Filamin A

A regulator of blood-testis barrier assembly during post-natal development

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Filamins are a family of actin-binding proteins composed of filamin A, B and C. Besides of their ability to induce perpendicular branching of F-actin filaments via their actin binding domains near the N-terminus, filamins can regulate multiple cellular functions because of their unique ability to recruit more than 90 protein binding partners to their primary sequences which are having highly diversified cellular functions. However, this family of proteins has not been examined in the testis until recently. Herein, we highlight recent findings in the field regarding the role of these proteins in cell epithelia, and based on recent data in the testis regarding their role on spermatogenesis, this review provides the basis for future functional studies.

Introduction

Filamin A [formerly known as actin-binding protein 280 (ABP280)] is a non-muscle actin filament cross-linking protein first identified in macrophages in 1975.[1] Since then, three isoforms of filamins, known as filamin A, B and C, which are products of distinct genes have been identified in different mammalian epithelia.[2,3] Studies from the past 36 years have shown that filamins play multiple cellular roles, serving as organizers of cell structure (e.g., cytoskeleton) and function, regulating cell signaling, transcription, cell adhesion, focal adhesion assembly, cell apoptosis and organ development.[4-8] A recent study has demonstrated that filamin A serves as a central mechanotransduction element of the cytoskeleton.[9] In short, filamin A working with FilGAP (an filamin A-binding GTPase-activating protein specific for Rac GTPase) and β-integrin acts as a "molecular switch" that converts mechanical stimuli into chemical signals[9] to elicit cellular responses in response to changes in environment, growth and/or development. While the filamin protein family is composed of only three proteins, however, each filamin is known to serve as scaffolds for multiple proteins, and more than 90 binding partners of filamins have been identified to date, ranging from cell adhesion proteins (e.g., β1-, β3- and β7-integrin, ICAM-1), cytoskeletons (e.g., F-actin and vimentin), GTPases (e.g., Cdc42, Rho and Rac), GTPase regulatory proteins (e.g., FilGAP), cytokines (e.g., interferonα), adaptors (e.g., vinculin), ion channels (e.g., K+ channel), receptors (e.g., interferon receptor, dopamine receptor, insulin receptor), signaling proteins (e.g., MEKK1, MKK4 and JNK), protein kinases (e.g., PKCα, ROCK, p21 activated kinase 1 or Pak1), endocytic vesicle-mediated protein trafficking-related proteins (e.g., caveolin-1), proteases (e.g., caspase), polarity proteins (e.g., 14-3-3) and even transcription factors (e.g., androgen receptor, Smads).[5,8] Interestingly, while many of these molecules are intimately related to spermatogenesis (e.g., vinculin, 14-3-3, JNK, ROCK, PKC, Pak1, Smads, caspase and caveolin-1), there is no report in the literature, investigating the role of filamins on spermatogenesis and testicular function except a recent study.[10] Herein, we provide an update on filamins, in particular filamin A and how this protein
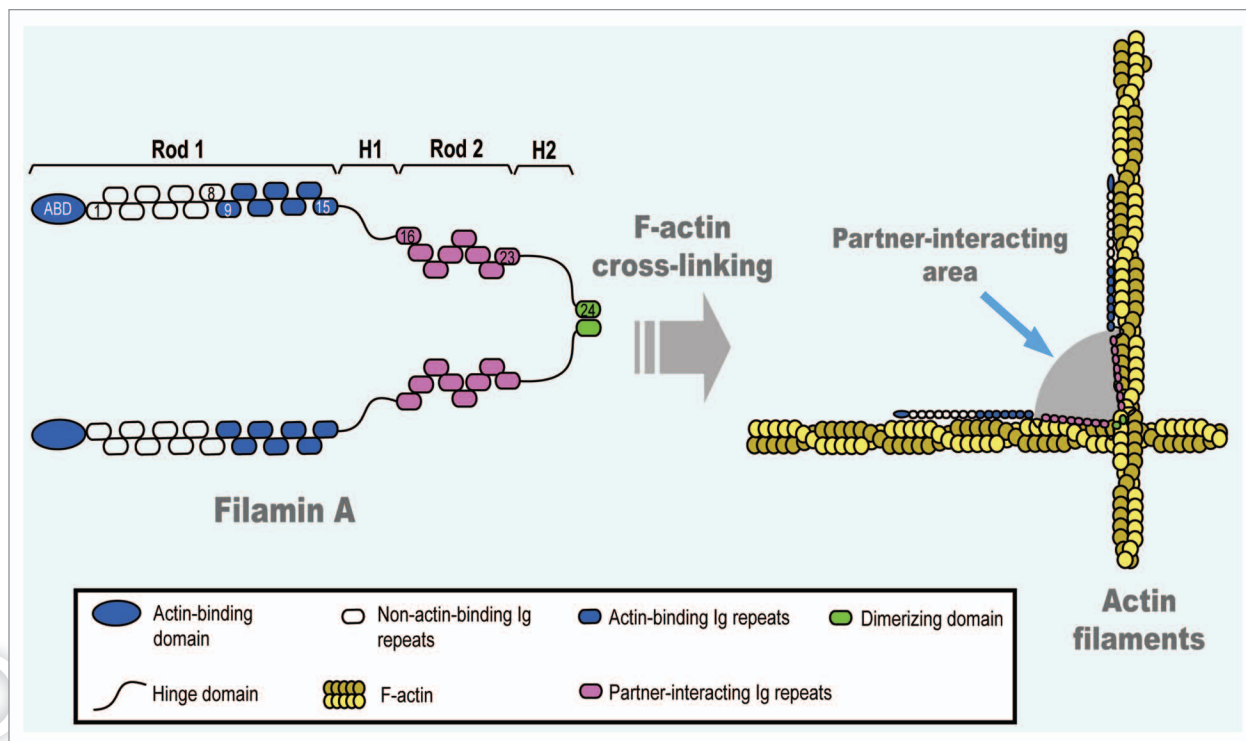
Figure 1. A schematic molecular model of filamin A illustrating its role as a F-actin cross-linker. The actin-binding domain (ABD) of filamin A is located at its N-terminus, which is followed by the 9–15 immunoglobulin (Ig) repeats, constituting the Rod 1, which is capable of binding one F-actin filament. The hing region (H1), the Rod 2 region and the H2 region are also shown. The 24 Ig repeat is the dimerizing domain where two subunits of filamin A are dimerized via the two 24 Ig repeats through non-covalent interactions. The shaded "gray" area between the two Rod 2 domains of two filamin A subunits is the region where filamin A interacts with its binding partners, such as integrins, Rho, Rac, Cdc42, ROCK, Pak1, FiGAP, dopamine receptor and others.

relates to cell adhesion function at the ectoplasmic specialization (ES) at the Sertoli cell-elongating spermatid interface (known as apical ES) and at the Sertoli-Sertoli cell interface at the blood-testis barrier (BTB) (known as basal ES),[11,12] and how filamins can likely be working with other actin binding (e.g., drebrin E)[13,14] and regulatory proteins (e.g., Arp2/3 complex,[15] N-WASP,[15,16] Eps8 [17]).[18,19] This information should be helpful to investigators in the field seeking to study the impact of actin dynamics on different cellular events of spermatogenesis, including spermatogonial stem cell/spermatogonial renewal, germ cell differentiation, meiosis, spermiogenesis and spermiation.[20-24]

Structure of Filamins

Each mammalian filamin is composed of two polypeptide chains of ~280 kDa that self-associate to form a V-shaped dimeric protein,[25] with these two polypeptides being non-covalently linked via their dimerizing domain at the C-terminus (**Fig. 1**), such that each filamin subunit binds to only one F-actin (**Fig. 2**).[4] Each monomer of filamins is composed of an F-actin-binding domain (ABD) at its N-terminus and a rod segment consisting of 24 homologous repeats of ~96 amino acid residues in each repeat [Repeats 1–8 are known to bind vimentin and PKC;[26] Repeats 9–15 that binds F-actin; Repeats 16–23 that binds dopamine receptor, GTPases, β-integrins and Pak1, and Repeat 24 (the dimerizing domain that also binds ROCK) at the C-terminus] that adopts an immunoglobulin-like fold (Ig repeats[27]) (**Fig. 1**). Two calpain-sensitive hinge domain regions that separate the 24 Ig repeats into two large rod domains (Rod 1: Repeats 1–15 and Rod 2: Repeats 16–23) between Repeats 15 and 16 (known as Hinge 1, H1) and between Repeats 23 and 24 at the C-terminus (known as Hinge 2, H2) (**Fig. 1**). Thus, the binding of a V-shaped dimeric filamin molecule[25] to two filamentous actin (F-actin) filaments favors perpendicular (i.e., at 90°) branching of F-actin (**Fig. 1**). Rod 1 domain is mostly used for actin-binding (see **Fig. 1**) while Rod 2 domain associate mostly with other partner proteins[28] (see **Fig. 1**) for filamins.

Filamin A, Ectoplasmic Specialization (ES) and Cell Adhesion

ES is an atypical adherens junction (AJ) type uniquely found in the mammalian testis.[11,29-32] It is limited to the interface of Sertoli cells and spermatids (steps 8–19 spermatids in rats) during spermiogenesis known as the apical ES, but once it appears, it is the only anchoring junction that supersedes desmosome and gap junction (restricted to steps 1–7 spermatids in rats) that anchor developing spermatids to the Sertoli cell in the seminiferous epithelium until it undergoes degeneration at spermiation to allow the release of spermatozoa into the tubule lumen.[11,24,30,33,34] However, ES is also found at the Sertoli-Sertoli cell
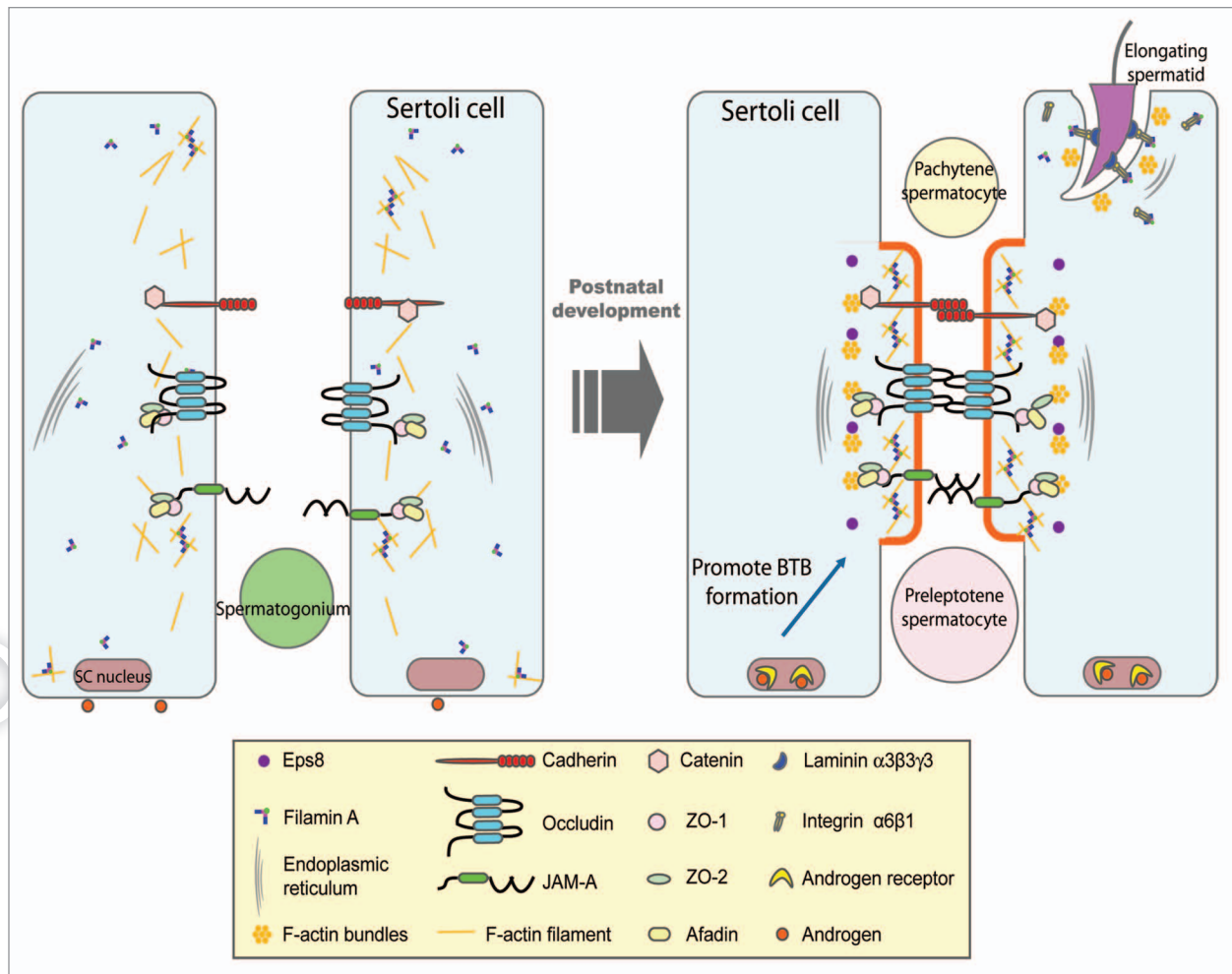
Figure 2. A schematic drawing illustrating the role of filamin A in the assembly of a functional BTB during postnatal development. In immature rat testes, cell adhesion protein complexes (e.g., occludin-ZO-1, cadherin-catenin, JAM-A-ZO-1) cannot be recruited to the BTB site to assemble the functional TJ-permeability barrier (see left part). At age 17–25 d postpartum, the expression of filamin A increases, the functional filamin A recruits the assembly of actin filament network at the BTB site, which in turn, recruits cell adhesion protein complexes. This process is facilitated by androgen, which induces cross-linking of F-actin filaments mediated by filamin A to form rigid scaffold underneath cell membrane, which can lead to membrane protrusion at cell-cell interface to facilitate adhesion formation (see right part). Also, during spermiogenesis, the assembly of cell adhesion protein complexes (e.g., integrin-laminin) at the Sertoli-spermatid interface, namely the apical ectoplasmic specialization (apical ES), is likely facilitated by the recruitment of integrins to the apical ES via interactions between integrins (e.g., α6-integrin, β1-integrin) and filamin A (right part).

interface at the BTB, known as the basal ES.[30] But unlike the apical ES, the basal ES coexists with tight junction (TJ) and gap junction (GJ); and together with desmosome, all these junctions constitute the BTB (since the endothelial TJ-barrier of the microvessels located in the interstitium contribute relatively little to the barrier function of the BTB in the testis), so that post-meiotic spermatid development takes place in the adluminal (or apical) compartment behind the BTB in an immune-privileged site,[30,35-37] segregated from the host immune system in the mammalian body.[30,38,39]

ES is typified by the presence of tightly packed actin filament bundles sandwiched between cisternae of endoplasmic reticulum and the opposing plasma membranes of either the Sertoli cell and the elongating/elongated spermatid (at the apical ES) or the two adjacent Sertoli cells (at the basal ES),[11,30] which is also the hallmark ultrastructure of the ES, making this anchoring junction type in the testis different from all other anchoring junctions in the mammalian body. However, at the apical ES, this typical ultrastructural features, namely the actin filament bundles that lie perpendicular to the plasma membrane is limited only to the

Sertoli cells without comparable ultrastructures visible in the spermatid, but the actin filament bundles are found on both sides of the Sertoli cells at the basal ES.[30,33,40] Recent studies using a mechanical device to pull attached spermatids from the Sertoli cell epithelium in order to estimate the strength of the apical ES have shown that this is one of the strongest anchoring junctions, significantly stronger than desmosome in the testis,[41,42] and it is noted that desmosome is considered to be a very strong adhesion junction.[43,44] Based on these findings, it was postulated that the unusual adhesive strength of the ES (e.g., apical ES) is the result of these actin

filament bundles.[30,40] Indeed, the BTB is one of the tightest blood-tissue barriers in the mammalian body, which is also largely contributed by the tightly packed actin filament bundles at the basal ES coexisting with TJ.[30] Since filamins (e.g., filamin A) induce perpendicular branching of F-actin filaments, the presence of filamin A in Sertoli cells of the rat testis[10] seemingly suggest that it is being used to induce changes in the conformation of the tightly packed actin filament bundles which are necessary to maintain the morphology of apical and basal ES in the seminiferous epithelium, to a "branched" state, facilitating ES restructuring during spermiogenesis. This possibility is physiologically necessary since spermatids are not anchored statically in a specific location in the epithelium during spermiogenesis. Instead, developing spermatids are moving "up-and-down" the epithelium during the epithelial cycle, perhaps to "acquire" necessary signals and nutrients from the Sertoli cells at the cell-cell interface, namely the apical ES, for their development during spermiogenesis which is composed of a series of dynamic changes, both in cell shape, morphology, biochemically, and at the molecular level. On the other hand, the basal ES at the BTB is also not a static ultrastructure even the barrier function conferred by the BTB cannot be compromised, event transiently, during spermatogenesis. This is because preleptotene spermatocytes connected in "clones" via intercellular bridges must traverse the BTB at stage VIII of the epithelial cycle to enter the adluminal compartment to continue their development, such as meiosis I and II, and spermiogenesis. Thus, the basal ES is also a highly dynamic ultrastructure. In short, ES provides unusual adhesive strength to the developing spermatids during spermiogenesis via apical ES, and to the Sertoli cell at the BTB via basal ES, this ultrastructure requires intricate regulation so that the actin filament bundles can be "switched" on-and-off between the "bundled" and "branched" state such that the adhesive function can be constantly regulated during the epithelial cycle. The fact that filamins can rapidly induce branched actin filaments, it is likely that filamins are working in concert with the Arp2/3/N-WASP protein complex that is known to confer branched actin polymerization, these proteins thus provide an efficient molecular mechanism to alter the "fluidity" and "rigidity" of the actin filament bundles at the ES during spermatogenesis. In short, filamins are important cell adhesion regulators based on their intrinsic actin binding activity, along with their protein binding partners, so that multiple regulatory proteins can be recruited to the actin filament bundles at the ES during spermatid movement throughout spermatogenesis (**Fig. 2**). For instance, recent studies have shown that filamin A can recruit integrins to the specific cellular domains via a unqiue mechanism,[45] and β1-integrin is a known constituent cell adhesion protein at the apical ES;[46-48] and a filamin A-integrin receptor complex has been identified in epithelial cells to elicit changes in cell adhesion,[49] and filamin B was found to be involved in the assembly of focal adhesion complex (or focal contact, a cell-matrix anchoring junction type).[8] These latter findings thus illustrate that filamins can regulate changes in cell adhesion via their indirect effects on cell adhesion proteins (e.g., integrins) at the apical ES (**Fig. 2**), besides the actin-based cytoskeleton and the vimentin-based intermediate filament cytoskeleton.

Filamin A and Blood-Testis Barrier Function in the Testis

In light of the findings regarding the role of filamins as a cell organizer and a regulator of cytoskeletal function, it is not unusual that filamin A is predominantly found in Sertoli cells in the testis since germ cells lack the extensive actin filament networks.[10] Interestingly, filamin A was predominantly expressed in developing testes, in particular at the BTB at ~15–25 d postpartum and tightly co-localized with the F-actin network,[10] at the time the BTB was being assembled.[50] Furthermore, a knockdown of filamin A by RNAi using specific siRNA duplexes in Sertoli cells was found to perturb the TJ-permeability barrier function due to a disruption of actin dynamics.[10] This result was consistent with a recent report using human coronary artery endothelial cells (HCECs) in which a knockdown of filamin A was found to reduce the vascular permeability in vitro.[51] Proteomics analysis using bovine brain capillary endothelial cells (BBCEC) also revealed the participation of filamin A in establishing the blood brain barrier phenotype.[52] More important, the knockdown of filamin A in vivo was found to significantly delay the BTB assembly in developing rat testes, which was caused, at least in part, by the inability of BTB proteins (e.g., occludin, N-cadherin) to be recruited to localize properly at the BTB site to induce necessary cell adhesion at the Sertoli-Sertoli cell interface (**Fig. 2**),[10] demonstrating for the first time that filamin A is a functionally significant actin-binding and cross-linking protein crucial for the assembly of the BTB, which is necessary for initiation of spermatogenesis, such as differentiation of type A spermatogonia to B type to initiate cell cycle progression in the testis.[50,53]

Concluding Remarks and Future Perspectives

The recent identification of filamin A in Sertoli cells and its involvement in regulating BTB assembly during postnatal development in rats[10] has added a new member to the growing list of actin regulatory proteins in the testis, which include Eps8, Arp3, N-WASP and drebrin E.[13,14,16,18] It is obvious that this list will be rapidly growing in the years to come. Furthermore, future investigations need to include studies to assess how these proteins are working with other protein partners in the testis to regulate spermatogenesis, such as polarity proteins (e.g., PAR6, 14-3-3).[19] Additionally, future studies should take advantage of what are known in the field regarding the roles of these actin binding and/or regulatory proteins in other epithelia, so that better functional experiments can be designed to understand the intricate actions of these proteins to regulate different distinctive cellular events in the seminiferous epithelium pertinent to spermatogenesis.

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