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p70 S6 kinase and actin dynamics
A perspective

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p70 S6 kinase (p70^{S6K}), a member of the AGC serine/threonine kinase family, was initially identified as a key player, together with its downstream effector S6, in the regulation of cellular growth and survival. The p70^{S6K} protein has emerged in recent years as a multifunctional protein which also regulates the actin cytoskeleton and thus plays a role in cell migration. This new function is through two important activities of p70^{S6K}, namely actin cross-linking and Rac1 and Cdc42 activation. The testis is critically dependent on an intricate balance of fundamental cellular processes such as adhesion, migration, and differentiation. It is increasingly evident that Rho GTPases and actin binding proteins play fundamental roles in regulating spermatogenesis within the testis. In this review, we will discuss current findings of p70^{S6K} in the control of actin cytoskeleton dynamics. In addition, the potential role of p70^{S6K} in spermatogenesis and testicular function will be highlighted.

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Introduction

Cell migration is an essential component of a variety of processes including wound repair,[1] angiogenesis,[2] immunity,[3] and metastasis.[4] Coordinated changes in actin cytoskeleton reorganization in response to microenvironmental signals result in migration. Thus, much effort has been made to understand the molecular machinery that drives the movement of the cell and has focused on the nature of cytoskeletal structures. Indeed, the actin cytoskeleton is essential and central to every step of the migration process.[5] The 70 kDa ribosomal S6 kinase (p70^{S6K}) is a serine/threonine kinase with a well-established role in protein synthesis.[6] Although it was originally described as being exclusively involved in cell growth, our laboratory has recently published data describing p70^{S6K} in other key aspects of cell functions, such as cellular migration. In this new role for p70^{S6K}, the protein interacts with the actin cytoskeleton and activates the Rho GTPases to catalyze the formation of lamellipodia and filopodia.[7] In this article, we will review the current data that may provide new insights to the potentially important role for p70^{S6K} as a possible regulator of actin dynamics in the testis.

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Overview of p70^{S6K}

p70^{S6K} belongs to the AGC subfamily of serine/threonine kinases, which also includes other important signaling molecules like Akt, protein kinase A and protein kinase C. p70^{S6K} is encoded by the *ribosomal protein S6 kinase, 70 kDa, polypeptide 1* gene (*RPS6KB1*), which is located on chromosome 17q23.1 in humans. p70^{S6K}, with an apparent electrophoretic mobility of 70 kDa,[8] consists of 502 amino acids and a molecular weight of 56,153 Da. The amino acid sequence of p70^{S6K} has 100% similarity in all mammalians so far examined.[9] *S6K* gene has also been identified in several invertebrate species, including *Drosophila melanogaster*[10,11] and *Caenorhabditis elegans*.[12] A novel *S6K* was recently found in the yeast *Schizosaccharomyces pomba*.[13] All these indicate that *S6K* is evolutionary conserved among eukaryotes and therefore may represent a significant functional component.

Structure of p70^{S6K}. p70^{S6K} can be divided into five functional domains/regions: (1) the amino (N)-terminal domain, (2) the AGC-kinase conserved catalytic domain, (3) the linker region, (4) the putative autoinhibitory domain, and (5) the carboxyl (C)-terminal domain.[14] At least eight phosphorylation sites have been mapped in endogenous kinase, including Ser^{411}, Ser^{418}, Thr^{421} and Ser^{424} in the autoinhibitory domain,[15,16] Thr^{229} in the catalytic domain[17] and Ser^{371}, Thr^{389} and Ser^{404} in the linker region (**Fig. 1**).[15] The kinase exists in two conformations, inactive and active state. In the inactive state of p70^{S6K}, the carboxyl-terminal autoinhibitory domain, which has sequence similarity to the substrate region of the S6 protein, may act as a pseudo-substrate and interacts with the N-terminus (**Fig. 1**).[14] According to the current model, p70^{S6K} activation is initiated by the release of the autoinhibition exerted by the autoinhibitory domain.[18] This is then followed by a series of phosphorylation of eight or more serine or threonine residues at the autoinhibitory domain, the linker region, and then the catalytic domain, to obtain full kinase activation.[6,19-23]

Regulation of p70^{S6K}. The activity of p70^{S6K} is regulated through phosphorylation/dephosphorylation events. The phosphorylation events are stimulated by a variety of mitogenic factors.[24,25] Several upstream in vivo signaling pathways have been identified to regulate the phosphorylation and activation of p70^{S6K}. One pathway that has been widely accepted is the phosphatidylinositol 3-kinase (PI3K)/Akt pathway.[26-28] Following stimulation, PI3K is recruited to plasma membrane and activated by G-protein coupled receptors or receptor tyrosine kinase.

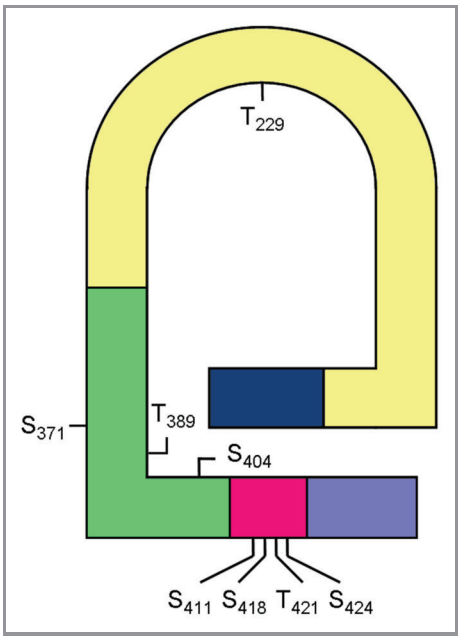

Figure 1. A model to illustrate domains and phosphorylation sites of p70^{S6K}. p70^{S6K} can be divided into five functional domains/regions: (1) the amino (N)-terminal domain (blue), (2) the AGC-kinase conserved catalytic domain (yellow), (3) the linker region (green), (4) the putative autoinhibitory domain (red), and (5) the carboxyl (C)-terminal domain (purple). Eight phosphorylation sites have been mapped.

Active PI3K then phosphorylates the membrane lipid phosphatidylinositol 4,5-biphosphate PIP_2 to produce phosphatidylinositol 3,4,5-biphosphate PIP_3 which recruits and activates 3-phosphoinositide-dependent kinase 1 (PDK1). PDK1 has been shown to markedly phosphorylate p70^{S6K} to acquire full kinase activation.[18,26,29,30] Alternatively, PDK1 phosphorylates and activates Akt, which is recruited by PIP_2/PIP_3 to plasma membrane, and active Akt subsequently activates p70^{S6K}.[31] Mammalian target of rapamycin (mTOR) is another signaling protein downstream of PI3K pathway that phosphorylates p70^{S6K} in vivo.[20,32] Dunfner et al. has proposed that an additional signaling pathway is required for full p70^{S6K} activation as PI3K, PDK1, and mTOR only partial activate p70^{S6K} in vivo.[26] Extracellular signal-regulated kinases 1/2 (ERK1/2) under the mitogen-activated protein kinase (MAPK) signaling has been shown to phosphorylate p70^{S6K} in vivo.[33] However, the involvement of ERK1/2 in p70^{S6K} activation is controversial since there are studies providing evidence that ERK1/2 is neither necessary nor sufficient for p70^{S6K} activation.[34,35] As the serine/threonine sites in the autoinhibitory domain of p70^{S6K} are of consensus sequences similar to those recognized by MAPKs and that phosphorylation at the autoinhibitory domain is an early step required for p70^{S6K} activation, MAPK has been suggested to be essential for p70^{S6K} activation similar to PI3K.[27,36] Moreover, there are reports revealing that p70^{S6K} can be activated by the Raf/MEK/ERK signaling under specific physiological conditions.[27,37] In addition to ERK1/2, p38 MAPK and Jun N-terminal kinase (JNK) are also putative kinases regulating p70^{S6K} activation in the cell.[18,38]

Functions of p70^{S6K}. *1. Protein synthesis.* Ribosomal S6 protein (S6), a component of the 40S ribosomal subunit, is the first identified downstream target of p70^{S6K}.[6] Through phosphorylation of S6, p70^{S6K} regulates the translation of a subset of mRNA containing an oligopyrimidines tract at the 5' untranslated region, 5' TOP mRNA.[39] These 5'TOP mRNAs account for 20% of total cellular mRNA and most of them encode ribosomal proteins, elongation factors, and poly(A)-binding protein, which are essential components for the translation machinery.[40,41] In addition to S6, p70^{S6K} also regulates both the initiation and elongation phases of translation by phosphorylating eukaryotic translation initiation factor 4B (eIF4B), a cofactor of an RNA helicase, eukaryotic translation initiation factor 4A (eIF4A).[42] Phosphorylation of eIF4B leads to the assembly of eIF4A to form the translation initiation complex, which subsequently increases the rate of translation. Furthermore, p70^{S6K} can regulate translation elongation by inhibiting the kinase activity of eukaryotic elongation factor 2 kinase (eEF2K), a negative regulator of eukaryotic elongation factor 2 (eEF2).[43]

2. Cell growth and cell size. The regulation of p70^{S6K} on cell growth has been studied in Drosophila and mice. Montagne et al. has demonstrated that loss of *S6K* gene is semi-lethal to Drosophila, in which the few surviving adults had a severely reduced body size due to a decrease in cell size rather than a decrease in cell number.[44] However, in mice the deficiency of the *S6K* gene is not lethal, although their body size at birth was reduced due to a decrease in organ weight, including the testis.[45] Cell growth modulated by p70^{S6K} may be independent of S6-mediated protein translation because the level of S6 phosphorylation remains intact in the *S6K* knockout mice.[45] S6K1 Aly/REF-like target (SKAR), a mRNA processing protein, is later revealed as a downstream regulator of p70^{S6K} on cell growth.[46]

3. Cell cycle progression. Evidence for a role of p70^{S6K} in cell cycle progression comes from studies using the p70^{S6K} neutralizing antibodies[47] or the immunosuppressant rapamycin.[48-50] These inhibitory effects cause G_1 arrest and significantly delay S phase entry. p70^{S6K} may also promote G_1 to S phase transition by increasing the translation of cyclin D1 and p21 which are critical proteins forming the cyclin-CDK complex in G_1 phase required for Rb phosphorylation and the subsequent entry of S phase.[51]

4. Cell survival. Bcl-2-associated death promoter (BAD), a proapoptotic protein, is a downstream effector of p70^{S6K} that regulates cell survival. The apoptotic effect of BAD can be abrogated upon phosphorylation.[52] BAD would be hypophosphorylated in the absence of p70^{S6K}.[53] Moreover, overexpression of p70^{S6K} can rescue cell from apoptosis by expressing either BAD wildtype or S112A.[53] p70^{S6K} may also promote cell survival through phosphorylation of Mdm2, a p53 ubiquitin ligase.[54]

In addition to the major functions mentioned above, p70^{S6K} can regulate other cellular functions through downstream effectors, such as cAMP-responsive element modulator τ (CREMτ) for gene transcription[55] and insulin receptor substrate 1 (IRS1) for homeostasis.[56]

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Actin Filament Dynamics

A dynamic actin cytoskeleton is essential for many cellular functions, such as maintenance of cell shape,[57] cell junction,[57] and cell motility.[5] Actin in cells exists in three different forms: monomeric (globular-actin; G-actin), oligomeric, and polymeric (filamentous actin; F-actin).[58] F-actin is polarized with a fast-growing plus end, also known as barbed end, and a slow-grown minus end, also known as pointed end.[59] Reorganization of the actin cytoskeleton, including polymerization, depolymerization, nucleation, bundling/ cross-linking, capping, severing, and branching, is facilitated by the actin binding proteins.[58] The Rho family GTPases, including Rac1–3, Cdc42, and RhoA-C, play a central role in coordinating the actin binding proteins for cytoskeleton reorganization.[60] Rac1 and Cdc42 transduce signals to the actin binding proteins through two major types of interacting proteins: (1) Wiskott-Aldrich syndrome protein (WASP)/ suppressor of cAMP receptor (Scar)/WASP family verprolin homology protein (WAVE) and (2) p21-activated kinase 1 (PAK1). WASP/Scar/WAVE promotes F-actin nucleation upon Rac1 and Cdc42 activation. While WASP family protein is activated by Cdc42,[61,62] Scar/WAVE family protein is indirectly activated by Rac1 through the Nck-adaptor complex.[63,64] Active WASP/WAVE then undergoes conformational change and binds with the ATP-G-actin binding protein, profilin, and the actin-related protein2/3 (Arp2/3) complex. These work synergistically to speed up actin branching and polymerization through actin nucleation of ATP-G-actin to the pre-existing actin filaments,[65,66] thereby facilitating the building of the dendritic actin network.[67] On the other hand, PAK1, a downstream effector of Rac1 and Cdc42, phosphorylates and activates LIM kinase (LIMK). LIMK phosphorylates cofilin, an actin filament serving protein,[68,69] leading to its inactivation, which in turn inhibits depolymerization and severing of actin filaments. LIMK can also be phosphorylated by p160 Rho-associated coiled-coil-containing protein kinase (ROCK), a downstream effector of Rho.[70] Apart from the LIMK, Rho can also regulate actin polymerization through another downstream effector diaphanous-related formin, which promotes the polymerization of unbranched filaments.[71,72]

p70^{S6K} in the control of actin cytoskeleton. The Rho GTPases and p70^{S6K} were first shown to coexist in the same pathway in a study by Chou et al.[73] Rac1 and Cdc42, but not RhoA, complex with and activate p70^{S6K}, which can be blocked by the mTOR inhibitor, rapamycin and the PI3K inhibitor, wortmannin.[73] A role of p70^{S6K} in actin cytoskeleton reorganization was further hinted by Berven et al. and through to colocalize with the stress fibers and actin arc at the leading edge of Swiss3T3 fibroblasts under growth factor stimulation.[74] This colocalization of p70^{S6K} and stress fibers was suggested to regulate actin polymerization as rapamycin treatment could inhibit the elongation and organization of actin stress fibers via inhibition of p70^{S6K}.[75] However, the biological function of such an interaction is not known. Recently, our lab not only identified p70^{S6K} as a critical regulator of the actin cytoskeleton but also showed that it is pivotal for the directional migration of cancer cells, which is a prerequisite of metastasis (**Fig. 2**).[7] Our findings provide several insights into the regulation of p70^{S6K} on the actin cytoskeleton. First, we have demonstrated for the first time that p70^{S6K} can directly bind with and cross-link F-actin in vitro. Moreover, active p70^{S6K} colocalizes with the actin filaments at the leading edge of motile cells in vivo and p70^{S6K}-F-actin colocalization is cytochalasin D-sensitive. However, unlike some actin bundling/cross-linking proteins,

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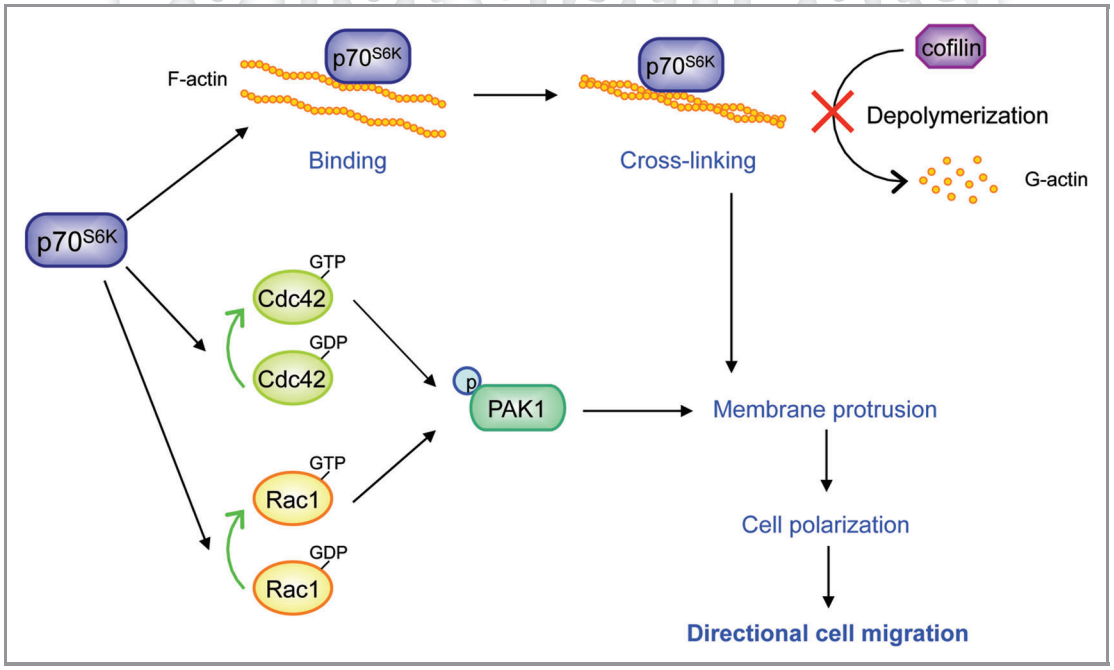

Figure 2. Schematic illustration on the mechanism by which p70^{S6K} regulates actin cytoskeleton in ovarian cancer cells. p70^{S6K} directly binds/cross links with F-actin and activates PAK1 through Rac1 and Cdc42 to modulate actin cytoskeleton dynamics for cell migration.

p70^{S6K} does not change the rate of actin polymerization, but stabilizes actin filaments by decreasing the rate and extent of ADP/cofilin-dependent actin depolymerization.

Second, our results suggest that the correlation of p70^{S6K} with Rho GTPases and cell migration depends on the cell type or on the signaling context, leading to differential functions. In fibroblasts, p70^{S6K} acts as a downstream effector of Rac1 and Cdc42. It is interesting to note that this activation of p70^{S6K} by Rac1 and Cdc42 appears to be independent of the ability of these Rho GTPases to regulate the cytoskeleton during cell cycle progression.[73] By contrast, in carcinomas and epithelial cells, we have shown that p70^{S6K} functions upstream of both Rac1 and Cdc42 to regulate actin cytoskeleton reorganization and thus cell migration, in which Rac1 and Cdc42 are known to function. We also show an essential role for PAK1 in this process.

Third, the regulation of the actin cytoskeleton by p70^{S6K} reveals that many oncogenic signals could mediate cancer cell invasion and metastasis by modulating p70^{S6K} activity. For example, p70^{S6K} is a downstream effector of the PI3K/Akt pathway, which is frequently activated in human cancers. Moreover, p70^{S6K} is also well known to be activated by hormones, cytokines, and growth factors. Our finding that p70^{S6K} binding to the actin cytoskeleton is more effective in the presence than in the absence of p70^{S6K} phosphorylation indicates that it is a dynamic regulation of the actin cytoskeleton, reinforcing the notion that cell migration is a finely tuned event. This also implies that the actin-binding domain in p70^{S6K} in an inactive conformation may be unseen and suggests an additional regulation inside the cell.

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Actin Cytoskeleton Reorganization and Spermatogenesis

Spermatogenesis is a process in which diploid spermatogonia (germ cells) go through a series of stages and differentiate into mature spermatozoa between Sertoli cells within the seminiferous tubule. Many cellular events are involved in this process, including cell division, differentiation, cell movement, reconstructing of cell junctions,[76] changes in cell shape and size such as differentiation of elongated spermatids from round spermatocytes, all of which require dramatic reorganization of the actin cytoskeleton. Since actin cytoskeleton dynamics and some of the regulatory proteins in spermatogenesis have been comprehensively reviewed,[76,77] we will focus on the activities of actin bundling/cross-linking and Rho GTPases.

Actin cytoskeletal network in Sertoli cell. Sertoli cells in the seminiferous tubule extend from the basal lamina to the lumen of the tubule and are able to alter their cell shape to accommodate morphological changes of germ cells, thereby providing both structural and nutritional support to the germ cells throughout their development.[78] In Sertoli cells, F-actin are abundantly detected and are concentrated at the adherens junctions (AJ), the ectoplasmic specialization (ES) and the tubulobulbar complex (TBC).[79] The arrangement of F-actin in the AJs, ES and TBC, is drastically different from that in other polarized epithelia which will be discussed in the following sections.

Ectoplasmic specialization (ES). ESs are localized at two sites in seminiferous epithelium: (1) junctions between adjacent Sertoli cells near the basal lamina of the seminiferous epithelium, namely basal ES; (2) and adhesion between Sertoli cells and elongating/elongated spermatids at the apical region of the seminiferous epithelium, namely apical ES. F-actin at the ES forms a hexagonal array between the plasma and endoplasmic reticulum membranes of Sertoli cells.[80] Although myosin VIIa, an actin motor protein, has been found to be enriched at the apical ES, the actin bundles at ES are thought to be non-contractile. Instead, these actin bundles may structurally contribute to the stability of the intercellular adhesion at ES. The mechanisms underlying the above processes are largely unknown. However, based on the actin bundle structure at the ES, formin that has been shown to be abundant in Sertoli cells has been proposed to be important in the regulation of actin polymerization at the ES.[79,81] Ena/VASP family proteins that promote actin elongation at pointed end by tethering the filaments to sites of active actin assembly may also be involved. While the localization of formin and Ena/VASP at the ES is still unknown, several actin bundling/crosslinking proteins have been found at the ES site. These include espin,[82] fimbrin,[83] vinculin,[83] Eps8,[84] and α-actinin.[85] The temporal and spatial expression of Eps8 and Arp2 at the Sertoli cell-spermatid interface (apical ES) coincides with the onset of spermatid elongation.[82] The expression of Eps8 at the blood-testis barrier (BTB) is high at all stages of the epithelial cycle, except at stage VIII when the BTB undergoes extensive restructuring to facilitate the transit of preleptotene spermatocytes.[84] Interestingly, the expression of Arp3 is also significantly induced at the BTB at stage VIII of the spermatogenesis cycle.[86] Although the activity of actin bundling proteins at the ES has not been directly demonstrated, treatment of developing rats with adjudin, a drug that disrupts AJ at the Sertoli-germ cell interface, showed that espin appears to function with establishment of BTB.[87] Small Rho GTPases known to regulate actin are also implicated in regulating spermatogenesis within the testis. For example, RhoB was found at the apical ES in region in association with elongating spermatids. RhoB expression was decreased markedly surrounding the elongated spermatids at late stages VII to VIII of the cycle.[88]

Tubulobulbar complex (TBC). Similar to ES, TBC is localized to both the basal and apical region of the Sertoli cell: (1) basal TBC is between Sertoli cells near the basal lamina of the seminiferous epithelium; (2) apical TBC is at adhesions between Sertoli cells and the concave side of the head of elongated spermatids at the apical region of the seminiferous epithelium and appears just a few days before spermiation at late stage VIII.[89] Various functions of TBC has been proposed, such as to anchor spermatids to the Sertoli cells, to remove cytoplasm from spermatids,[90] to facilitate shaping of spermatid heads,[91] and to internalize junction proteins during movement of spermatocytes through basal TBC and the release of sperms.[92] F-actin at the TBC exists as three-dimensional highly branched networks.[79,93] Although the detailed mechanism mediating the actin network at TBC is still unknown, Arp2/3-based branching of actin filaments has been proposed to play a role in the formation of the actin network. Arp3 and WASP that are essential for actin branching have been shown to localize to the

apical TBC,[94] supporting the proposed mechanism of the actin network formation. Rac1 and Cdc42 that activate the Arp2/3 mechanism have been detected in the cytoplasm of Sertoli cells around the spermatid head[95]and inactivation of Rho GTPases by toxin A causes disaggregation of actin cytoskeleton in Sertoli cells.[96] In addition to the actin binding proteins mentioned above, cofilin,[97] and Eps8[84] have also been shown to localize to the TBC, suggesting that actin bundling and actin severing and/or depolymerization are required in the formation of actin network at the TBC.

Actin regulatory proteins in germ cells. During spermatogenesis, round germ cells undergo dramatic morphological changes and remodel into mature spermatozoa with head and tail. F-actin in spermatids is concentrated in the intercellular junctions, the subacrosomal space, the acroplaxome, and the manchette. Actin and its regulatory proteins therefore have important functions in regulating the development and morphogenesis of germ cells. The actin bundling protein testis fascin has been shown to express in the head of elongating spermatids. Another actin bundling protein Eps8 has also been detected in germ cells although its expression is less than that in Sertoli cells.[84] In addition to the actin bundling proteins, the actin polymerization and branching promoters mDia1/2[81] and WAVE1[98] have also been detected in spermatocytes or spermatids, suggesting actin bundling, polymerization, and branching may be involved in spermatid development.

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p70^{S6K} in Spermatogenesis

Several studies have suggested that p70^{S6K} may have a role in spermatogenesis by regulating the development of primary Sertoli cells under follicle stimulating hormone (FSH) and luteinizing hormone (LH) stimulation.[99,100] Although there are no studies demonstrating that p70^{S6K} may play a role in actin cytoskeleton reorganization in Sertoli cells, a report by Riera et al., which focused on the interleukin (IL)-1β-stimulated lactate production in Sertoli cells,[101] may provide hints on this role. The results showed that IL-1β increases phosphorylation of p70^{S6K}, but the activation is not related to lactate production. Recent studies have also shown that IL-1 can regulate the dynamics of actin cytoskeleton and cell junctions in addition to its well-known role in innate immunity[102] and tissue homeostasis,[103] suggesting that p70^{S6K} may be a regulator of IL-1 on actin cytoskeleton reorganization. Some other cytokines and growth factors which are potent activator of p70^{S6K} in other cell types, such as transforming growth factor (TGF)-β, and hepatocyte growth factor (HGF), also have important functions in testicular development and spermatogenesis (**Table 1**). Cytokines involved in spermatogenesis have been reviewed by Xia et al.[89] These cytokines mostly activate p70^{S6K} through PI3K/Akt and MAPK pathways, which have been shown to regulate AJ dynamics and spermatogenesis. The expression or activities of the key components in PI3K/Akt and MAPK pathways, including the 85α and p110α subunits of PI3K, Akt, and ERK1/2, have been shown to increase during the AJ assembly of Sertoli cells and germ cells (i.e., apical ES).[104] Both PI3K and active Akt are abundant at the site of apical ES from stages IV to VIII and are detected at the basal ES.[104] Inhibition of PI3K using inhibitors is able to disrupt the AJ.[105] All these suggest that the PI3K/Akt and MAPK pathways may be required for AJ assembly (for ERK pathway review, ref. 106). Siu et al. has also shown the expression of PAK2 during AJ assembly and suggested a role of PAK in AJ formation. Although the expression of PAK2 but not PAK1 increases during AJ assembly, the activity of PAK1 was not detected in the study.[104] Recently, Wong et al. has revealed that Cdc42 mediates TGF-β3-induced BTB disruption by enhancing endocytosis of integral membrane proteins at BTB.[107] This suggests a possible mechanism by which p70^{S6K} may regulate the BTB restructuring at stage VIII through mediating

Table 1. Potent activators of p70^{S6K} in testis

	Function of cytokines in testis	Activation of p70^{S6K} in testis	References
Hormones			
FSH	Regulate the development of Sertoli cells	Yes	100
Cytokines			
IL-1α	Regulate Sertoli-germ cell adhesion	n.d.	99,100
IL-1β	Regulate lactate production in Sertoli cells	Yes	101
BMP-4	Maintain spermatogenesis; promote differentiation of spermatogonia	n.d.	110–112
Growth factors			
EGF	Enhance spermatogonia proliferation and differentiation	n.d.	113,114
FGF2	Induce testosterone production in Leydig cells	Yes	115
HGF	Modulate Sertoli-Sertoli tight junction dynamics; increase steroidogenetic activity of Leydig cell	n.d.	116–118
PDGF	Regulate the development of the Leydig cell lineage and spermatogenesis	n.d.	110,111
SCF	Promote spermatogonia proliferation	Yes	109
TGF-β1	Inhibit steroidogenesis in Leydig cells	n.d.	119,120

Abbreviations: BMP-4, bone morphogenetic protein-4; EGF, epidermal growth factor; FGF2, fibroblast growth factor 2; FSH, follicle stimulating hormone; HGF, hepatocyte growth factor; IL-1β, interleukin-1β; n.d., not determined; PDGF, platelet-derived growth factor; SCF, stem cell factor; TGF β1, transforming growth factor-β1.

the activation of Cdc42. Thus, extracellular stimuli may activate p70^{S6K} via PI3K/Akt and MAPK pathways, which in turn may lead to actin cytoskeleton reorganization at AJs and BTB restructuring through Rac1/Cdc42 activation. A possible perspective of p70^{S6K} in regulating the actin cytoskeleton dynamics of spermatogenesis in Sertoli cells is shown in **Figure 3**.

In germ cells, the expression of p70^{S6K} is relatively constant during its maturation; however, the kinase activity of p70^{S6K} is increased. Immunohistochemistry detection of p70^{S6K} also showed that there is a nucleus-to-cytoplasm translocation of p70^{S6K} during spermatogenesis.[108] Moreover, p70^{S6K} has been shown to mediate cytokine-induced signaling to stimulate proliferation of type A spermatogonia, which may play a role in the biosynthesis and preparation of germ cells for fertilization.[109]

Concluding Remarks and Perspectives

The regulation of ES recently has received a great deal of attention because they may shed light on male contraceptive development. However, the signaling pathways that regulate actin polymerization and depolymerization at the ES have not been studied in detail. It is suspected that different cytokines and hormones may be involved in changes in the dynamics of actin filaments in the testis. Our new findings have linked p70^{S6K} to the actin cytoskeleton and have led to the suggestion that this widely studied kinase may play a key role in epithelial cell motility. Altered expression of p70^{S6K} and several actin bundling/cross-linking proteins are being reported in the testis. Rho GTPases activities are important for the maintenance and formation of the actin cytoskeleton in Sertoli cells. Further demonstration of this intriguing phenomenon of a new role for p70^{S6K} in regulating the actin dynamics in the testis would have interesting and important consequences. In addition, a better understanding of how the different networks of p70^{S6K} functional interactions are orchestrated in a stimulus or context-specific way, as well as the functional roles on actin reorganization in specific cellular and animal experimental models are essential. This knowledge likely will contribute to a new and important piece in the complex jigsaw of spermatogenesis and male infertility.

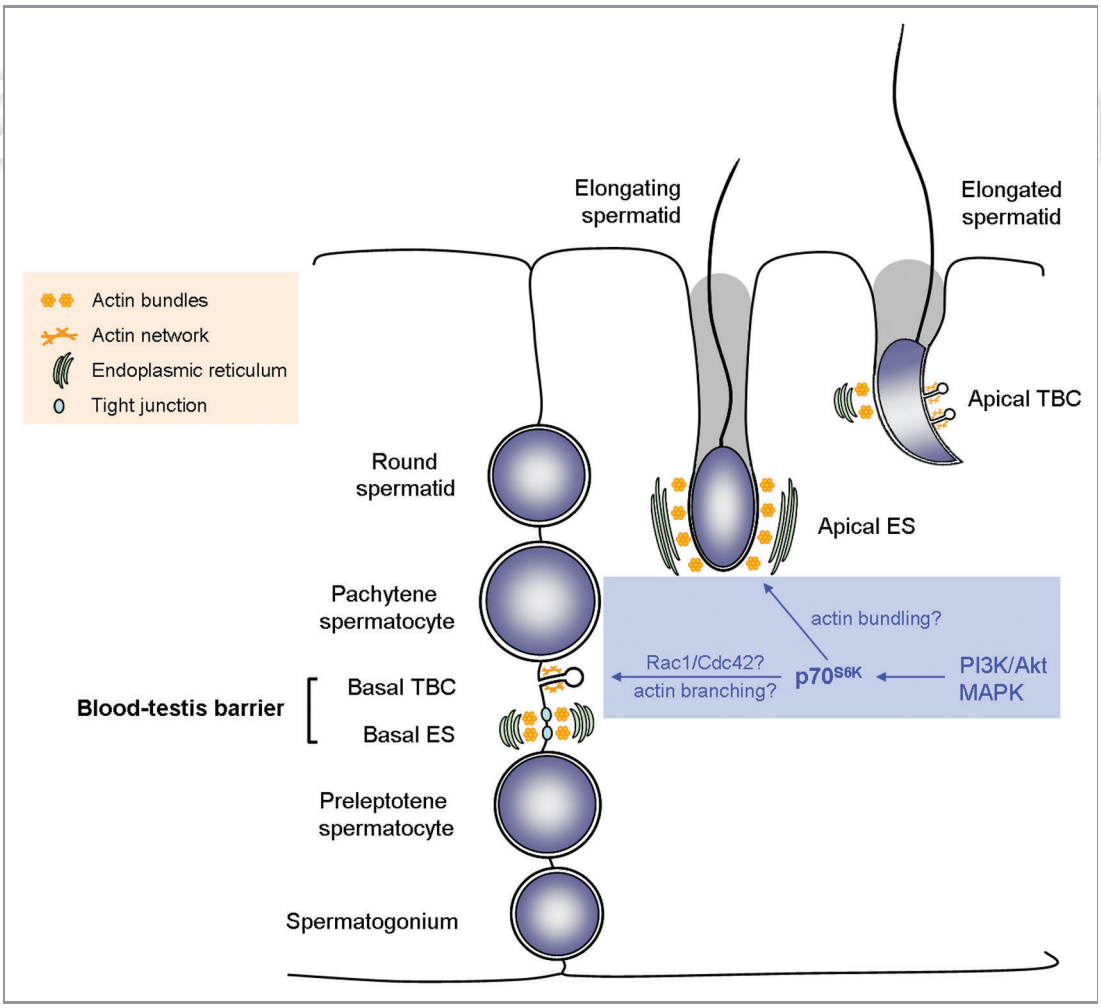

Figure 3. Schematic perspective of p70^{S6K} on spermatogenesis regulation in Sertoli cells. p70^{S6K} activation through the PI3K/Akt or MAPK pathways may have a possible role in regulating the actin cytoskeleton at AJ and BTB restructuring through Rac1/Cdc42-activating activities.

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