Dynamins, Spermatogenesis and Contraceptive Development

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Abstract: Dynamins are large GTPases of ~100 kDa known to participate in endocytosis and interact with the actin-based cytoskeletal network in multiple tissues. Recent studies have shown that dynamins play a critical role in the internalization of integral membrane proteins *via* either clathrin-mediated or clathrin-independent endocytosis. Furthermore, recent studies have shown that dynamin II interacts with junctional complex adaptors, namely ZO-1 and β-catenin, at the blood-testis barrier in the seminiferous epithelium of adult rat testes. This interaction may be responsible for pulling away tight junction- and adherens junction-based protein complexes, thereby facilitating blood-testis barrier opening to permit preleptotene and leptotene spermatocyte migration, which is a critical event in spermatogenesis occurring at stage VIII of the seminiferous epithelial cycle. In this short review, we highlight some of the latest findings on dynamins in the field, and discuss how this information can be used to further expand the functional studies to tackle the role of dynamins in spermatogenesis. It is likely that dynamins *per se* or their interacting protein partners can become a target for male contraceptive research to compromise spermatogenesis, leading to transient male infertility without perturbing the hypothalamic-pituitary-testicular axis.

Key Words: Testis, GTPases, dynamins, blood-testis barrier, ectoplasmic specialization, tubulobulbar complex, adherens junction, anchoring junction, Sertoli cells.

INTRODUCTION

During spermatogenesis, developing germ cells migrate progressively across the seminiferous epithelium from the basal to the adluminal compartment. To accommodate germ cell migration, the seminiferous epithelium undergoes extensive junction restructuring throughout the seminiferous epithelial cycle, especially at the sites of the blood-testis barrier (BTB) and the apical ectoplasmic specialization (ES) [1-3]. Besides the rapid turnover of junctions, extensive restructuring of the cytoskeleton also takes place in Sertoli cells, and perhaps germ cells [4]. Together with the reorganization of the cytoskeleton, changes in the steady-state levels of junctional integral membrane proteins lead to the assembled or disassembled states of the BTB and the apical ES. However, the regulatory mechanisms controlling cytoskeletal organization and the levels of junction proteins remain largely unexplored in male reproductive physiology. Obviously, if these mechanisms are known, many of the regulatory molecules can become the targets of contraceptive development, as the disruption of germ cell movement or cytoskeletal reorganization can lead to the arrest of spermatogenesis. The use of this strategy will not affect the hypothalamic-pituitary-testicular axis, thus any side-effects would be minimal. Since recent studies have shown that dynamins participate in different aspects of spermatogenesis, it may be a potential candidate for male contraceptive development.

Dynamins are large GTPases that belong to the dynamin superfamily. The first discovered isoform, dynamin I, was isolated from microtubule-associated calf brain extract about two decades ago [5]. The extracted 100 kDa protein was designated "dynamin", and was proposed to be a mechanoenzyme mediating microtubule sliding. However, the physiological significance of its *in vitro* microtubule related activities remains unresolved. Instead, the role of dynamin as a key player in endocytosis began to unfold shortly after its discovery. This was first shown by the classical study on the *Drosophila* gene *shibire*, which demonstrated that *shibire* encodes multiple forms of dynamins [6], while this locus was previously shown to control synaptic vesicle recycling in *Drosophila* [7]. Since then, much investigation has been dedicated to unveiling the role of dynamin in endocytosis (reviewed in [8-10]). To date, three dynamin isoforms are found, and extensive studies have shown that they play multiple roles in many seemingly unrelated processes, such as actin dynamics, apoptosis, and membrane fusion [11-13].

Members of the dynamin superfamily include three classical dynamins and a number of dynamin-like proteins, such as the yeast Vps1 protein for vacuolar protein sorting, and Mx protein with antiviral activities [14]. The three classical dynamins, namely dynamins I, II and III, have distinct tissue distributions [15] and physiological functions (reviewed in [8-10]). For instance, dynamin I is the neural specific isoform involved in synaptic vesicle recycling, and is not found in organs other than the brain. Dynamin II, with a well-studied role in clathrin mediated endocytosis, is ubiquitously expressed with the highest expression in the testis [16] . Dynamin III has only been found in a few organs including the testis [15].

In earlier studies it was found that dynamins II and III are differentially localized within the seminiferous tubules [17,18]. It is obvious that both isoforms are involved in spermatogenesis, but their precise physiological functions remain elusive. In light of their roles in promoting protein internalization and regulating actin dynamics in other epithelia, dynamins are potential regulators of cell junction and cytoskeletal dynamics during spermatogenesis. It is likely that novel targets for male contraceptive development will emerge when more is known about the functions and interacting protein partners of dynamins in the testis. This article serves to recapitulate the properties and functions of dynamins known to date, and discuss their possible role in spermatogenesis.

DYNAMINS – THE BASICS

1. Structure and Domains

Dynamins are 100 kDa globular proteins which exist as tetramers in solution [19]. The three classical dynamins have similar architecture with five domains. The GTPase domain is located near the N-terminus, followed by the middle domain, the pleckstrin-homology (PH) domain, the GTPase effector domain (GED), and finally the proline-rich domain (PRD) at the C-terminus (reviewed in [9,10,20]). The GTPase domain mediates GTP binding and hydrolysis, and the GED serves to stimulate GTPase activity by interacting with the GTPase domain [19]. This mode of internal activation is not common among GTPases, and will be further discussed in the next section. The middle domain is critical for the tetramerization and further assembly of dynamin [21]. The PH domain and the PRD interact with external factors that may regulate the activity of dynamin or target dynamin to different organelles (reviewed in

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[9]). For instance, the PH domain interacts with acidic phospholipids especially phosphoinositides [22,23], while the PRD binds a large variety of proteins containing the Src homology 3 (SH3) domain (see Table **1**).

2. Regulation of GTPase Activity

Until recently, the only identified "GTPase activating protein" (GAP) for dynamin was GED itself [19], which is viewed as the "internal GAP". It is now generally accepted that activation of dynamin's GTPase activity requires GED and is driven by self-polymerization [24]. The assembly of dynamin tetramers into rings or helices may permit a concerted interaction between neighboring GTPase domains and GEDs [19]. However, the precise interaction is still unclear. Interestingly, the first inter-molecular GAP for dynamin has been proposed lately. The phox homology domain of phospholipase D was shown to activate dynamin's GTPase activity much more efficiently than GED [25]. Importantly, the phospholipase D phox homology domain activates dynamin via a direct interaction with the dynamin GTPase domain. This is unlike other proteins which mediate activation by promoting dynamin assembly, and thereby driving the intrinsic activation described previously. It is suggested that the presence of an inter-molecular GAP may provide a tighter regulation in non-constitutive processes [26].

CELLULAR FUNCTIONS OF DYNAMINS

1. Membrane Remodeling

1.1. Endocytosis

The best known function of dynamins is their involvement in the severance of membrane vesicles during endocytosis. The endocytic processes shown to require dynamin include the well characterized clathrin-mediated endocytosis [27] and also clathrin-independent mechanisms, such as caveolae-mediated endocytosis and pinocytosis [28-30]. Despite ongoing rigorous studies for more than a decade, the detailed mechanism(s) by which dynamins facilitate the formation of endocytic vesicles is still uncertain, but the efforts made so far gave rise to several models. Opinions are still divided regarding whether dynamin serves as a mechanoenzyme [8,9,31] or a regulatory molecule [10,32] in endocytosis.

1.1.1. Mechanoenzyme Model

For those studies supporting dynamin as a mechanoenzyme, the common idea is that dynamin molecules self-polymerize and as-

Table 1. Proteins and Molecules Known to Interact with Dynamins and Their Reported and/or Possible Physiological Functions*

Isoform	Domain	Binding Partner	Function	Possible Function of Interaction/Remarks	Refs.
		Actin binding proteins			
Dyn II**	PRD	Cortactin	Activation of actin nucleation	*In vitro* influence of actin assembly	[57]
Dyn I, isoform specific	PRD	Profilin II	Regulation of actin polymerization		[58]
Dyn I/II	PRD	Mammalian Abp1	Actin regulation in response to signaling	Link cortical actin to endocytosis	[59]
		Scaffold in actin regulatory machinery			
n.d.	n.d.	Intersectin-1	Involved in activation of actin nucleation	Link Dyn to actin assembly machinery	[60]
Dyn I	n.d.	Tuba	Involved in activation of actin nucleation	Link Dyn to actin assembly machinery	[61]
		Microtubules and associated proteins			
Dyn I[+]	PRD	Microtubules	Component of cytoskeleton	*In vitro* stimulation of Dyn GTPase activity	[62,63]
Dyn II	middle	γ-tubulin	Involved in microtubule nucleation	Centrosome cohesion	[64]
		Endocytic proteins			
Dyn I	PRD	Syndapin I	Couples actin regulation to CME		[65]
Dyn 1, GTP bound	GED	Auxilin 1	Cochaperone of Hsc70 during CME	Regulation of vesicle fission by Dyn	[66]
Dyn 1, GTP bound	n.d.	Hsc70	Uncoating of clathrin during CME	Regulation of vesicle fission by Dyn	[66]
Dyn II	PRD	Caveolin-1	Organize signaling molecules on caveolae	Affinity is distinct for different Dyn spliced forms	[28]
Dyn I	PRD	Amphiphysin	Fission of synaptic vesicles	Recruitment of dynamin to membrane with appropriate curvature	[67,68]
		Signal transduction proteins			
Dyn I	PRD	Phospholipase C γ	Signal transduction during RTK activation	*In vitro* binding is growth factor inducible	[69,70]
Dyn I	PRD	Growth factor receptor-bound protein 2	Mitogenic signal transduction	*In vitro* stimulation of Dyn GTPase by promoting self assembly	[69-71]
n.d.	GTPase	Phospholipase D	Generate phosphatidic acid for signaling	GAP for Dyn	[25]
		Others			
Dyn I/II	PH	Phosphoinositides	Component of membrane lipids	Oligomerization dependent membrane binding; *In vitro* stimulation of Dyn GTPase activity	[22,72]

*This list was compiled based on selected studies in different tissues and animal models. The listing is not intended to be exhaustive due to page limits, but our goal is to use this table as a reference guide in particular for new investigators in the field.

**Abbreviations used: CME, clathrin-mediated endocytosis; Dyn, dynamin; GAP, GTPase activating protein; GED, GTPase effector domain; Hsc70, heat shock cognate 70; n.d., not determined; PH, pleckstrin homology; PRD, proline-rich domain; RTK, receptor protein tyrosine kinase.

[+]*Dyn I* (italicized dynamin I) indicates the study was conducted at the time before the other two isoforms were identified.

semble into a spiral around the neck of budding vesicles to form a collar. Next, their GTPase activity helps the vesicles to detach from the membrane. There are two alternative hypotheses for this step. The first one is the "pinchase model", where the mechanical constriction of the dynamin collar leads to the pinching off of the endocytic vesicle. It was demonstrated that dynamin alone could assemble spontaneously on a lipid bilayer forming membrane tubules *in vitro*, and upon the addition of GTP, the dynamin helix constricted and induced vesiculation [27]. Alternatively, in a later proposed "poppase model", it is thought that a sudden spring-like extension of the dynamin collar causes the vesicle to pop off [33]. This was illustrated by a study using lipid nanotubules as a membrane template for dynamin to polymerize and assemble into spirals. It was visualized that GTP-bound dynamins form tightly packed rings, and upon GTP hydrolysis, the spacing between the rings nearly doubled. Although this did not drive vesicle fission in the nanotubules, it was suggested that during *in vivo* condition, the force generated by the dynamin "spring" can be acted upon the budding vesicle and the plasma membrane at its two ends, and thus causes mechanical breakage. In light of a recent study, these two alternatives are seemingly working in concert to facilitate endocytosis. For instance, the addition of GTP to dynamin-coated lipid tubules induced twisting and supercoiling of the tubules [31]. It was argued that constriction alone is not sufficient for vesicle fission, the longitudinal tension produced by twisting was also necessary. Thus, it is likely that dynamin confers both "pinchase" (neck constriction) and "poppase" (longitudinal tension) functions to facilitate vesicle formation during endocytosis.

In addition to vesicle scission, it has recently been proposed that dynamin also participates in an earlier step during vesicle formation. The use of a newly screened dynamin inhibitor called dynasore caused the accumulation of incomplete vesicles. It was surprising that not only O-shaped undetached vesicles, but also U-shaped half formed vesicles accumulated, suggesting that dynamin's GTPase activity may be needed at a critical step when the vesicle assumes its shape [34].

1.1.2. Regulatory Model

On the other hand, a number of studies supported dynamin as a regulatory molecule. It was proposed that the GTP-bound form of dynamin recruits downstream effectors to the site, instead of executing the reaction itself (reviewed in [10]). This idea is supported by a study using dynamin mutants with an impaired GED [35]. These mutants are defective in GTPase activation upon self assembly. However, it was shown that rather than inhibiting endocytosis, their overexpression stimulated receptor-mediated endocytosis. Thus, the "regulatory model" proposes that GTP-bound dynamins are active and serve to recruit downstream partners for vesicle scission. Once the recruitment is complete, dynamin assembles and undergoes GTP hydrolysis, returning to its inactive state. Recently, a potential candidate target of dynamin has been identified [32]. It was suggested that the chaperone activity of Hsc70/auxilin during clathrin-coated pit constriction is subjected to regulation by dynamin.

1.2. Membrane Tubulation

It was first observed that by treating nerve terminals with the unhydrolysable GTP analog GTP-γ S, dynamin coated tubular membrane invaginations were formed. This suggested that dynamin can mediate membrane tubulation when its GTP-bound form is stabilized [36]. This observation was somewhat contradicting with a later study [27], where dynamin molecules spontaneously formed tubes from liposomes without the addition of GTP. Interestingly, a recent study on BAR/F-BAR protein mediated membrane tubulation showed antagonizing effects between actin and dynamin [37]. Membrane tubule formation was enhanced by actin cytoskeleton disruption, but inhibited by dynamin, presumably due to the encouragement of vesicle scission from the tubule by dynamin. Taken together, these results suggest that under physiological conditions, the GTPase cycle of dynamins may be subjected to upstream regulation, so the controlled GTPase activity can result in either membrane tubulation or vesicle fission.

Besides the above artificially induced tubulation events, dynamin II was demonstrated to be necessary for the formation of tubular invaginations in podosomes [38]. Mutant forms of dynamin II either abolished podosome formation, or delayed the normally rapid actin turnover. This thus illustrates a functional relationship between dynamin and actin in membrane tubulation.

2. Regulation of Actin Dynamics

While the mechanisms of dynamins' membrane remodeling activities are still not fully understood, another role in cell physiology has been attributed to dynamins – regulating actin dynamics. In a study using the actin comet model, which is a well established *in vivo* system for studying the regulation of actin nucleation, overexpression of dynamin mutants inhibited the formation of actin tails [11]. This verified that a functional link between dynamin and actin dynamics indeed exists. In addition, an increasing number of key regulatory proteins in actin assembly have been found capable of binding to dynamin at its PRD (reviewed in [39]). One notable example is cortactin, which is a cofactor of Arp2/3 complex mediating *de novo* actin assembly. It was found that dynamin II can influence actin nucleation by purified Arp2/3 complex and cortactin *in vitro* [40]. A later *in vivo* study showed that dynamin II cooperates with cortactin and Arp2/3 complex to reorganize actin filaments in migrating cells following growth factor stimulation [41]. Some other examples of dynamin ligands related to actin regulation include syndapin, intersectin and Tuba (reviewed in [39]). However, it is not yet known whether the binding of dynamin to these proteins can influence actin dynamics.

Nevertheless, this emerging role of actin regulation is not surprising considering that dynamin is a key player in membrane remodeling processes, as actin and membrane dynamics are closely related. Both endocytosis and membrane tubulation discussed in the previous section are tightly associated with actin dynamics (reviewed in [42]). Back to the scenario of endocytosis, it is conceivable that dynamin may coordinate vesicle fission and polymerization of an actin meshwork to aid the propulsion of the vesicle away from the membrane [11]. In this context, dynamin can be a mechanoenzyme, as well as a regulatory molecule.

DYNAMINS AND SPERMATOGENESIS

1. Possible Role in Cell Junction Restructuring

In other epithelia, a number of studies have shown that the disassembly of tight and adherens junctions is accompanied by the internalization of junction proteins *via* clathrin- or caveolae-mediated endocytosis [43-45]. The participation of dynamin II is evident in these events. Overexpression of mutant dynamin, dynamin II^{K44A}, blocked the induced internalization of E-cadherin [43] and occludin [45] in MDCK cells.

In the seminiferous epithelium of mammalian testes, mechanisms must be in place for facilitating junction restructuring that occur during different stages of the seminiferous epithelial cycle. For instance, inter-Sertoli cell junctions at the blood-testis barrier (BTB) created by adjacent Sertoli cells near the basement membrane must disassemble to facilitate the passage of preleptotene/leptotene spermatocytes at stage VIII of the epithelial cycle in adult rat testes. At the same time, the apical ES must also disassemble to release fully developed spermatids (i.e., spermatozoa) into the tubule lumen during spermiation (reviewed in [2]). It would be physiologically uneconomical for continuous degradation of junction proteins to occur each time the junctions need to disassemble. Thus, the internalization and recycling of junction proteins is an attractive mechanism to facilitate junction restructuring during

spermatogenesis, and this is supported by recent findings [46] illustrating that the internalization of integral membrane proteins occludin and N-cadherin at the BTB is regulated by CNP. As a key player of endocytosis, it is conceivable that dynamin II may assist the internalization of tight junction (TJ) and adherens junction (AJ) proteins during spermatogenesis.

1.1. The Blood-Testis Barrier

The involvement of dynamin II in BTB dynamics has been demonstrated in our previous study using the adjudin model [47] (see Fig. **1** for localization of dynamin II at the BTB). In the testis of adjudin treated rats [48], the BTB remains intact [2], while junction disassembly occurs at the ectoplasmic specializations (ES), which are hybrid junctions containing AJ, TJ and focal contact component proteins. After adjudin treatment, dynamin II was found to associate more tightly with the adaptors of AJ and TJ, namely β-catenin and zonula occludens-1 (ZO-1), respectively. Based on the previously proposed engagement/disengagement mechanism, the adaptors of TJ and AJ may dissociate from each other during AJ restructuring, so that TJ can transiently maintain BTB integrity, while preleptotene spermatocytes are allowed to migrate across the BTB [49]. It was proposed that dynamin II may serve to pull the adaptors away from each other, causing disengagement of the AJ- and TJ-integral membrane proteins at the BTB. However, the mechanism of this phenomenon is still poorly understood. It is possible that dynamin II may influence these actin-based junctions indirectly by acting on the actin scaffold. While these findings were based on the use of an *in vivo* animal model, they have important implication in the normal physiology of spermatogenesis, during which extensive junction restructuring takes place in the seminiferous epithelium. Germ cells must traverse the epithelium throughout their development, causing rapid anchoring junction disassembly and reassembly at the Sertoli-germ cell interface. Such extensive anchoring junction restructuring, however, must not affect the integrity of the BTB, which is analogous to the adjudin model [48, 49]. Thus, the role of dynamin II in disengaging the cadherin- and occludin-based protein complexes at the time of extensive anchoring junction restructuring is a novel mechanism to preserve the BTB integrity while facilitating germ cell movement across the epithelium.

1.2. The Apical ES

In our previous study, stage-specific staining of dynamin II was found in the apical compartment [47]. In stages VII to VIII tubules, intense staining of dynamin II was seen on the convex side of spermatid heads, at the site of the apical ES (see Fig. **1**). The apical ES is a testis-specific actin-based hybrid junction type (for a review, see [52]). Apical ES are formed at the Sertoli-spermatid interface when spermatids (step 8 and beyond) begin to elongate, and they anchor developing spermatids to Sertoli cells until their disappearance at stage VIII of the seminiferous epithelial cycle, to be replaced by apical tubulobulbar complex (apical TBC, another testis-specific adherens junction type) until spermiation (for a review, see [2]).

Although the function of dynamin II in apical ES dynamics remains largely unexplored to date, a recent study on fibroblasts [50] may shed light on its possible involvement in apical ES disassembly even though fibroblasts do not possess the ES. In this study, it was demonstrated that dynamin II is necessary for microtubule-targeted focal adhesion disassembly, and it is recruited to the site by tyrosine phosphorylated focal adhesion kinase (p-FAK). The mechanism of dynamin's action has not been investigated in this study, but it was suggested that integrins may be internalized by dynamin-mediated endocytosis, or that the connection between integrins and actin stress fibers may be altered by dynamin. Since p-FAK was shown to associate with the apical ES in the seminiferous epithelium of adult rat testes [51], it is likely that this non-receptor tyrosine kinase also recruits dynamin to the site at specific stages of the epithelial cycle, which in turn regulates ES dynamics.

While the above study was on focal adhesions, the apical ES possesses certain properties and components of focal adhesions normally found in cell-matrix focal adhesions, such as the integrin-laminin-cSrc-pFAK complex. In a previous study from our laboratory [51], it has been shown that the sequence of target protein activation (e.g., FAK) during adjudin-induced apical ES disassembly is strikingly similar to the above study on focal adhesions. For instance, it was shown that during the adjudin-induced disruption of apical ES, the level of pFAK transiently surged prior to the depletion of spermatids from the epithelium [51]. If this drug model reflects the normal physiology of spermatogenesis regarding the regulation of anchoring junction restructuring, it is a compelling proposal that pFAK may recruit dynamin II to mediate apical ES disassembly during spermiation.

2. Other Functions

2.1. Dynamin II in Acrosome Development/Reaction

Studies by immunofluorescence have shown that the expression of dynamin II is very intense in the developing acrosomes of spermatids during spermiogenesis (see also Fig. **1**), where it was found to co-localize and interact with SNARE regulatory proteins, complexins I and II [53]. The authors suggested that dynamin II may play a role in acrosome formation, such as the vesicular transport of acrosome contents from the Golgi to the developing acrosome system. In order to account for the interaction of dynamin II with complexins, it was speculated that dynamin II may also participate in acrosome reaction of spermatozoa during fertilization, possibly by regulating actin assembly or disassembly at the SNARE complex. However, considering a previous study on the yeast form of dynamin Vps1p [13], it is also possible that dynamin II could influence the SNARE cycle directly. SNAREs, which stands for "soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors", are critical for membrane fusion. V-SNARE on vesicle membrane and t-SNARE on the target membrane assemble into a SNARE core complex, so as to drive membrane fusion [54]. During the activation of a new round of membrane fusion, the SNARE core complex from the previous fusion event is dissociated to liberate t-SNAREs. The yeast dynamin Vps1p was shown to trap t-SNAREs after this dissociation, which may help to a create t-SNAREs hot spot and prepare the membrane for fusion [13,55]. Based on the yeast model, it is possible that dynamin II could participate in SNARE activation during the exocytosis of acrosomes, when the vesicular membrane fuses to the plasma membrane. This possibility is worth exploring in future studies.

2.2. Dynamin III in Tubulobulbar Complex Morphogenesis

Dynamin III has a distinct localization versus dynamin II in the seminiferous epithelium [18], and is only expressed in a few organs besides the testis, suggesting it has a special function in the seminiferous epithelium. Recently, dynamin III was found to be a major component in tubulobulbar complexes (TBCs) [56]. TBCs are testis-specific structures containing actin-based junction complexes found at inter-Sertoli cell interface (known as basal TBC at the BTB) and Sertoli cell-elongate spermatid interface (known as apical TBC, which appears at stage VIII of the seminiferous epithelial cycle prior to spermiation, concomitant with the disappearance or dissolution of apical ES) (reviewed in [2]). They are thought to internalize intact cell junctions by the budding of double membrane vesicles into one of the apposing Sertoli cells to facilitate spermiation. In MDCK cells stably transfected with nectin-2, an integral membrane protein found in both Sertoli cells and elongating spermatids, the overexpression of dynamin III induced the formation of membrane tubules containing nectin-2 [56]. Thus, the authors speculated that dynamin III may participate in membrane tubulation during the morphogenesis of TBCs. However, it must be noted that the model used in this study was based on a kidney cell line. Although this cell line is capable of forming impermeable tight junctions *in vitro* as in cultured Sertoli cells, it cannot mimic other phe-

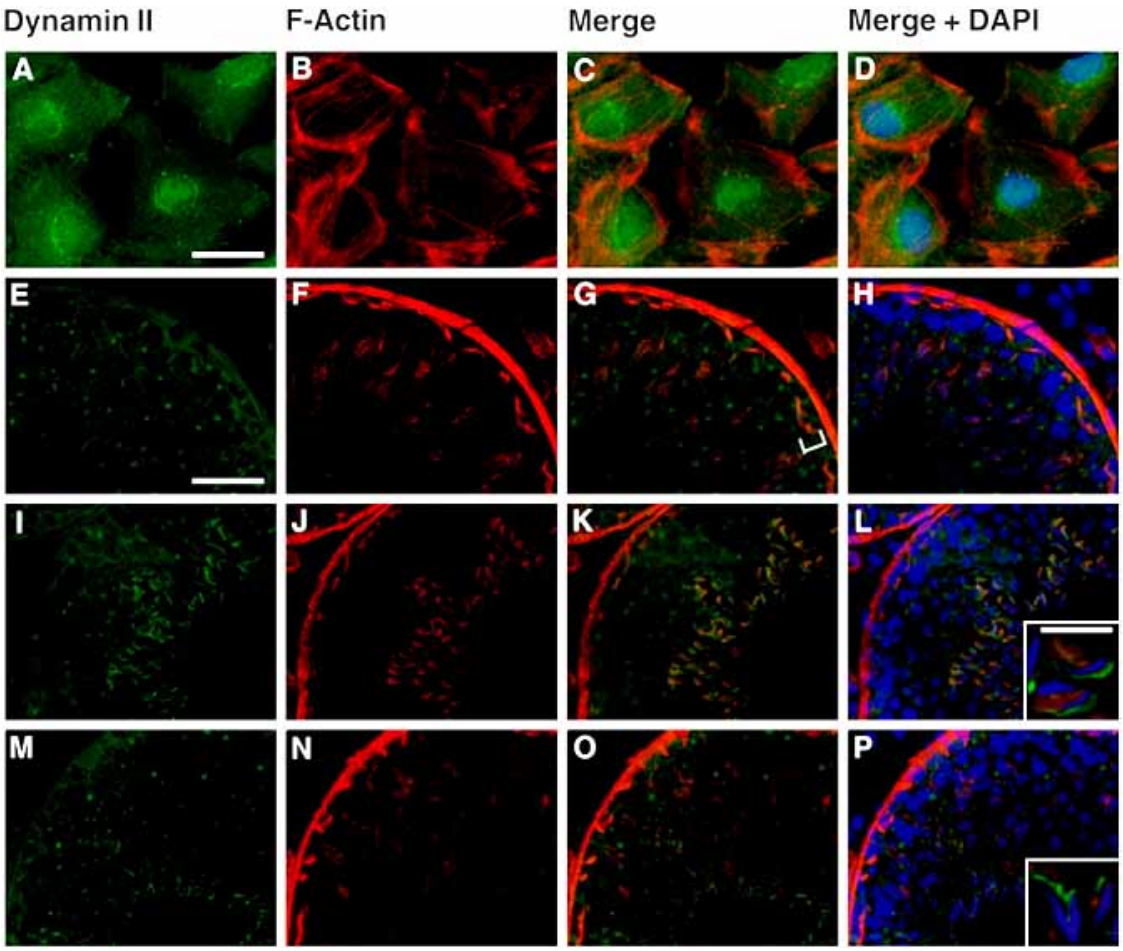

Fig. (1). A study to examine the cellular localization of dynamin II in Sertoli cells *in vitro* (A-D) and the seminiferous epithelium of adult rat testes *in vivo* (E-P). (A-D). Co-localization of dynamin II and F-actin in cultured Sertoli cells isolated from 20-day-old rat testes with a cell density of ~0.025 x 10^6 cells/cm^2. Cells with functional anchoring junctions after being cultured at 35 ^oC for 5 days were used for fixation and staining. In cultured Sertoli cells, dynamin II stained strongly around and near the nucleus, presumably in the trans-Golgi network (A). Furthermore, visible staining of dynamin II (green fluorescence) was detected at the cell-cell contact site (A), where it co-localized with the meshwork of F-actin (stained by using rhodamine phallodin, red fluorescence) (see B and the merged images of A and B in C and D). Where dynamin II and F-actin co-localized, the overlapping images appeared as orange fluorescence. DAPI (4',6-diamidino-2-phenylindole hydrochloride) stained nuclei which appeared in blue. (E-P) Co-localization of dynamin II and F-actin in the seminiferous epithelium using frozen sections of adult rat testes. As illustrated by E-H, dynamin II staining was found at the site of the BTB in all examined tubules (see the white bracket in G). Dynamin II co-localized with F-actin at the BTB, which appeared as orange fluorescence. In I-L, this is a stage VII seminiferous tubule, dynamin II was found surrounding the heads of elongating spermatids, in particular at the convex side of the spermatid heads (see enlarged view in L), consistent with its localization at the apical ES. Some dynamin II staining was also detected and co-localized with F-actin at the concave side. In M-P, this is a stage VIII tubule, dynamin II was still detectable at the convex side of spermatid heads (see enlarged view in P), although the signal was not as strong as in a stage VII tubule (M-P versus I-L). Interestingly, the amount of F-actin around the spermatid heads appeared to be subsided, illustrating that the spermatids were ready for spermiation. An intense staining of F-actin was visible at the tunica propria in all tubules examined. Methods: Cells and frozen sections (~7 μm thick sections obtained with a cryostat) were fixed in 3.7% paraformaldehyde in PBS (10 mM sodium phosphate, 0.15M NaCl, pH 7.4 at 22 ^oC), to be followed by permeabilization by 0.1% Triton X100 in PBS, and rehydration in PBS. Cells and sections were incubated with rabbit anti-dynamin II antibody (Abcam, Inc.; Cat.#: ab3457; Lot 202136) overnight at room temperature, followed by a 30 minute incubation of FITC-conjugated goat anti-rabbit IgG (Zymed, Invitrogen; Cat.#: 62-6111; Lot 40789033) and rhodamine phalloidin (Molecular Probes, Invitrogen; Cat.#: R-415; Lot 41B1-6). Bar in A = 12 μm, which applies also to B-D; bar in E = 80 μm, which applies also to F-P; bar in inset in L = 25 μm also applies to inset in P.

notypic aspects of Sertoli and germ cells, particularly elongated spermatids. Thus, it remains to be shown if dynamin III indeed is involved in TBC formation during spermatogenesis.

CONCLUDING REMARKS

While emerging evidence suggests the significance of dynamins in spermatogenesis (summarized in Fig. **2**), much of this information was obtained by corroborative studies. Future studies should include the use of primary cultures of Sertoli cells or Sertoli-germ cell co-cultures to assess the function of dynamins based on well defined physiological endpoints. Functional studies can be conducted by the use of RNA silencing techniques, transient overexpression and animal models such as knockout mice. The resulting phenotypic changes can be correlated with the defined physiological endpoints. For instance, it will be crucial to assess if the silencing of dynamins can block the endocytosis and/or recycling of integral membrane proteins in Sertoli cell cultures with functional tight junctions, adherens junctions, and/or blood-testis barrier under different experimental conditions.

Apart from the recently described hypothesis that dynamin II may serve to maintain BTB integrity during AJ restructuring [44], other novel functions of dynamins pertinent to junction restructuring in the seminiferous epithelium during spermatogenesis may be uncovered within the next decade. These findings will likely unfold novel targets for male contraceptive development. For instance, spermatogenesis may be perturbed by a disruption of dynamin

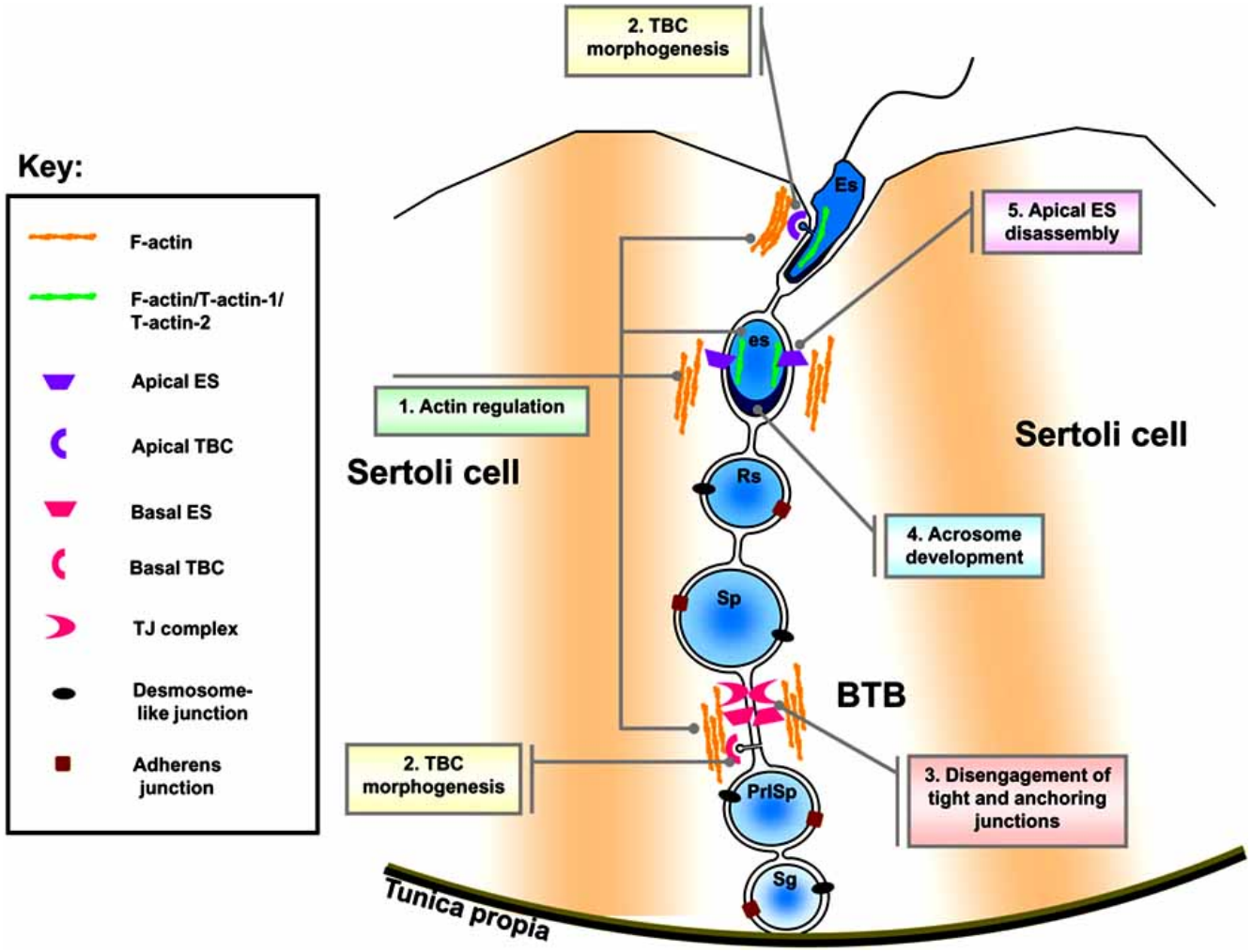

Fig. (2). This is a schematic drawing of the seminiferous epithelium in adult rat testes illustrating the possible sites of action of dynamins regarding their role in spermatogenesis. Between the two adjacent Sertoli cells, germ cells (in blue) at different stages of development are shown: namely spermatogonium (Sg), preleptotene spermatocyte (PrlSp), pachytene spermatocyte (Sp), round spermatid (Rs), elongating spermatid (es, such as a step 8 spermatid) and elongate spermatid (Es, such as a step 18 spermatid) from tunica propria and up. Based on the available information in the literature as reviewed herein (see text), dynamins may play a role in the following events pertinent to spermatogenesis. 1) Actin regulation in the seminiferous epithelium, especially at the actin rich sites, such as the BTB, the apical ES and the apical TBC. 2) Based on the study of Vaid *et al.* [56], dynamin III may facilitate membrane tubulation during the formation of membrane extensions in TBCs. 3) Dynamin II was suggested to maintain BTB integrity during its disassembly, such as at stage VIII of the seminiferous epithelial cycle when the BTB has to open to facilitate preleptotene spermatocyte migration, by aiding the disengagement of junctional complex adaptors [47, 49]. 4) Zhao *et al.* [53] proposed that dynamin II may participate in the development of acrosomes in spermatids. 5) The stage-specific localization of dynamin II in the apical ES (see Fig. **1**) leads to the speculation of its possible involvement in ES disassembly, plausibly by facilitating endocytosis of integral membrane proteins at the ES, thus destabilizing adhesion at the ES. ES, ectoplasmic specialization; TBC, tubulobulbar complex; TJ, tight junction; BTB, blood-testis barrier.

Table 2. Proteins Known to Interact with Dynamin II in Adult Rodent Testes

Isoform Studied	Domain	Interacting Proteins in the Testis	Function	Possible Function of Interaction	References
Dyn II**	n.d.	Complexins I/II	SNARE regulatory proteins	acrosome reaction	[53]
Dyn II	N/A	Occludin	Transmembrane protein in tight junctions	junction restructuring	[47]
Dyn II	N/A	Zonula occludens-1 (ZO-1)	Adaptor protein in tight junctions	junction restructuring	[47]
Dyn II	N/A	N-cadherin	Transmembrane protein in adherens junctions	junction restructuring	[47]
Dyn II	N/A	β-catenin	Adaptor protein in adherens junctions	junction restructuring	[47]

**Abbreviation used: Dyn, dynamin; n.d., not determined; N/A, not applicable since interaction is indirect.

related protein trafficking and/or protein-protein interactions which are uniquely important to spermatogenesis. Since these changes occur locally in the seminiferous epithelium, any side-effects on the hypothalamic-pituitary-testicular axis would be minimized. Thus, studies can be designed to block either dynamins or one of the associated partner molecules to induce reversible male contraception.

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GLOSSARY

Arp2/3 complex. A macromolecular complex containing two actin related proteins. It is the central machinery initiating *de novo* actin nucleation.

Auxilin. A member of the J-protein family. During clathrin-mediated endocytosis, auxilin is associated with the adaptor complex of the coated vesicle, and it acts as the cofactor for the chaperone activity of Hsc70 by recruiting Hsc70 for clathrin uncoating.

Acrosome. An ultrastructure surrounding the heads of developing spermatids (from step 7 through step 19 spermatids in rats) containing acrosin as the major proteolytic enzyme, which is used by mature spermatozoa to digest cellular materials surrounding the ovum during fertilization.

Blood-testis barrier (BTB). An impermeable barrier created by adjacent Sertoli cells near the basal lamina in the seminiferous epithelium of adult mammalian testes containing co-existing tight junctions, basal ectoplasmic specializations, desmosome-like junctions, and gap junctions. It also confers cell polarity and serves as an immunological barrier, segregating post-meiotic germ cell development from the systemic circulation.

Clathrin. A protein with membrane deforming power composed of three 180 kDa and three 34 kDa chains (a hexamer). Clathrin molecules assemble on the plasma membrane to introduce curvature, which serves as a site for the formation of clathrin coated vesicles.

Clathrin-mediated endocytosis. The internalization of clathrin-coated vesicles, usually for regulated transport of transmembrane receptors or integral membrane proteins.

Caveolae. Flask shaped pits on the plasma membrane implicated in endocytosis and signal transduction events. The major protein component of caveolae is consisted of 21-24 kDa caveolins, which also associate with heterotrimeric G-proteins.

Caveolins. Membrane proteins of 21-24 kDa present in caveolae, and are associated with heterotrimeric G-proteins. They comprise the principle structural component of caveolae, and are also implicated in scaffolding function for signal transduction. Caveolins do not associate with clathrin.

Complexins. Small proteins that bind to and stabilize the SNARE core complex during membrane fusion (see SNARE). They were initially found in nerve cells as nerve terminal syntaxin (t-SNARE)-binding proteins.

Cortactin. A cortical actin binding protein. It regulates the actin nucleating activity of Arp2/3 complex.

C-type natriuretic peptide (CNP). CNP is one of the peptide hormones (e.g., atrial natriuretic peptide, ANP and brain natriuretic peptide) with vasodilatory properties. It has recently been shown to be a product of Sertoli and germ cells and can regulate BTB dynamics. CNP binds to and activates natriuretic peptide receptor (NPR)-B but not NPR-A.

Ectoplasmic specialization (ES). A hybrid testis-specific actin-based anchoring junction type restricted to the Sertoli-Sertoli cell interface at the BTB (basal ES) and the interface of Sertoli cells and elongating spermatids of step 8 and beyond (apical ES). It possesses the properties and constituent proteins of adherens junctions, focal contacts, gap junctions and tight junctions.

GTPase-activating proteins (GAP). GTPase regulating proteins containing SH2 and SH3 domains (see Src homology domain). They are capable of stimulating the enzymatic activity of GTPases and thus switching them to an inactive state (for small GTPases which act as molecular switch). GAP can be regulated by phospholipids and tyrosine phosphorylation.

Hsc70. A molecular chaperone with ATPase activity. It mediates clathrin uncoating during clathrin mediated endocytosis.

Intersectin. A guanine nucleotide exchange factor that influences actin assembly through multiple mechanisms.

Phospholipase D. An enzyme that hydrolyzes the ester bonds of the major membrane phospholipid, phosphatidylcholine, into phosphatidic acid and choline. It participates in signal transduction in multiple processes.

Phosphorylated focal adhesion kinase (pFAK). pFAK, the activated form of FAK, recruits cytosolic proteins to the site of focal adhesions for signal transduction. Although it is normally found at focal adhesions, it is largely restricted to the apical ES in the testis (see ectoplasmic specialization).

Phox homology domain. Also called PX domain. It possesses the key residues for binding phosphoinositide.

Pinocytosis. The uptake of extracellular fluid *via* the internalization of small membrane vesicles (endocytosis). Macro- and micro-pinocytosis are distinctive ATP-dependent and –independent cellular processes, respectively.

Podosomes. Dynamic punctate structures at the cell-matrix interface originally described in osteoclasts, and are also found in motile cells during tissue invasion. Podosomes have a specialized molecular architecture, with an F-actin-core surrounded by a ring of structural proteins such as talin, fimbrin and vinculin.

SNARE. A superfamily of small proteins with a central role in membrane fusion, e.g., vesicle fusion to a target membrane or homotypic organelle fusion. SNAREs from two opposing membranes bind to each other to form a stable core complex, and thus bring the membranes into close apposition for fusion. For a new round of membrane fusion, the core complex is dissociated by the SNARE ligands, NSF (*N*-ethylmaleimide-sensitive factor) and SNAPs (soluble *N*-ethylmaleimide-sensitive factor attachment proteins).

Src homology domain (SH domain). A domain in a protein that shares homology with src (src is a family of non-receptor protein tyrosine kinases such as c-Src, Fyn, Yes, Fgr, and Lyn). Examples of SH domains include SH2 domain, which interacts with protein ligands rich in phosphorylated tyrosine residues, and SH3 domain, which interacts with proline-rich ligands.

Syndapin. Also called PACSIN. It serves as a partner of dynamin to mediate vesicle fission, and is implicated in linking endocytosis to actin dynamics by interacting with actin regulatory molecules.

Tuba. A guanine nucleotide exchange factor specific for the GTPase Cdc42, which regulates actin cytoskeleton dynamics.

Tubulobulbar complex (TBC). TBC is a testis-specific structure containing actin-based junction complexes found at the BTB (basal TBC), and at the interface of Sertoli cells and late elongated spermatids prior to spermiation, near the concave side of the elongated spermatid heads (apical TBC). Apical TBC replaces apical ES as the only anchoring device for elongated spermatids about several hours prior to spermiation.

ABBREVIATIONS

AJ	=	Adherens junction
Arp2/3	=	Actin related protein-2/3
BTB	=	Blood-testis barrier
CNP	=	C-type natriuretic peptide
ES	=	Ectoplasmic specialization
GAP	=	GTPase activating protein
GED	=	GTPase effector domain
Hsc 70	=	Heat shock cognate 70
pFAK	=	Phosphorylated focal adhesion kinase
PH	=	Pleckstrin homology
PRD	=	Proline-rich domain
SH3	=	Src homology 3
SNARE	=	Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor
TBC	=	Tubulobulbar complex
TJ	=	Tight junction
ZO-1	=	Zonula occludens-1

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