Published in final edited form as:

Biochem J. 2011 January 15; 433(2): 253–262. doi:10.1042/BJ20101267.

The myotubularin family of lipid phosphatases in disease and in spermatogenesis

Dolores D. Mruk and C. Yan Cheng

Center for Biomedical Research, Population Council, 1230 York Avenue, New York, NY 10065, U.S.A.

Abstract

The MTM (myotubularin)/MTMR (myotubularin-related) protein family is comprised of 15 lipid phosphatases, of which nine members are catalytically active. MTMs are known to play a fundamental role in human physiology as gene mutations can give rise to X-linked myotubular myopathy or Charcot–Marie–Tooth disease, which manifest in skeletal muscle or in peripheral neurons respectively. Interestingly, studies have shown *MTMR2* and *MTMR5*, two MTM family members, to be highly expressed in the testis, particularly in Sertoli and germ cells, and knockout of either gene resulted in spermatogenic defects. Other studies have shown that *MTMR2* functions in endocytosis and membrane trafficking. In the testis, *MTMR2* interacts and co-localizes with c-Src/phospho-Src-(Tyr⁴¹⁶), a non-receptor protein tyrosine kinase that regulates the phosphorylation state of proteins at the apical ES (ectoplasmic specialization), a unique type of cell junction found between Sertoli cells and elongating/elongated spermatids. In the present review, we highlight recent findings that have made a significant impact on our understanding of this protein family in normal cell function and in disease, with the emphasis on the role of MTMs and MTMRs in spermatogenesis. We also describe a working model to explain how *MTMR2* interacts with other proteins such as c-Src, dynamin 2, EPS8 (growth factor receptor pathway substrate 8) and ARP2/3 (actin-related protein 2/3) at the apical ES and the apical TBC (tubulobulbar complex; tubular-like invaginations that function in the disassembly of the apical ES and in the recycling of its components) to regulate spermiation at late stage VIII of the seminiferous epithelial cycle.

Keywords

endocytosis; lipid second messenger; myotubularin (MTM); phosphatidylinositol 3-phosphatase; spermatogenesis; testis

INTRODUCTION

Spermatogenesis in mammals is a complicated process in which spermatogonia undergo a series of differentiation steps to become spermatozoa that are released into the tubule lumen at late stage VIII of the seminiferous epithelial cycle. This entire process, beginning with

one spermatogonium and ending with 256 spermatozoa, requires ~74 and ~58 days in the human and rat respectively, and it is under the regulation of autocrine, paracrine and endocrine factors [1–4]. During this time, an enormous amount of restructuring takes place in the seminiferous epithelium, in particular at the BTB (blood–testis barrier) when spermatocytes have to gain entry into the adluminal compartment for continued development, and at the luminal edge when spermatozoa have to be released [5,6]. Of equal importance is the necessity that these two events, which occur at opposite ends of the seminiferous epithelium, are strictly co-ordinated with germ cell development to ensure that spermatogenesis and fertility are not perturbed. For instance, a unique signalling cascade likely follows spermiation to trigger BTB restructuring and germ cell movement across the barrier. However, a better understanding of this cross-talk is needed.

Previous *in vitro* studies from our laboratory have shown cytokines and androgens to facilitate disassembly of the Sertoli cell barrier (i.e. the BTB) by regulating the kinetics of endocytosis of integral membrane proteins such as occludin and N-cadherin [7,8]. The BTB is a unique structure and different from other blood–tissue barriers such as the blood–brain barrier. It comprises several testis-specific cell junctions [i.e. tight junctions, basal ES (ectoplasmic specializations), desmosome-gap junctions and basal TBCs (tubulobulbar complexes)] that co-exist and co-function with one another, suggesting that a novel endocytic mechanism is behind the disassembly of these junctions when germ cells traverse the BTB. *In vivo* studies have shown that endocytosis also occurs at the luminal edge prior to spermiation and that different proteins are involved in this event [9–11]. In the present review, we discuss how the MTM (myotubularin) protein family functions in endocytosis and in cell junction dynamics with emphasis on the role of these proteins in spermatogenesis. General sections on MTM structure and function are also included, as well as a section on MTMs in disease. Finally, we describe how MTMR2 (myotubularin-related 2) interacts with other proteins such as c-Src, dynamin 2, EPS8 (growth factor receptor pathway substrate 8) and ARP2/3 (actin-related protein 2/3) at the apical-ES–apical-TBC interface to regulate spermiation at late stage VIII of the seminiferous epithelial cycle.

MTM/MTMR STRUCTURE

MTMs/MTMRs comprise a large family of lipid phosphatases that contain the Cys-Xaa₅-Arg protein tyrosine phosphatase active site. The MTM family in the human consists of 15 members, namely MTM1 and MTMRs 1–14, and each MTM/MTMR exhibits a unique and non-overlapping function within cells. Nine members of this protein family (i.e. MTM1, MTMR1, MTMR2, MTMR3, MTMR4, MTMR6, MTMT7, MTMR8 and MTMT14) possess catalytic activity, dephosphorylating PtdIns3P and PtdIns(3,5)P₂ to PtdIns and PtdIns5P respectively [12–16] (Figure 1), whereas the remaining members of this family are not catalytically active because they lack the conserved cysteine residue in the protein tyrosine phosphatase active site. An exception to the above rule is MTMR7 for which Ins(1,3)P₂ is the preferred substrate [17]. Moreover, all MTM proteins (except MTMR14) contain four functional domains that mediate protein–protein and protein–lipid interactions: (i) PH (pleckstrin homology)-GRAM (glucosyltransferase, Rab-like GTPase activator and myotubularin); (ii) RID (Rac-induced recruitment domain), (iii) PTD (protein tyrosine phosphatase domain); and (iv) SID (SET-interacting domain) [18–20] (Figure 2). The

GRAM domain, which overlaps with a PH motif whose role is to target proteins to specific cell membranes, such as the plasma membrane or the endosome [19], has been proposed to function in protein–protein and protein–lipid interactions [21]. In essence, the PH-GRAM domain binds to phosphoinositides [22,23]. Similarly, the RID domain is characterized as a membrane-targeting motif [24], whereas the SID domain is known to mediate protein–protein interactions [25,26]. Common to the PTD domain is the Cys-Xaa₅-Arg motif discussed above [18,27]. In addition, most MTM proteins also contain a coiled-coil domain, which is critical for homo- [28] and/or hetero- [29,30] dimerization, as well as a PDZ-binding motif, which is also critical for protein–protein interactions (Figure 2). Another important characteristic of MTM function is the ability of these proteins to assemble as heterodimers, thereby increasing the catalytic activity of the active member and targeting this complex to a specific cellular localization [31]. For instance, by co-immunoprecipitation and MS studies, MTM1 has been shown to heterodimerize with MTMR12 [also known as 3-PAP (3-phosphatase-associated protein)], a catalytically inactive lipid phosphatase of unknown function, in K562 cells [26]. Moreover, MTMR2 binds to MTMR5 [30], MTMR2 binds to MTMR13 [32], MTMR6 binds to MTMR9 [33,34] and MTMR7 binds to MTMR9 [17]. MTMs/MTMRs can also assemble as homodimers [32a]. These results clearly indicate that MTMs/MTMRs are rich in protein–protein and protein–lipid interactions.

MTM/MTMR FUNCTION

Within cells, each MTM/MTMR appears to regulate a specific pool of PtdIns3P (whose cellular concentration is $\sim 200 \mu\text{M}$) and PtdIns(3,5)P₂ (Figure 1), which in turn regulates endo-cytosis and membrane trafficking. This appears to be the most important and the best studied function of MTMs/MTMRs. However, these lipid phosphatases can also regulate many other processes including cell proliferation, differentiation, survival, autophagy, cytokinesis, cytoskeletal and cell junction dynamics, and motility [35–38]. For example, numerous *in vitro* studies have demonstrated that MTM1 functions in intracellular membrane trafficking and vesicle transport because it was shown to have activity towards PtdIns3P and PtdIns(3,5)P₂, two important regulators of these processes [35,39]. In line with this observation, PtdIns3P and PtdIns(3,5)P₂ have both been reported to be enriched in endocytic vesicles [16,40,41]. Furthermore, overexpression of *MTM1* in *Schizosaccharomyces pombe* resulted in the formation of large vacuoles, as well as in a concomitant decrease in PtdIns3P [12,42]. These observations are consistent with *MTM1* overexpression studies performed in epithelial cells [31,43,44], which demonstrated the relocalization of EEA1 (early endosome antigen 1), which binds PtdIns3P via its FYVE zinc-finger motif [45,46], from the early endosome in Cos-7 and L6 [44], and BHK cells [47]. Equally important, MTM1 has been reported to localize to early endosomes and at least partially to late endosomes, and to associate with the VPS (vacuolar protein sorting) 15–VPS34 protein complex in A431 cells [47]. VPS34, a PI3K (phosphoinositide 3-kinase) first identified in *Saccharomyces cerevisiae*, is known to synthesize PtdIns3P and to associate with VPS15, which regulates protein sorting [48]. Thus MTM1 is thought to regulate the PtdIns3P pool within cells via the VPS15–VPS34 protein complex, thereby affecting the spatiotemporal localization of PtdIns3P-binding proteins and activating important signalling cascades. What remains to be investigated is whether this kinase-

phosphatase duo (i.e. VPS–MTM1) is concomitantly active or whether the activity of one is dependent on the activity of the other. Alternatively, MTMR2 is believed to function in late endocytosis and not in early endocytosis. For instance, MTMR2 was shown to co-localize with RAB7, a late endosomal marker [49]. Moreover, its knockdown by RNAi (RNA interference), elevated PtdIns3P levels and resulted in the accumulation of EGFR (epidermal growth factor receptor) within late endosomes [49]. These results demonstrate that MTMs/MTMRs are critical for endocytosis.

MTMs/MTMRs also function in cytoskeletal and cell junction dynamics, and both PtdIns3P and PtdIns(3,5)P₂ have been shown to be present at the plasma membrane [50]. In line with these observations, the MTMR6–MTMR9 protein complex was reported to distribute along the plasma membrane in HeLa cells [34]. Likewise, MTM1 was shown to display a cytosolic staining pattern in various cells, but localization to the plasma membrane, where it supposedly induces membrane remodelling, was also reported following its overexpression [12,15,42,43] or co-transfection with constitutively active Rac1 [24], illustrating that MTM1 also participates in cytoskeletal dynamics. Indeed, Rac1 is a major regulator of the cytoskeleton and of cell junctions in various epithelia and endothelia [51–53], but it is not yet known if Rac1 associates with MTM1 at the plasma membrane. Interestingly, co-expression of *MTM1* with *MTMR12* in Cos-7 cells completely abolished the formation of membrane projections and resulted in the cytoplasmic localization of MTM1 [26]. It is possible that transit of MTM1 between the plasma membrane and the cytosol may be critical for cell junction assembly and disassembly, two events that rely on endocytosis and membrane trafficking [54–56]. Nevertheless, these findings illustrate that MTMR12 can regulate the subcellular localization of MTM1, in addition to up-regulating the latter MTM's lipid phosphatase activity. Other examples of MTM participation in cytoskeletal and cell junction dynamics exist as well. In zebrafish embryos, *mtmr8* loss-of-function affected the actin cytoskeleton and muscle development [57]. In another study in Schwann cells, MTMR2 was reported to interact with DLG1 (Discs large 1) [58,59], a PDZ-containing membrane-associated MAGUK (membrane-associated guanylate kinase) protein critical for cell polarity, proliferation and tumorigenesis [60,61]. In Schwann cells from *Mtmr2*-null mice, DLG1 localization was altered, thereby affecting cell junction integrity and membrane remodelling. Although additional studies are needed to expand the role of MTMs in cytoskeletal and cell junction dynamics, the relative importance of MTM/MTMRs in several critical cellular processes is apparent.

MTMs/MTMRs IN DISEASE

Mutations in active or inactive *MTM* or *MTMR* genes result in two severe disorders: XLMTM (X-linked myotubular myopathy; a type of centronuclear myopathy) or CMT (Charcot–Marie–Tooth) disease which manifest in skeletal muscle or in peripheral neurons respectively [18,62,63]. XLMTM is a congenital disease arising from a mutation in *MTM1* [64,65], resulting in either the loss of MTM function or the absence of MTM protein. It was previously hypothesized that XLMTM in humans causes an arrest in myogenesis during embryogenesis because biopsies from affected individuals showed skeletal muscle fibre hypotrophy and cells with centrally located nuclei resembling fetal myotubes [65a]. However, other investigators have suggested that XLMTM may cause defects much later in

development such as during muscle cell differentiation or maintenance [66]. At the cellular level, XLMTM likely results from the improper regulation of PtdIns3P and/or PtdIns(3,5)P₂ (Figure 1), thereby disrupting vesicle trafficking in the skeletal muscle of affected individuals. Incidence of XLMTM is one in approx. 50000 male births, and female carriers of XLMTM are usually asymptomatic. Moreover, presentation of the disease varies significantly from individual to individual, and this is due largely to the type of mutation manifested. Although exceptions are known to exist, truncation and splice-site mutations generally associate with the most severe forms of the disease and result in death at infancy. On the other hand, missense mutations, especially those that occur outside of MTM functional domains (Figure 2), generally result in moderate and mild disease phenotypes. Thus far, more than 200 disease-causing mutations in the *MTM1* gene have been reported [63]. In addition, mutations in BIN1 (bridging integrator 1, also known as amphiphysin 2; a protein with roles in membrane dynamics, cell polarity, tumour suppression and endocytosis [67]), which in turn disturbed its interaction with DYNAMIN 2 (a GTPase that functions in endocytosis [68,69]), resulted in autosomal recessive centronuclear myopathy [70,71].

CMT disease is an inherited disorder that affects motor and sensory neurons, causing patients to slowly lose normal use of their extremities [72]. CMT disease can affect the myelin sheath, which surrounds the peripheral nerve axon, or it can affect the axon itself. Intermediate forms of the disease have also been described in which both the myelin sheath and the axon are affected. Incidence of CMT disease is one in approx. 2500 births, making it one of the most common inheritable diseases. Although many different types of CMT disease (i.e. CMT1, CMT2, CMT3, CMT4 and CMTX) have been described, all types present with similar symptoms: progressive muscle weakness and atrophy in feet, legs, hands and arms [72a]. Moreover, mutations in multiple genes have been linked to this disease. For example, mutations in either *MTMR2* or *MTMR13* cause CMT4B [73,74], mutations in either *Rab7* (a GTPase that regulates late-endosomal traffic [75,76]) or *DYNAMIN 2* cause CMT2B [77–80] and mutations in *SH3TC2* [SH3 (Src homology 3) domain and tetratricopeptide repeats 2; a protein hypothesized to function as an adaptor or docking molecule) cause CMT4C [81,82]. Interestingly, all of these genes encode proteins with functions in endocytosis and membrane trafficking, suggesting that defects in these cellular processes may contribute to the pathogenesis of CMT disease. Finally, mutations in *GJB1* (gap junction protein β 1; also known as connexin 32 [83,84]) also result in CMT disease [85,86].

MTMs/MTMRs AND THE TESTIS

MTMs/MTMRs also play a critical role in the testis. The first report to illustrate their importance in spermatogenesis was made available in 2002 when it was demonstrated that *Mtmr5*-null mice were infertile as a result of germ-cell loss from the seminiferous epithelium [87]. Although no changes in serum FSH (follicle-stimulating hormone), LH (luteinizing hormone) and testosterone levels were noted, *Mtmr5*-deficient mice clearly displayed seminiferous tubule disorganization, Sertoli cell vacuolization (this appeared to be the initial effect), germ cell apoptotic bodies and Leydig cell hyperplasia [87], illustrating that the loss of a single *Mtmr* gene can cause profound changes in the testis. In this same study, *Mtmr5* [also known as Sbf1 (SET-binding factor 1)] was shown to be highly

expressed in the testis with specific expression in Sertoli cells, spermatogonia and pachytene spermatocytes, but not round spermatids [87]. As discussed above, catalytically inactive MTMR5 is known to bind to catalytically active MTMR2, which also plays an important role in the testis. For instance, spermatogenesis was disrupted in *Mtmr2*-knockout mice when spermatocytes and spermatids were found in the seminiferous tubule lumen [87a], suggesting that *Mtmr2* may be needed for some aspect of Sertoli-germ cell adhesion or cytoskeletal regulation. Moreover, this is in agreement with the only documented case of azoospermia in a patient having CMT4B [18].

The participation of *Mtmr2* in cytoskeletal and cell junction dynamics in the testis was further emphasized by studies from our laboratory {it should be noted that rat *Mtmr2* was referred to as rat *Mtm1* in our initial studies [88,89] because the mouse and rat *Mtmr2* cDNAs (GenBank® accession numbers AY055832 and NM_001108123 respectively) were not available at that time}. Similar to *Mtmr5*, *Mtmr2* was highly expressed in the rat testis, and its expression in this organ increased during development [88,89]. Culturing Sertoli cells alone at high density or co-culturing Sertoli cells with germ cells *in vitro* up-regulated *Mtmr2* expression. These changes coincided with a late stage of cell–cell junction assembly [88,89]. However, the MTMR2 level also increased when Sertoli cell–germ cell junctions were being restructured *in vivo* by adjuvin, a compound that affects the adhesion of germ cells to Sertoli cells [90–93], illustrating that this lipid phosphatase has diverse roles in cell junction dynamics. Furthermore, immunolocalization studies in the adult rat testis revealed *Mtmr2* to be present within Sertoli and germ cells [88,90]. Specifically, *Mtmr2* was found to be present at the BTB in all stages of the seminiferous epithelial cycle except at stages IX and X when its level diminished greatly [90] (Figure 3). MTMR2 also localized to the apical ES (possibly to the apical TBC as well, but additional studies will be needed to confirm this localization) predominantly at stage VIII. The ES is a unique type of Sertoli cell adhesive junction found at two distinct sites within the testis. The basal ES is found basolaterally within Sertoli cells, and it co-exists with other junction types (i.e. tight junctions, desmosome-gap junctions and basal TBCs) to constitute the BTB, whereas the apical ES is found between Sertoli cells and elongating/elongated spermatids where it functions as the only adhesive device found between these cells [94–96]. Prior to spermiation, the apical ES is disassembled and replaced by a morphological structure known as the apical TBC, tubular-like invaginations that function in the disassembly of the apical ES and in the recycling of its components [97,98]. In essence, the apical TBC functions as an endocytic device to ‘clean up’ and to prepare the luminal edge for spermiation. As important, MTMR2 was shown by co-immunoprecipitation to interact with Src when testis lysates were used [90], the latter of which also associated with N-cadherin, β -catenin and actin [99] (Figure 3). Src is a non-receptor protein tyrosine kinase with ubiquitous expression that functions in cell proliferation, survival, cytoskeletal and cell junction dynamics, and motility [100,101]. In many tumours, Src is often overexpressed or activated [100,102,103]. In the testis, both Src and phosphorylated Src (phospho-Src-Tyr⁴¹⁶) localized predominantly to the apical ES at stage VIII of the seminiferous epithelial cycle [90,99,104,105], and the latter protein co-localized with MTMR2 at this site [90] (Figure 3). At present, it is believed that Src (within the Src–MTMR2 protein complex) determines the phosphorylation state of N-cadherin and β -catenin and that MTMR2 plays a critical role in regulating ES and TBC dynamics. Indeed,

in another *in vitro* system, unrelated to the testis, Src was shown to phosphorylate β -catenin on Tyr⁶⁵⁴ [106] which disrupted its binding to N-cadherin [107] and perturbed cell–cell adhesion [108]. The delicate balance between protein phosphorylation and dephosphorylation also regulates tight junction dynamics. For instance, treating Sertoli cells *in vitro* with protein tyrosine phosphatase inhibitors was shown to adversely affect the tight junction permeability barrier when its integrity was assessed by measuring transepithelial electrical resistance across the epithelium [88,109]. These results demonstrate that an increase in intracellular phosphoprotein content can negatively affect the assembly and maintenance of Sertoli cell–Sertoli cell tight junctions [88,109]. An increase in phosphoprotein content may also facilitate spermiation given that several phosphorylated proteins were found to localize at the luminal edge of stage VIII seminiferous tubules. However, the role of protein phosphorylation and dephosphorylation in junction dynamics still remains to be fully understood. In addition, the significance of the MTMR2–Src interaction should be further investigated since MTMR2 is a lipid phosphatase but Src is a protein kinase.

In addition to its function in cytoskeletal and cell junction dynamics, the MTMR2–Src protein complex may also play a role in endocytosis in the testis. As previously discussed, MTMs are important regulators of endocytosis and membrane trafficking, and it now seems that Src may have a similar role. For instance, c-Src was shown to co-localize with RhoB [110] and RhoD [111] in endosomes, two GTPases that are known to regulate various aspects of endocytosis [112]. In another report, Src was shown to travel within endosomes from the perinuclear region (where it was inactive) to the plasma membrane (where it became active), after being stimulated by cytokines [100]. Once at the plasma membrane, Src activation brought about the detachment of loaded caveolae [113], plasma membrane invaginations rich in proteins and lipids [114]. Although additional data is needed to better understand the function of Src in the testis, these findings suggest that the MTMR2–Src protein complex may be recruited to Sertoli cell–Sertoli cell and Sertoli cell–germ cell adhesion sites where cytokines are known to facilitate the endocytosis of integral membrane proteins which results in junction disassembly [115,116] (Figure 3). It is also possible that the loss of *Mtmr2* and *Mtmr5* in mice disrupted endocytosis (e.g. by affecting the cellular localization of endocytic vesicles or the recruitment of adaptor proteins) in the testis, which then resulted in the disassembly of Sertoli cell–germ cell junctions and in the loss of germ cells from the seminiferous epithelium [59,87]. We arrive at this conclusion based on recent *in vitro* studies which showed cytokines [e.g. TGF- β (transforming growth factor- β)] and androgens (e.g. testosterone) to increase upon the internalization of integral membrane proteins (e.g. occludin) at the site of the BTB with one important difference: testosterone, and not TGF- β , accelerated the recycling of occludin back to the plasma membrane so that it could be used for junction reassembly (Figure 3). Irrespective of this difference, both factors increased the endocytosis of integral membrane proteins, and this affected the integrity of Sertoli cell junctions [7,8].

It is also possible that Src is involved in the phosphorylation of endocytic proteins [117]. Indeed, dynamin 2 was shown to be phosphorylated by Src, and phosphorylated dynamin 2, whose role is to pinch off vesicles from the plasma membrane, was essential for caveolin-

mediated endocytosis and cell function [118]. A recent study also reported that Vps34 is phosphorylated by Src [119]. In the testis, it is not clear whether dynamin 2 is present at the TBC. Vaid et al. [120], reported that dynamin 2 is not a major component of the TBC, but Kusumi et al. [10], demonstrated that dynamin 2 co-localized with amphiphysin 1 and actin at the TBC and was critical for spermiation. However, we found dynamin 2 was not localized at the apical TBC, but at the apical ES and the BTB instead [121]. Other endocytic proteins that have localized to the apical TBC include dynamin 3 [120] and ARP2/3 [122] (Figure 3). Presently, it is not known whether the MTMR2–Src protein complex includes other proteins such as dynamin, amphiphysin 1 or ARP2/3, but it is possible that these proteins together regulate the timely release of elongated spermatids at late stage VIII of the seminiferous epithelial cycle. Thus additional studies would be needed to determine whether Src phosphorylates these endocytic proteins.

At this point, we ask: what is the functional significance of the MTMR2–Src interaction at the apical-ES–apical-TBC and at the BTB? As discussed above, both MTMR2 and c-Src/phospho-Src-Tyr⁴¹⁶ localize predominantly to the apical ES at stage VIII of the seminiferous epithelial cycle, as well as to the BTB, albeit more weakly [90,99,104,105] (Figure 3). Our results seem to indicate that c-Src phosphorylates apical-ES and BTB proteins (e.g. N-cadherin and β -catenin), thereby resulting in the loss of protein–protein interactions and in the internalization of proteins because phosphorylated junctional proteins are generally believed to move away from sites of cell contact [123–125]. At the same time, Src-mediated phosphorylation may bring endocytic proteins together to form a functional multi-protein complex which may facilitate endocytosis at both sites. Endocytosed proteins that are not destined for immediate degradation may be recycled to other key sites within the seminiferous epithelium such as to the BTB (Figure 3). We hypothesize that MTMR2, dynamin 2 [121], 14-3-3 proteins (a protein family with roles in protein trafficking, apoptosis, cell cycle regulation and cell junction dynamics [126]; MTMR12/3-PAP contains a motif that is thought to interact with 14-3-3 proteins [127]) [128] and EPS8 (a protein that regulates Rac-mediated actin dynamics when it is in a complex with SOS (son of sevenless) and ABI-1 (Abelson interactor protein-1) [129,130]) [131] participate in these events, and this leads to apical ES disassembly [132]. For instance, EPS8 was recently reported to associate with early and late endosomes in different cells [133,134], indicating that this protein has roles outside of actin dynamics. These events are then followed by the assembly of (or transformation into) the apical TBC at stages VII and VIII of the seminiferous epithelial cycle, which involves ARP2/3 to branch actin filaments and dynamin 3 to facilitate endocytosis, thereby triggering the cascade of events leading to spermiation. Interestingly, this coincides with the loss of EPS8 at stage VIII [131] (it should be noted that EPS8 also bundles actin filaments at the apical ES [135]). Following spermiation, the BTB restructures to allow the entry of spermatocytes into the adluminal compartment of the seminiferous epithelium, illustrating that there is co-ordination between apical-ES–apical-TBC disassembly and barrier remodelling (Figure 3). As such, this provides an efficient mechanism to explain how the apical ES is restructured into the apical TBC to facilitate spermiation.

NEXT FRONTIERS

Currently, there is no cure for XLMTM and CMT disease because the underlying causes of these disorders are genetic. Moreover, it is difficult to pinpoint a feature of the protein that one day may become a drug target. For instance, most disease-causing mutations have been described to occur in the PTD domain (Figure 2), resulting in the inactivation of MTM1 enzymatic activity and XLMTM, whereas other domains are important in regulating the cellular localization of MTM1. For instance, the coiled-coil domain can mediate interactions between active and inactive MTM family members, thereby regulating the cellular distribution of the active member [17,29,30]. Likewise, the PH-GRAM domain of MTM1 binds both $\text{PtdIns}(3,5)\text{P}_2$ [22] and $\text{PtdIns}5\text{P}$ [23], and these interactions are also known to regulate the cellular localization of MTM1 [22,23,28] (Figure 2). Collectively, these observations suggest that cellular localization plays a critical role in MTM1 function, and chemical entities that would correct missense mutations occurring within these functional domains may alleviate adverse effects associated with the disease. At present, treatment attempts to minimize medical complications which may arise from the disease and to provide the best quality of life possible to those inflicted with XLMTM and CMT disease.

Ataluren (alternatively known as PTC124[®], 3-[5-(2-fluorophenyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid; $\text{C}_{15}\text{H}_9\text{FN}_2\text{O}_3$, PTC Therapeutics) is an orally administered investigational drug that has shown promise in the treatment of individuals with cystic fibrosis and Duchenne muscular dystrophy arising from nonsense mutations [136]. Ataluren allows muscle cells to ignore nonsense mutations in mRNA, thereby allowing translation of full-length and functional protein. Ataluren does not prevent the translation machinery from reading genuine termination codons. Although this drug cannot fix an individual's disease-causing nonsense mutations, it can provide a limited quantity of functional protein. In turn, this may eliminate many of the symptoms associated with the disease and improve the quality of life. *In vitro* and *in vivo* studies of Ataluren have been encouraging [137–140], and Ataluren is currently in Phase III clinical trials for cystic fibrosis. Ataluren was assigned orphan drug status in December 2004, and licensing of this drug for use in individuals with cystic fibrosis and Duchenne muscular dystrophy was anticipated in 2009 [141]. However, the drug is presently only available via a clinical trial. Finally, it is not yet known whether Ataluren has any effects on spermatogenesis and fertility. However, it is worth noting that most individuals diagnosed with cystic fibrosis produce functional spermatozoa and maintain normal hormonal profiles, but that they are nevertheless infertile due to complete obstruction or absence of the vas deferens [142], the tube that transports spermatozoa out of the testis. Regardless, these findings imply that Ataluren can be used for any disease caused by a nonsense mutation, and it may have future clinical promise in the treatment of XLMTM or CMT disease.

Gene therapy is defined as the transfer of genetic material for the purpose of curing a disease caused by mutation(s), thereby replacing the defective gene. The basic concept of gene therapy is to use a viral or non-viral vector to deliver a normal copy of a gene into the nucleus, followed by its incorporation into the cell's existing DNA, transcription and the synthesis of the target gene into a protein [143]. Although the ability to fix a defective gene has great clinical promise, this therapy has been met with several challenges in the past. For

instance, the overall level of transcriptional activity in most target tissues does not appear to be high enough to correct the deficiency in the target protein with obvious clinical benefits. Interestingly, chimaeric RNA–DNA oligonucleotides targeting a single point mutation in the dystrophin gene, which results in Duchenne muscular dystrophy, were able to induce gene repair in mature myofibres and muscle precursor cells in the *Mdx* mouse (an X-linked myopathic mutant model) [144,145], seemingly suggesting that gene therapy may become a viable treatment approach for individuals with XLMTM or CMT disease in the future.

Stem cell therapy may offer an additional treatment option to individuals with XLMTM or CMT disease in coming years. The goal of stem cell therapy is to replace defective cells with stem cells that will produce functional protein. Skeletal muscle is known to have enormous regenerative capacity, and several studies have shown that transplantation of bone marrow-derived [146,147] or haemopoietic [148] stem cells can give rise to differentiated skeletal muscle cell fibres. However, stem cell therapy is far from being tested in a clinical environment.

CONCLUDING REMARKS

In the present review, we discussed the roles of MTMs/MTMRs in disease and in spermatogenesis. In summary, mutations in or knockout of *MTM* or *MTMR* genes are known to result in severe neurological disorders and/or spermatogenic defects. In this review, we have also provided a model to explain how disassembly of the apical ES is co-ordinated with assembly of the apical TBC, which facilitates spermiation at late stage VIII of the seminiferous epithelial cycle. Additional studies are needed to address the role of MTMs in the testis because several important questions remain unanswered. For example, what triggers the apical ES to disassemble and the apical TBC to assemble prior to spermiation, two transiently occurring events that involve extensive restructuring and endocytosis of key proteins? Is there a functional loop originating from the BTB to regulate apical-ES–apical-TBC dynamics? Do MTMs participate in these events by regulating the levels of PtdIns3P and/or PtdIns(3,5)P₂ at the luminal edge? Do MTMs work in concert with cytokines and androgens to facilitate spermiation? Are other *MTM* genes expressed by the testis, besides those already discussed above, and if so, what are their roles in spermatogenesis? As a first step, *in vitro* functional experiments should be designed using highly pure Sertoli cells isolated from immature rats. It would be interesting to determine whether knockdown of *Mtmr2* by RNAi can affect Sertoli cell endocytosis and cell junction integrity. Finally, many of the studies discussed in the present review should be expanded by using Sertoli cell/germ cell co-cultures. Taken collectively, these findings would be welcomed in the field because they would significantly contribute to our understanding of spermatogenesis.

Acknowledgments

FUNDING

The work of the authors' laboratory was supported by grants from the National Institutes of Health (National Institute of Child Health and Human Development) [grant numbers U54 HD029990 Project 5 (to C.Y.C), R01 HD056034 (to C.Y.C), R03 HD061401 (to D.D.M.)].

Abbreviations used

ARP2/3	actin-related protein 2/3
BTB	blood–testis barrier
CMT	Charcot–Marie–Tooth
DLG1	Discs large 1
EPS8	growth factor receptor pathway substrate 8
ES	ectoplasmic specialization
GRAM	glucosyltransferase, Rab-like GTPase activator and myotubularin
MTM	myotubularin
MTMR	myotubularin-related
PH	pleckstrin homology
PTD	protein tyrosine phosphatase domain
RID	Rac-induced recruitment domain
RNAi	RNA interference
SID	SET-interacting domain
TBC	tubulobulbar complex
TGF-β	transforming growth factor- β
VPS	vacuolar protein sorting
XLMTM	X-linked myotubular myopathy

REFERENCES

1. Amann RP. The cycle of the seminiferous epithelium in human: a need to revisit? *J. Androl.* 2008; 29:469–487. [PubMed: 18497337]
2. Ehmcke J, Schlatt S. A revised model for spermatogonial expansion in man: lessons from non-human primates. *Reproduction.* 2006; 132:673–680. [PubMed: 17071768]
3. Clermont Y. Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal. *Physiol. Rev.* 1972; 52:198–235. [PubMed: 4621362]
4. Clermont Y, Perey B. Quantitative study of the cell population of the seminiferous tubules in immature rats. *J. Anat.* 1957; 100:241–267.
5. de Kretser, DM.; Kerr, JB. The cytology of the testis. In: Knobil, E.; Neill, JB.; Ewing, LL.; Greenwald, GS.; Markert, CL.; Pfaff, DW., editors. *The Physiology of Reproduction*. New York: Raven Press; 1988. p. 837-932.
6. Kerr, JB.; Loveland, KL.; O'Bryan, MK.; de Kretser, DM. Cytology of the testis and intrinsic control mechanisms. In: Neill, JD., editor. *In Knobil and Neill's Physiology of Reproduction*. New York: Elsevier; 2006. p. 827-947.
7. Yan HHN, Mruk DD, Lee WM, Cheng CY. Blood-testis barrier dynamics are regulated by testosterone and cytokines via their differential effects on the kinetics of protein endocytosis and recycling in Sertoli cells. *FASEB J.* 2008; 22:1945–1959. [PubMed: 18192323]
8. Xia W, Wong EWP, Mruk DD, Cheng CY. TGF- β and TNF α perturb blood-testis barrier (BTB) dynamics by accelerating the clathrin-mediated endocytosis of integral membrane proteins: a new

- concept of BTB regulation during spermatogenesis. *Dev. Biol.* 2009; 327:48–61. [PubMed: 19103189]
9. Guttman JA, Takai Y, Vogl AW. Evidence that tubulobulbar complexes in the seminiferous epithelium are involved in the internalization of adhesion junctions. *Biol. Reprod.* 2004; 71:548–559. [PubMed: 15084482]
10. Kusumi N, Watanabe M, Yamada H, Li SA, Kashiwakura Y, Matsukawa T, Nagai A, Nasu Y, Kumon H, Takei K. Implication of amphiphysin 1 and dynamin 2 in tubulobulbar complex formation and spermatid release. *Cell Struct. Funct.* 2007; 32:101–113. [PubMed: 17785912]
11. Young JS, Guttman JA, Vaid KS, Shahinian H, Vogl AW. Cortactin (CTTN), N-WASP (WASP) and clathrin (CLTC) are present at podosome-like tubulobulbar complexes in the rat testis. *Biol. Reprod.* 2009; 80:153–161. [PubMed: 18799755]
12. Blondeau F, Laporte J, Bodin S, Superti-Furga G, Payrastre B, Mandel JL. Myotubularin, a phosphatase deficient in myotubular myopathy, acts on phosphatidylinositol 3-kinase and phosphatidylinositol 3-phosphate pathway. *Hum. Mol. Genet.* 2000; 9:2223–2229. [PubMed: 11001925]
13. Taylor GS, Maehama T, Dixon JE. Inaugural article: myotubularin, a protein tyrosine phosphatase mutated in myotubular myopathy, dephosphorylates the lipid second messenger, phosphatidylinositol 3-phosphate. *Proc. Natl. Acad. Sci. U.S.A.* 2000; 97:8910–8915. [PubMed: 10900271]
14. Schaletzky J, Dove SK, Short B, Lorenzo O, Clague MJ, Barr FA. Phosphatidylinositol-5-phosphate activation and conserved substrate specificity of the myotubularin phosphatidylinositol-3-phosphatases. *Curr. Biol.* 2003; 13:504–509. [PubMed: 12646134]
15. Taylor GS, Maehama T, Dixon JE. Myotubularin, a protein tyrosine phosphatase mutated in myotubular myopathy, dephosphorylates the lipid second messenger, phosphatidylinositol 3-phosphate. *Proc. Natl. Acad. Sci. U.S.A.* 2000; 97:8910–8915. [PubMed: 10900271]
16. Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. *Nature.* 2006; 443:651–657. [PubMed: 17035995]
17. Mochizuki Y, Majerus PW. Characterization of myotubularin-related protein 7 and its binding partner, myotubularin-related protein 9. *Proc. Natl. Acad. Sci. U.S.A.* 2003; 100:9768–9773. [PubMed: 12890864]
18. Laporte J, Bedez F, Bolino A, Mandel JL. Myotubularins, a large disease-associated family of cooperating catalytically active and inactive phosphoinositides phosphatases. *Hum. Mol. Genet.* 2003; 12:R285–R292. [PubMed: 12925573]
19. Begley MJ, Dixon JE. The structure and regulation of myotubularin phosphatases. *Curr. Opin. Struct. Biol.* 2005; 15:614–620. [PubMed: 16289848]
20. Clague MJ, Lorenzo O. The myotubularin family of lipid phosphatases. *Traffic.* 2005; 6:1063–1069. [PubMed: 16262718]
21. Doerks T, Strauss M, Brendel M, Bork P. GRAM, a novel domain in glucosyltransferases, myotubularins and other putative membrane-associated proteins. *Trends Biochem. Sci.* 2000; 25:483–485. [PubMed: 11050430]
22. Tsujita K, Itoh T, Ijuin T, Yamamoto A, Shisheva A, Laporte J, Takenawa T. Myotubularin regulates the function of the late endosome through the GRAM domain-phosphatidylinositol 3,5-biphosphate interaction. *J. Biol. Chem.* 2004; 279:13817–13824. [PubMed: 14722070]
23. Lorenzo O, Urbe S, Clague MJ. Analysis of phosphoinositide binding domain properties within the myotubularin-related protein MTMR3. *J. Cell Sci.* 2005; 118:2005–2012. [PubMed: 15840652]
24. Laporte J, Blondeau F, Gansmuller A, Lutz Y, Vonesch JL, Mandel JL. The PtdIns3P phosphatase myotubularin is a cytoplasmic protein that also localizes to Rac1-inducible plasma membrane ruffles. *J. Cell Sci.* 2002; 115:3105–3117. [PubMed: 12118066]
25. Cui X, De Vivo I, Slany R, Miyamoto A, Firestein R, Cleary ML. Association of SET domain and myotubularin-related proteins modulates growth control. *Nat. Genet.* 1998; 18:331–337. [PubMed: 9537414]
26. Nandurkar HN, Layton M, Laporte J, Selan C, Corcoran L, Caldwell KK, Mochizuki Y, Majerus PW, Mitchell CA. Identification of myotubularin as the lipid phosphatase catalytic subunit

- associated with the 3-phosphatase adapter protein, 3-PAP. *Proc. Natl. Acad. Sci. U.S.A.* 2003; 100:8660–8665. [PubMed: 12847286]
27. Wishart MJ, Dixon JE. Gathering STYX: phosphatase-like form predicts functions for unique protein-interaction domains. *Trends Biochem. Sci.* 1998; 23:301–306. [PubMed: 9757831]
 28. Berger P, Schaffitzel C, Berger I, Ban N, Suter U. Membrane association of myotubularin-related protein 2 is mediated by a pleckstrin homology-GRAM domain and a coiled-coil dimerization module. *Proc. Natl. Acad. Sci. U.S.A.* 2003; 100:12177–12182. [PubMed: 14530412]
 29. Robinson FL, Dixon JE. The phosphoinositide-3-phosphatase MTMR2 associates with MTMR13, a membrane-associated pseudophosphatase also mutated in type 4B Charcot-Marie-Tooth disease. *J. Biol. Chem.* 2005; 280:31699–31707. [PubMed: 15998640]
 30. Kim SA, Vacratis PO, Firestein R, Cleary ML, Dixon JE. Regulation of myotubularin-related (MTMR)2 phosphatidylinositol phosphatase by MTMR5, a catalytically inactive phosphatase. *Proc. Natl. Acad. Sci. U.S.A.* 2003; 100:4492–4497. [PubMed: 12668758]
 31. Lorenzo O, Urbe S, Clague MJ. Systematic analysis of myotubularins: heteromeric interactions, subcellular localisation and endosome-related functions. *J. Cell Sci.* 2006; 119:2953–2959. [PubMed: 16787938]
 32. Berger P, Berger I, Schaffitzel C, Tersar K, Volkmer B, Suter U. Multi-level regulation of myotubularin-related protein-2 phosphatase activity by myotubularin-related protein-13/set-binding factor-2. *Hum. Mol. Genet.* 2006; 15:569–579. [PubMed: 16399794]
 - 32a. Berger P, Schaffitzel C, Berger I, Ban N, Suter U. Membrane association of myotubularin-related protein 2 is mediated by a pleckstrin homology-GRAM domain and a coiled-coil dimerization module. *Proc. Natl. Acad. Sci. U.S.A.* 2003; 100:12177–12182. [PubMed: 14530412]
 33. Dang H, Li Z, Skolnik EY, Fares H. Disease-related myotubularins function in endocytic traffic in *Caenorhabditis elegans*. *Mol. Biol. Cell.* 2004; 15:189–196. [PubMed: 14565969]
 34. Zou J, Chang SC, Marjanovic J, Majerus PW. MTMR9 increases enzyme activity, stability, and role in apoptosis. *J. Biol. Chem.* 2009; 284:2064–2071. [PubMed: 19038970]
 35. Michell RH, Heath VL, Lemmon MA, Dove SK. Phosphatidylinositol 3,5-bisphosphate: metabolism and cellular functions. *Trends Biochem. Sci.* 2006; 31:52–63. [PubMed: 16364647]
 36. Haucke V, Di Paolo G. Lipids and lipid modifications in the regulation of membrane traffic. *Curr. Opin. Cell Biol.* 2007; 19:426–435. [PubMed: 17651957]
 37. Montagnac G, Chavrier P. Abscission accomplished by PtdIns(3)P. *Nat. Cell Biol.* 2010; 12:308–310. [PubMed: 20360746]
 38. Burman C, Ktistakis NT. Regulation of autophagy by phosphatidylinositol 3-phosphate. *FEBS Lett.* 2010; 584:1302–1312. [PubMed: 20074568]
 39. Backer JM. Phosphoinositide 3-kinases and the regulation of vesicular trafficking. *Mol. Cell Biol. Res. Commun.* 2000; 3:193–204. [PubMed: 10891392]
 40. Gillooly DJ, Morrow IC, Lindsay M, Gould R, Bryant NJ, Gaullier JM, Parton RG, Stenmark H. Localization of phosphatidylinositol 3-phosphate in yeast and mammalian cells. *EMBO J.* 2000; 19:4577–4588. [PubMed: 10970851]
 41. Roth MG. Phosphoinositides in constitutive membrane traffic. *Physiol. Rev.* 2004; 84:699–730. [PubMed: 15269334]
 42. Laporte J, Liaubet L, Blondeau F, Tronchere H, Mandel JL, Payraastre B. Functional redundancy in the myotubularin family. *Biochem. Biophys. Res. Commun.* 2002; 291:305–312. [PubMed: 11846405]
 43. Kim SA, Taylor GS, Torgersen KM, Dixon JE. Myotubularin and MTMR2, phosphatidylinositol 3-phosphatases mutated in myotubular myopathy and type 4B Charcot-Marie-Tooth disease. *J. Biol. Chem.* 2002; 277:4526–4531. [PubMed: 11733541]
 44. Chaussade C, Pirola L, Bonnafous S, Blondeau F, Brenz-Verca S, Tronchere H, Portis F, Rusconi S, Payraastre B, Laporte J, Van Obberghen E. Expression of myotubularin by an adenoviral vector demonstrates its function as a phosphatidylinositol 3-phosphate [PtdIns(3)P] phosphatase in muscle cells: involvement of PtdIns(3)P in insulin-stimulated glucose transport. *Mol. Endocrinol.* 2003; 17:2448–2460. [PubMed: 14500759]

45. Lemmon MA. Phosphoinositide recognition domains. *Traffic*. 2003; 4:201–213. [PubMed: 12694559]
46. Gaullier JM, Ronning E, Gillooly DJ, Stenmark H. Interaction of the EEA1 FYVE finger with phosphatidylinositol 3-phosphate and early endosomes. Role of conserved residues. *J. Biol. Chem.* 2000; 275:24595–24600.
47. Cao C, Laporte J, Backer JM, Wandinger-Ness A, Stein MP. Myotubularin lipid phosphatase binds the hVPS15/hVPS34 lipid kinase complex on endosomes. *Traffic*. 2007; 8:1052–1067. [PubMed: 17651088]
48. Backer JM. The regulation and function of Class III PI3Ks: novel roles for Vps34. *Biochem. J.* 2008; 410:1–17. [PubMed: 18215151]
49. Cao C, Backer JM, Laporte J, Bedrick EJ, Wandinger-Ness A. Sequential actions of myotubularin lipid phosphatases regulate endosomal PI(3)P and growth factor receptor trafficking. *Mol. Biol. Cell.* 2008; 19:3334–3346. [PubMed: 18524850]
50. Pendaries C, Tronchere H, Plantavid M, Payrastre B. Phosphoinositide signaling disorders in human disease. *FEBS Lett.* 2003; 546:25–31. [PubMed: 12829232]
51. Beckers CML, van Hinsbergh VWM, van Nieuw Amerongen GP. Driving Rho GTPase activity in endothelial cells regulates barrier integrity. *Thromb. Haemost.* 2010; 103:40–55. [PubMed: 20062930]
52. Nelson WJ. Regulation of cell-cell adhesion by the cadherin-catenin complex. *Biochem. Soc. Trans.* 2008; 36:149–155. [PubMed: 18363555]
53. Vandenbroucke E, Mehta D, Minshall R, Malik AB. Regulation of endothelial junctional permeability. *Ann. N. Y. Acad. Sci.* 2008; 1123:134–145. [PubMed: 18375586]
54. Griffiths GM, Tsun A, Stinchcombe JC. The immunological synapse: a focal point for endocytosis and exocytosis. *J. Cell Biol.* 2010; 189:399–406. [PubMed: 20439993]
55. Troyanovsky SM. Regulation of cadherin-based epithelial cell adhesion by endocytosis. *Front. Biosci.* 2009; 1:61–67.
56. Komarova Y, Malik AB. Regulation of endothelial permeability via paracellular and transcellular transport pathways. *Annu. Rev. Physiol.* 2010; 72:463–493. [PubMed: 20148685]
57. Mei J, Li Z, Gui JF. Cooperation of Mtmr8 with PI3K regulates actin filament modeling and muscle development in zebrafish. *PLoS One.* 2009; 4:e4979. [PubMed: 19325702]
58. Bolis A, Coviello S, Visigalli I, Taveggia C, Bachi A, Chishti AH, Hanada T, Quattrini A, Previtali SC, Biffi A, Bolino A. Dlg1, Sec8, and Mtmr2 regulate membrane homeostasis in Schwann cell myelination. *J. Neurosci.* 2009; 29:8858–8870. [PubMed: 19587293]
59. Bolino A, Bolis A, Previtali SC, Dina G, Bussini S, Dati G, Amadio S, Del Carro U, Mruk DD, Feltri ML, et al. Disruption of *Mtmr2* produces CMT4B1-like neuropathy with myelin outfolding and impaired spermatogenesis. *J. Cell Biol.* 2004; 167:711–721. [PubMed: 15557122]
60. Humbert P, Russell S, Richardson H. Dlg, Scribble and Lgl in cell polarity, cell proliferation and cancer. *BioEssays.* 2003; 25:542–553. [PubMed: 12766944]
61. Humbert PO, Grzeschik NA, Brumby AM, Galea R, Elsum IA, Richardson HE. Control of tumorigenesis by the Scribble/Dlg/Lgl polarity module. *Oncogene.* 2008; 27:6888–6907. [PubMed: 19029932]
62. Robinson FL, Dixon JE. Myotubularin phosphatases: policing 3-phosphoinositides. *Trends Cell Biol.* 2006; 16:403–412. [PubMed: 16828287]
63. Nicot AS, Laporte J. Endosomal phosphoinositides and human disease. *Traffic.* 2008; 9:1240–1249. [PubMed: 18429927]
64. Thomas NS, Williams H, Cole G, Roberts K, Clarke A, Liechti-Gallati S, Braga S, Gerber A, Meier C, Moser H, Harper PS. X-linked centronuclear/myotubular myopathy: evidence for linkage to Xq28 marker loci. *J. Med. Genet.* 1990; 27:284–287. [PubMed: 2352256]
65. Liechti-Gallati S, Mueller B, Grimm T, Kress W, Mueller C, Boltshauser E, Moser H, Braga S. X-linked centronuclear myopathy: mapping the gene to Xq28. *Neuromuscul. Disord.* 1991; 1:239–245. [PubMed: 1822801]
- 65a. van Wijngaarden GK, Fleury P, Bethlem J, Meijer AE. Familial ‘myotubular’ myopathy. *Neurology.* 1969; 19:901–908. [PubMed: 5816884]

66. Buj-Bello A, Laugel V, Messaddeq N, Zahreddine H, Laporte J, Pellissier JF, Mandel JL. The lipid phosphatase myotubularin is essential for skeletal muscle maintenance but not for myogenesis in mice. *Proc. Natl. Acad. Sci. U.S.A.* 2002; 99:15060–15065. [PubMed: 12391329]
67. Prendergast GC, Muller AJ, Ramalingam A, Chang MY. BAR the door: cancer suppression by amphiphysin-like genes. *Biochim. Biophys. Acta.* 2009; 1795:25–36. [PubMed: 18930786]
68. Kasai K, Shin HW, Shinotsuka C, Murakami K, Nakayama K. Dynamin II is involved in endocytosis but not in the formation of transport vesicles from the *trans*-Golgi network. *J. Biochem.* 1999; 125:780–789. [PubMed: 10101292]
69. McClure SJ, Robinson PJ. Dynamin, endocytosis and intracellular signalling. *Mol. Membr. Biol.* 1996; 13:189–215. [PubMed: 9116759]
70. Nicot SA, Toussaint A, Tosch V, Kretz C, Wallgren-Pettersson C, Iwarsson E, Kingston H, Garnier JM, Biancalana V, Oldfors A, et al. Mutations in amphiphysin 2 (*BIN1*) disrupt interactions with dynamin 2 and cause autosomal recessive centronuclear myopathy. *Nat. Genet.* 2007; 39:1134–1139. [PubMed: 17676042]
71. Bitoun M, Maugenre S, Jeannet PY, Lacene E, Ferrer X, Laforet P, Martin JJ, Laporte J, Lochmuller H, Beggs AH, et al. Mutations in dynamin 2 cause dominant centronuclear myopathy. *Nat. Genet.* 2005; 37:1207–1209. [PubMed: 16227997]
72. Jaradeh SS. Hereditary neuropathies. *J. Clin. Neuromuscul. Dis.* 2003; 5:72–80. [PubMed: 19078724]
- 72a. Banchs I, Casasnovas C, Alberti A, De Jorge L, Povedano M, Montero J, Martinez-Matos JA, Volpini V. Diagnosis of Charcot-Marie-Tooth disease. *J. Biomed. Biotechnol.* 2009; 2009:985415. [PubMed: 19826499]
73. Azzedine H, Bolino A, Taieb T, Birouk N, Di Duca M, Bouhouche A, Benamou S, Mrabet A, Hammadouche T, Chkili T, et al. Mutations in *MTMR13*, a new pseudophosphatase homologue of *MTMR2* and *Sbfl*, in two families with an autosomal recessive demyelinating form of Charcot-Marie-Tooth disease associated with early-onset glaucoma. *Am. J. Hum. Genet.* 2003; 72:1141–1153. [PubMed: 12687498]
74. Senderek J, Bergmann C, Weber S, Ketelsen UP, Schorle H, Rudnik-Schoneborn S, Buttner R, Buchheim E, Zerres K. Mutation of the *SBF2* gene, encoding a novel member of the myotubularin family, in Charcot-Marie-Tooth neuropathy type 4B2/11p15. *Hum. Mol. Genet.* 2003; 12:349–356. [PubMed: 12554688]
75. Stenmark H. Rab GTPases as coordinators of membrane traffic. *Nat. Rev. Mol. Cell Biol.* 2009; 10:513–525. [PubMed: 19603039]
76. Somsel Rodman J, Wandinger-Ness A. Rab GTPases coordinate endocytosis. *J. Cell Sci.* 2000; 113:183–192. [PubMed: 10633070]
77. Verhoeven K, De Jonghe P, Coen K, Verpoorten N, Auer-Grumbach M, Kwon JM, FitzPatrick D, Schmedding E, De Vriendt E, Jacobs A, et al. Mutations in the small GTPase late endosomal protein *RAB7* cause Charcot-Marie-Tooth type 2B neuropathy. *Am. J. Hum. Genet.* 2003; 72:722–727. [PubMed: 12545426]
78. Meggouh F, Bienfait HM, Weterman MA, de Visser M, Baas F. Charcot-Marie-Tooth disease due to a *de novo* mutation of the *RAB7* gene. *Neurology.* 2006; 67:1476–1478. [PubMed: 17060578]
79. Fabrizi GM, Ferrarini M, Cavallaro T, Cabrini I, Cerini R, Bertolasi L, Rizzuto N. Two novel mutations in dynamin-2 cause axonal Charcot-Marie-Tooth disease. *Neurology.* 2007; 69:291–295. [PubMed: 17636067]
80. Zuchner S, Noureddine M, Kennerson M, Verhoeven K, Claeys K, De Jonghe P, Merory J, Oliveira SA, Speer MC, Stenger JE, et al. Mutations in the pleckstrin homology domain of dynamin 2 cause dominant intermediate Charcot-Marie-Tooth disease. *Nat. Genet.* 2005; 37:289–294. [PubMed: 15731758]
81. Roberts RC, Peden AA, Buss F, Bright NA, Latouche M, Reilly MM, Kendrick-Jones J, Luzio JP. Mistargeting of *SH3TC2* away from the recycling endosome causes Charcot-Marie-Tooth disease type 4C. *Hum. Mol. Genet.* 2010; 19:1009–1018. [PubMed: 20028792]
82. Lupo V, Galindo MI, Martinez-Rubio D, Sevilla T, Vilchez JJ, Palau F, Espinos C. Missense mutations in the *SH3TC2* protein causing Charcot-Marie-Tooth disease type 4C affect its

- localization in the plasma membrane and endocytic pathway. *Hum. Mol. Genet.* 2009; 18:4603–4614. [PubMed: 19744956]
83. Laird DW. The gap junction proteome and its relationship to disease. *Trends Cell Biol.* 2010; 20:92–101. [PubMed: 19944606]
 84. Herve JC, Bourmeyster N, Sarrouilhe D. Diversity in protein-protein interactions of connexins: emerging roles. *Biochim. Biophys. Acta.* 2004; 1662:22–41. [PubMed: 15033577]
 85. Bergoffen J, Scherer SS, Wang S, Scott MO, Bone LJ, Paul DL, Chen K, Lensch MW, Chance PF, Fischbeck KH. Connexin mutations in X-linked Charcot-Marie-Tooth disease. *Science.* 1993; 262:2039–2042. [PubMed: 8266101]
 86. Braathen GJ, Sand JC, Bukholm G, Russell MB. Two novel connexin32 mutations cause early onset X-linked Charcot-Marie-Tooth disease. *BMC Neurol.* 2007; 7:19. [PubMed: 17620124]
 87. Firestein R, Nagy PL, Daly M, Huie P, Conti M, Cleary ML. Male infertility, impaired spermatogenesis, and azoospermia in mice deficient for pseudophosphatase Sbf1. *J. Clin. Invest.* 2002; 109:1165–1172. [PubMed: 11994405]
 - 87a. a Bolino A, Bolis A, Previtali SC, Dina G, Bussini S, Dati G, Amadio S, Del Carro U, Mruk DD, Feltri ML, et al. Disruption of Mtmr2 produces CMT4B1-like neuropathy with myelin outfoldings and impaired spermatogenesis. *J. Cell. Biol.* 2004; 167:711–721. [PubMed: 15557122]
 88. Li JC, Samy ET, Grima J, Chung SSW, Mruk D, Lee WM, Silvestrini B, Cheng CY. Rat testicular myotubularin, a protein tyrosine phosphatase expressed by Sertoli and germ cells, is a potential marker for studying cell-cell interactions in the rat testis. *J. Cell. Physiol.* 2000; 185:366–385. [PubMed: 11056007]
 89. Li JCH, Lee WM, Mruk DD, Cheng CY. Regulation of Sertoli cell myotubularin (rMTM) expression by germ cells *in vitro*. *J. Androl.* 2001; 22:266–277. [PubMed: 11229801]
 90. Zhang J, Wong CH, Xia W, Mruk DD, Lee NP, Lee WM, Cheng CY. Regulation of Sertoli-germ cell adherens junction dynamics via changes in protein-protein interactions of the N-cadherin- β -catenin protein complex which are possibly mediated by c-Src and myotubularin-related protein 2: an *in vivo* study using an androgen suppression model. *Endocrinology.* 2005; 146:1268–1284. [PubMed: 15591133]
 91. Cheng CY, Silvestrini B, Grima J, Mo MY, Zhu LJ, Johansson E, Saso L, Leone MG, Palmery M, Mruk DD. Two new male contraceptives exert their effects by depleting germ cells prematurely from the testis. *Biol. Reprod.* 2001; 65:449–461. [PubMed: 11466213]
 92. Grima J, Silvestrini B, Cheng CY. Reversible inhibition of spermatogenesis in rats using a new male contraceptive, 1-(2,4-dichlorobenzyl)-indazole-3-carbohydrazide. *Biol. Reprod.* 2001; 64:1500–1508. [PubMed: 11319158]
 93. Mruk DD, Wong CH, Silvestrini B, Cheng CY. A male contraceptive targeting germ cell adhesion. *Nat. Med.* 2006; 12:1323–1328. [PubMed: 17072312]
 94. Russell, LD. Morphological and functional evidence for Sertoli–germ cell relationships. In: Russell, LD.; Griswold, MD., editors. *The Sertoli Cell*. Clearwater: Cache River Press; 1993. p. 365–390.
 95. Mruk DD, Cheng CY. Cell-cell interactions at the ectoplasmic specialization in the testis. *Trends Endocrinol. Metab.* 2004; 15:439–447. [PubMed: 15519891]
 96. Cheng CY, Mruk DD. Cell junction dynamics in the testis: Sertoli-germ cell interactions and male contraceptive development. *Physiol. Rev.* 2002; 82:825–874. [PubMed: 12270945]
 97. Russell LD. Observations on the inter-relationships of Sertoli cells at the level of the blood-testis barrier: evidence for formation and resorption of Sertoli-Sertoli tubulobulbar complexes during the spermatogenic cycle of the rat. *Am. J. Anat.* 1979; 155:259–279. [PubMed: 474448]
 98. Russell LD. Further observations on tubulobulbar complexes formed by late spermatids and Sertoli cells in the rat testis. *Anat. Rec.* 1979; 194:213–232. [PubMed: 464323]
 99. Lee NP, Cheng CY. Protein kinases and adherens junction dynamics in the seminiferous epithelium of the rat testis. *J. Cell. Physiol.* 2005; 202:344–360. [PubMed: 15389520]
 100. Guarino M. Src signaling in cancer invasion. *J. Cell. Physiol.* 2010; 223:14–26. [PubMed: 20049846]
 101. Huveneers S, Danen EHJ. Adhesion signaling: crosstalk between integrins, Src and Rho. *J. Cell Sci.* 2009; 122:1059–1069. [PubMed: 19339545]

102. Kim LC, Song L, Haura EB. Src kinases as therapeutic targets for cancer. *Nat. Rev. Clin. Oncol.* 2009; 6:587–595. [PubMed: 19787002]
103. Benati D, Baldari CT. SRC family kinases as potential therapeutic targets for malignancies and immunological disorders. *Curr. Med. Chem.* 2008; 15:1154–1165. [PubMed: 18473810]
104. Wang W, Wine RN, Chapin RE. Rat testicular Src: normal distribution and involvement in ethylene glycol monomethyl ether-induced apoptosis. *Toxicol. Appl. Pharmacol.* 2000; 163:125–134. [PubMed: 10698670]
105. Wong CH, Xia W, Lee NPY, Mruk DD, Lee WM, Cheng CY. Regulation of ectoplasmic specialization dynamics in the seminiferous epithelium by focal adhesion-associated proteins in testosterone-suppressed rat testes. *Endocrinology.* 2005; 146:1192–1204. [PubMed: 15591141]
106. Piedra J, Martinez D, Castano J, Miravete S, Dunach M, Herreros AG. Regulation of β -catenin structure and activity by tyrosine phosphorylation. *J. Biol. Chem.* 2001; 276:20436–20443. [PubMed: 11279024]
107. Lilien J, Balsamo J. The regulation of cadherin-mediated adhesion by tyrosine phosphorylation/dephosphorylation of β -catenin. *Curr. Opin. Cell Biol.* 2005; 17:459–465. [PubMed: 16099633]
108. Matsuyoshi N, Hamaguchi M, Taniguchi S, Nagafuchi A, Tsukita S, Takeichi M. Cadherin-mediated cell-cell adhesion is perturbed by v-Src tyrosine phosphorylation in metastatic fibroblasts. *J. Cell Biol.* 1992; 118:703–714. [PubMed: 1639852]
109. Li JCH, Mruk DD, Cheng CY. The inter-Sertoli tight junction permeability barrier is regulated by the inter-play of protein phosphatases and kinases: an *in vitro* study. *J. Androl.* 2001; 22:847–856. [PubMed: 11545299]
110. Sandilands E, Cans C, Fincham VJ, Brunton VG, Mellor H, Prendergast GC, Norman JC, Superti-Furga G, Frame MC. RhoB and actin polymerization coordinate Src activation with endosome-mediated delivery to the membrane. *Dev. Cell.* 2004; 7:855–869. [PubMed: 15572128]
111. Gasman S, Kalaidzidis Y, Zerial M. RhoD regulates endosome dynamics through Diaphanous-related formin and Src tyrosine kinase. *Nat. Cell Biol.* 2003; 5:195–204. [PubMed: 12577064]
112. Ellis S, Mellor H. Regulation of endocytic traffic by Rho family GTPases. *Trends Cell Biol.* 2000; 10:85–88. [PubMed: 10675900]
113. Sverdlov M, Shajahan AN, Minshall RD. Tyrosine phosphorylation-dependence of caveolae-mediated endocytosis. *J. Cell. Mol. Med.* 2007; 11:1239–1250. [PubMed: 18205698]
114. Lajoie P, Nabi IR. Lipid rafts, caveolae and their endocytosis. *Int. Rev. Cell Mol. Biol.* 2010; 282:135–163. [PubMed: 20630468]
115. Stephan JP, Syed V, Jegou B. Regulation of Sertoli cell IL-1 and IL-6 production *in vitro*. *Mol. Cell. Endocrinol.* 1997; 134:109–118. [PubMed: 9426154]
116. Syed V, Stephan JP, Gerard N, Legrand A, Parvinen M, Bardin CW, Jegou B. Residual bodies activate Sertoli cell interleukin-1 α (IL-1 α) release, which triggers IL-6 production by an autocrine mechanism, through the lipoxygenase pathway. *Endocrinology.* 1995; 136:3070–3078. [PubMed: 7789334]
117. Korolchuk V, Banting G. Kinases in clathrin-mediated endocytosis. *Biochem. Soc. Trans.* 2003; 31:857–860. [PubMed: 12887321]
118. Shajahan AN, Timblin BK, Sandoval RM, Tirupathi C, Malik AB, Minshall RD. Role of Src-induced dynamin-2 phosphorylation in caveolae-mediated endocytosis in endothelial cells. *J. Biol. Chem.* 2004; 279:20392–20400. [PubMed: 15007081]
119. Hirsch DS, Shen Y, Dokmanovic M, Wu WJ. pp60^{S-Src} phosphorylates and activates vacuolar protein sorting 34 to mediate cellular transformation. *Cancer Res.* 2010; 70:5974–5983. [PubMed: 20551057]
120. Vaid KS, Guttman JA, Babyak N, Deng W, McNiven MA, Mochizuki N, Finlay BB, Vogl AW. The role of dynamin 3 in the testis. *J. Cell. Physiol.* 2007; 210:644–654. [PubMed: 17133358]
121. Lie PPY, Xia W, Wang CQ, Mruk DD, Yan HH, Wong CH, Lee WM, Cheng CY. Dynamin II interacts with the cadherin- and occludin-based protein complexes at the blood-testis barrier in adult rat testes. *J. Endocrinol.* 2006; 191:571–586. [PubMed: 17170215]

122. Lie PPY, Chan AYN, Mruk DD, Lee WM, Cheng CY. Restricted Arp3 expression in the testis prevents blood-testis barrier disruption during junction restructuring at spermatogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 2010; 107:11411–11416. [PubMed: 20534520]
123. Gumbiner BM. Regulation of cadherin adhesive activity. *J. Cell Biol.* 2000; 148:399–403. [PubMed: 10662767]
124. Kikyo M, Matozaki T, Kodama A, Kawabe H, Nakanishi H, Takai Y. Cell-cell adhesion-mediated tyrosine phosphorylation of nectin-2 δ , an immunoglobulin-like cell adhesion molecule at adherens junctions. *Oncogene.* 2000; 19:4022–4028. [PubMed: 10962558]
125. Mruk DD, Cheng CY. Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. *Endocr. Rev.* 2004; 25:747–806. [PubMed: 15466940]
126. Morrison DK. The 14-3-3 proteins: integrators of diverse signaling cues that impact cell fate and cancer development. *Trends Cell Biol.* 2008; 19:16–23. [PubMed: 19027299]
127. Nandurkar HH, Caldwell KK, Whisstock JC, Layton MJ, Gaudet EA, Norris FA, Majerus PW, Mitchell CA. Characterization of an adapter subunit to a phosphatidylinositol(3)P 3-phosphatase: identification of a myotubularin-related protein lacking catalytic activity. *Proc. Natl. Acad. Sci. U.S.A.* 2001; 98:9499–9504. [PubMed: 11504939]
128. Wong EWP, Sun SC, Li MWM, Lee WM, Cheng CY. 14-3-3 protein regulates cell adhesion in the seminiferous epithelium of rat testes. *Endocrinology.* 2009; 150:4713–4723. [PubMed: 19608648]
129. Di Fiore PP, Scita G. Eps8 in the midst of GTPases. *Int. J. Biochem. Cell Biol.* 2002; 34:1178–1183. [PubMed: 12127568]
130. Scita G, Nordstrom J, Carbone R, Tenca P, Giardina G, Gutkind S, Bjarnegard M, Betsholtz C, Di Fiore PP. EPS8 and E3B1 transduce signals from Ras to Rac. *Nature.* 1999; 401:290–293. [PubMed: 10499589]
131. Lie PPY, Mruk DD, Lee WM, Cheng CY. Epidermal growth factor receptor pathway substrate 8 (Eps8) is a novel regulator of cell adhesion and the blood-testis barrier integrity in the seminiferous epithelium. *FASEB J.* 2009; 23:2555–2567. [PubMed: 19293393]
132. Cheng CY, Mruk DD. A local autocrine axis in the testes that regulates spermatogenesis. *Nat. Rev. Endocrinol.* 2010; 6:380–395. [PubMed: 20571538]
133. Welsch T, Younsi A, Disanza A, Rodriguez JA, Cuervo AM, Scita G, Schmidt J. Eps8 is recruited to lysosomes and subjected to chaperone-mediated autophagy in cancer cells. *Exp. Cell Res.* 2010; 316:1914–1924. [PubMed: 20184880]
134. Lanzetti L, Rybin V, Malabarba MG, Christoforidis S, Scita G, Zerial M, Di Fiore PP. The Eps8 protein coordinates EGF receptor signalling through Rac and trafficking through Rab5. *Nature.* 2000; 408:374–377. [PubMed: 11099046]
135. Hertzog M, Milanese F, Hazelwood L, Disanza A, Liu HJ, Perlade E, Malabarba MG, Pasqualato S, Maiolica A, Confalonieri S, et al. Molecular basis for the dual function of Eps8 on actin dynamics: bundling and capping. *PLoS Biol.* 2010; 8:e1000387. [PubMed: 20532239]
136. Kuehn BM. Studies point way to new therapeutic prospects for muscular dystrophy. *JAMA, J. Am. Med. Assoc.* 2007; 298:1385–1386.
137. Hirawat S, Welch EM, Elfring GL, Northcutt VJ, Paushkin S, Hwang S, Leonard EM, Almstead NG, Ju W, Peltz SW, Miller LL. Safety, tolerability, and pharmacokinetics of PTC124, a nonaminoglycoside nonsense mutation suppressor, following single- and multiple-dose administration to healthy male and female adult volunteers. *J. Clin. Pharmacol.* 2007; 47:430–444. [PubMed: 17389552]
138. Du M, Liu X, Welch EM, Hirawat S, Peltz SW, Bedwell DM. PTC124 is an orally bioavailable compound that promotes suppression of the human CFTR-G542X nonsense allele in a CF mouse model. *Proc. Natl. Acad. Sci. U.S.A.* 2008; 105:2064–2069. [PubMed: 18272502]
139. Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P, Paushkin S, Patel M, Trotta CR, Hwang S, et al. PTC124 targets genetic disorders caused by nonsense mutations. *Nature.* 2007; 447:87–91. [PubMed: 17450125]
140. Kerem E, Hirawat S, Armoni S, Yaakov Y, Shoseyov D, Cohen M, Nissim-Rafinia M, Blau H, Rivlin J, Aviram M, et al. Effectiveness of PTC124 treatment of cystic fibrosis caused by

- nonsense mutations: a prospective phase II trial. *Lancet*. 2008; 372:719–727. [PubMed: 18722008]
141. Henderson, M. Daily pill to beat genetic diseases. 2007. *The Times* (<http://www.timesonline.co.uk/tol/news/science/article1690544.ece>)
142. Chan HC, Ruan YC, He Q, Chen MH, Chen H, Xu WM, Chen WY, Xie C, Zhang XH, Zhou Z. The cystic fibrosis transmembrane conductance regulator in reproductive health and disease. *J. Physiol.* 2009; 587:2187–2195. [PubMed: 19015188]
143. Parekh-Olmedo H, Kmiec EB. Progress and prospects: targeted gene alteration (TGA). *Gene Ther.* 2007; 14:1675–1680. [PubMed: 17972921]
144. Rando TA, Disatnik MH, Zhou LZ. Rescue of dystrophin expression in *mdx* mouse muscle by RNA/DNA oligonucleotides. *Proc. Natl. Acad. Sci. U.S.A.* 2000; 97:5363–5368. [PubMed: 10805797]
145. Bertoni C, Rando TA. Dystrophin gene repair in *mdx* muscle precursor cells *in vitro* and *in vivo* mediated by RNA-DNA chimeric oligonucleotides. *Hum. Gene Ther.* 2002; 13:707–718. [PubMed: 11936970]
146. LaBarge MA, Blau HM. Biological progression from bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. *Cell*. 2002; 111:589–601. [PubMed: 12437931]
147. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science*. 1998; 279:1528–1530. [PubMed: 9488650]
148. Camargo FD, Green R, Capetenaki Y, Jackson KA, Goodell MA. Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates. *Nat. Med.* 2003; 12:1520–1527. [PubMed: 14625546]

Figure 1. Phosphatidylinositol phosphate metabolism

Nine out of 15 members of the MTM/MTMR protein family possess catalytic activity, dephosphorylating PtdIns3P and PtdIns(3,5)P₂ to PtdIns and PtdIns5P respectively.

Figure 2. MTM/MTMR functional domains

All MTM proteins (except MTMR14) have four functional domains that mediate protein–protein and protein–lipid interactions: (i) PH-GRAM; (ii) RID; (iii) PTD; and (iv) SID. The GRAM domain, which overlaps with a PH motif, binds to phosphoinositides. The RID domain is a membrane-targeting motif, whereas the SID domain mediates protein–protein interactions. The Cys-Xaa₅-Arg protein tyrosine phosphatase active site is found within the PTD domain. In addition, most MTM proteins also contain a coiled-coil (CC) domain and a PDZ-binding motif.

Figure 3. A model to describe the role of MTM/MTMR proteins in apical ES–apical TBC and BTB dynamics at stages VII and VIII of the seminiferous epithelial cycle

The seminiferous epithelium in the adult rat testis is divided into a basal and an adluminal compartment by the BTB, which is situated near the basement membrane and comprised of co-existing tight junctions, basal ES, desmosome gap junctions and basal TBCs. Sitting atop the basement membrane are Sertoli cells and developing germ cells (i.e. spermatogonia, spermatocytes and spermatids) that line the entire length of the seminiferous epithelium from the basement membrane to the luminal edge. Depending on their developmental stage, germ cells can adhere to Sertoli cells via desmosome-gap junctions or apical ES. At stage

VII of the seminiferous epithelial cycle (left-hand panel), the apical ES is disassembled, resulting in actin remodelling involving ARP2/3. Apical ES disassembly is triggered by Src-mediated protein phosphorylation (e.g. of N-cadherin and β -catenin) which moves proteins away from sites of cell contact and into endocytic vesicles. Endocytosis at the apical ES involves the participation of MTMRs, as well as dynamin, EPS8 and 14-3-3 proteins. Endocytosed proteins that are not yet destined for degradation can be recycled/transcytosed to the site of the BTB, which remains 'closed' (under the influence of testosterone) until stage VIII at which time it will disassemble to allow spermatocytes to cross the barrier and enter into the adluminal compartment. Cytokines also appear to be involved in apical ES disassembly. At stage VIII of the seminiferous epithelial cycle (right-hand panel), the apical TBC is in place to prepare for spermiation. Here, MTMRs, Src, dynamin and 14-3-3 proteins are involved to mediate the endocytosis of phosphorylated and other cargo proteins which can be recycled/transcytosed to the site of the BTB (which reassembles underneath the next generation of preleptotene spermatocytes in due course) or degraded. ARP2/3 maintains actin as a branched network, whereas MTMRs, dynamin and 14-3-3 proteins facilitate endocytosis and membrane trafficking as the 'old' BTB disassembles. After the residual body has been phagocytosed and the apical TBC has 'cleaned up' the luminal edge of stage VIII seminiferous tubules, spermiation takes place. *De novo* protein synthesis, as well as cytokines and testosterone, also appear to be involved in these events. An animated version of this Figure is available at <http://www.BiochemJ.org/bj/433/0253/bj4330253add.htm>.