtubule-based cytoskeletons supports transport of spermatids and residual bodies/phagosomes during spermatogenesis in the rat testis. Endocrinology 157, 1644-1659.

Taxvig C., Rosenmai A.K. and Vinggaard A.M. (2014). Polyfluorinated alkyl phosphate ester surfactants - current knowledge and knowledge gaps. Basic Clin. Pharmacol. Toxicol. 115, 41-44.

Teng J., Takei Y., Harada A., Nakata T., Chen J. and Hirokawa N. (2001). Synergistic effects of MAP2 and MAP1b knockout in neuronal migration, dendritic outgrowth, and microtubule organization. J. Cell Biol. 155, 65-76.

Tögel M., Wiche. G. and Propst. F. (1998). Novel features of the light chain of microtubule-associated protein MAP1b: Microtubule stabilization, self interaction, actin filament binding, and regulation by the heavy chain. J. Cell Biol. 143, 695-707

Tovey Corinne A. and Conduit Paul T. (2018). Microtubule nucleation by γ-tubulin complexes and beyond. Essays Biochem. 62, 765-780.

Tsuda S. (2016). Differential toxicity between perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). J. Toxicol. Sci. 41, SP27-SP36.

Tymanskyj S.R., Yang B., Falnikar A., Lepore A.C. and Ma L. (2017). MAP7 regulates axon collateral branch development in dorsal root ganglion neurons. J. Neurosci. 37, 1648-1661.

van Beuningen S.F.B., Will L., Harterink M., Chazeau A., van Battum E.Y., Frias C.P., Franker M.A.M., Katrukha E.A., Stucchi R., Vocking K., Antunes A.T., Slenders L., Doulkeridou S., Sillevis Smitt P., Altelaar A.F.M., Post J.A., Akhmanova A., Pasterkamp R.J., Kapitein L.C., de Graaff E. and Hoogenraad C.C. (2015). TRIM46 controls neuronal polarity and axon specification by driving the formation of parallel microtubule arrays. Neuron 88, 1208-1226.

Vater I., Wagner F., Kreuz M., Berger H., Martin-Subero J.I., Pott C., Martinez-Climent J.A., Klapper W., Krause K., Dyer M.J., Gesk S., Harder L., Zamo A., Dreyling M., Hasenclever D., Arnold N. and Siebert R. (2009). Genechip analyses point to novel pathogenetic

mechanisms in mantle cell lymphoma. Br. J. Haematol. 144, 317-331.

Vershinin M., Carter B.C., Razafsky D.S., King S.J. and Gross S.P. (2007). Multiple-motor based transport and its regulation by tau. Proc. Natl. Acad. Sci. USA 104, 87-92.

Villarroel-Campos D., Henriquez D.R., Bodaleo F.J., Oguchi M.E., Bronfman F.C., Fukuda M. and Gonzalez-Billault C. (2016). Rab35 functions in axon elongation are regulated by p53-related protein kinase in a mechanism that involves Rab35 protein degradation and the microtubule-associated protein 1b. J. Neurosci. 36, 7298-7313.

Vogl A.W., Vaid K.S. and Guttman J.A. (2008). The Sertoli cell cytoskeleton. Adv. Exp. Med. Biol. 636, 186-211.

Volle J., Brocard J., Saoud M., Gory-Faure S., Brunelin J., Andrieux A. and Suaud-Chagny M.F. (2013). Reduced expression of STOP/MAP6 in mice leads to cognitive deficits. Schizophr. Bull. 39, 969-978.

Walczak C.E. and Shaw S.L. (2010). A MAP for bundling microtubules. Cell 142, 364-367.

Walters G.B., Gustafsson O., Sveinbjornsson G., Eiriksdottir V.K., Agustsdottir A.B., Jonsdottir G.A., Steinberg S., Gunnarsson A.F., Magnusson M.I., Unnsteinsdottir U., Lee A.L., Jonasdottir A., Sigurdsson A., Jonasdottir A., Skuladottir A., Jonsson L., Nawaz M.S., Sulem P., Frigge M., Ingason A., Love A., Norddhal G.L., Zervas M., Gudbjartsson D.F., Ulfarsson M.O., Saemundsen E., Stefansson H. and Stefansson K. (2018). MAP1b mutations cause intellectual disability and extensive white matter deficit. Nat. Commun. 9, 3456.

Wan H.T., Mruk D.D., Wong C.K.C. and Cheng C.Y. (2013). Targeting testis-specific proteins to inhibit spermatogenesis - lession from endocrine disrupting chemicals. Expert Opin. Ther. Targets 17, 839-855.

Wan H.T., Mruk D.D., Wong C.K.C. and Cheng C.Y. (2014). Perfluorooctanesulfonate (PFOS) perturbs male rat Sertoli cell blood-testis barrier function by affecting F-actin organization via p-FAK-Tyr407 - an *in vitro* study. Endocrinology 155, 249-262.

Weingarten M.D., Lockwood A.H., Hwo S.Y. and Kirschner M.W. (1975). A protein factor essential for micortubule assembly. Proc. Natl. Acad. Sci. USA 72, 1858-1862.

Wen Q., Tang E.I., Xiao X., Gao Y., Chu D.S., Mruk D.D., Silvestrini B. and Cheng C.Y. (2016). Transport of germ cells across the seminiferous epithelium during spermatogenesis-the involvement of both actin- and microtubule-based cytoskeletons. Tissue Barriers 4, e1265042.

Wen Q., Tang E.I., Lui W.Y., Lee W.M., Wong C.K.C., Silvestrini B. and Cheng C.Y. (2018). Dynein 1 supports spermatid transport and spermiation during spermatogenesis in the rat testis. Am. J. Physiol. Endocrinol. Metab. 315, E924-E948.

Wong E.Y., Tse J.Y., Yao K.M., Lui V.C., Tam P.C. and Yeung W.S. (2004). Identification and characterization of human VCY2-interacting protein: VCY2IP-1, a microtubule-associated protein-like protein. Biol. Reprod. 70, 775-784.

Wong E.W.P., Mruk D.D. and Cheng C.Y. (2008). Biology and regulation of ectoplasmic specialization, an atypical adherens junction type, in the testis. Biochem. Biophys. Acta 1778, 692-708.

Wszolek Z.K., Tsuboi Y., Ghetti B., Pickering-Brown S., Baba Y. and Cheshire W.P. (2006). Frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). Orphanet. J. Rare Dis. 1, 30.

Wu S., Yan M., Li L., Mao B., Wong C.K.C., Ge R., Lian Q. and Cheng C.Y. (2019). mTORC1/rpS6 and spermatogenic function in the testis - insights from the adjudin model. Reprod. Toxicol. 89, 54-66.

Xiang X., Qiu R., Yao X., Arst H.N., Jr., Penalva M.A. and Zhang J. (2015). Cytoplasmic dynein and early endosome transport. Cell. Mol. Life Sci. 72, 3267-3280.

Xie R., Nguyen S., McKeehan K., Wang F., McKeehan W.L. and Liu L. (2011). Microtubule-associated protein 1s (MAP1s) bridges autophagic components with microtubules and mitochondria to affect autophagosomal biogenesis and degradation. J. Biol. Chem. 286, 10367-10377.

Xu G., Yue F., Huang H., He Y., Li X., Zhao H., Su Z., Jiang X., Li W., Zou J., Chen Q. and Liu L. (2016). Defects in MAP1s-mediated autophagy turnover of fibronectin cause renal fibrosis. Aging 8, 977-985.

Yan M., Li L., Mao B.P., Li H., Li S.Y.T., Mruk D., Silvestrini B., Lian Q., Ge R. and Cheng C.Y. (2019). mTORC1/rpS6 signaling complex modifies btb transport function - an *in vivo* study using the adjudin model. Am. J. Physiol. Endocrinol. Metab. 317, E-121-E138.

Yang Y., Dowling J., Yu Q.C., Kouklis P., Cleveland D.W. and Fuchs E. (1996). An essential cytoskeletal linker protein connecting actin microfilaments to intermediate filaments. Cell 86, 655-665.

Yu W., Qiang L., Solowska J.M., Karabay A., Korulu S. and Baas P.W. (2008). The microtubule-severing proteins spastin and katanin participate differently in the formation of axonal branches. Mol. Biol. Cell 19, 1485-1498.

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