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14-3-3 and its binding partners are regulators of protein–protein interactions during spermatogenesis

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Abstract

During spermatogenesis, spermiation takes place at the adluminal edge of the seminiferous epithelium at stage VIII of the epithelial cycle during which fully developed spermatids (i.e. spermatozoa) detach from the epithelium in adult rat testes. This event coincides with the migration of preleptotene/leptotene spermatocytes across the blood–testis barrier from the basal to the apical (or adluminal) compartment. At stage XIV of the epithelial cycle, Pachytene spermatocytes (diploid, 2n) differentiate into diplotene spermatocytes (tetraploid, 4n) in the apical compartment of the epithelium, which begin meiosis I to be followed by meiosis II to form spermatids (haploid, 1n) at stage XIVof the epithelial cycle. These spermatids, in turn, undergo extensive morphological changes and traverse the seminiferous epithelium until they differentiate into elongated spermatids. Thus, there are extensive changes at the Sertoli–Sertoli and Sertoli–germ cell interface via protein 'coupling' and 'uncoupling' between cell adhesion protein complexes, as well as changes in interactions between integral membrane proteins and their peripheral adaptors, regulatory protein kinases and phosphatases, and the cytoskeletal proteins. These precisely coordinated protein–protein interactions affect cell adhesion and cell movement. In this review, we focus on the 14-3-3 protein family, whose members have different binding partners in the seminiferous epithelium. Recent studies have illustrated that 14-3-3 affects protein–protein interactions in the seminiferous epithelium, and regulates cell adhesion possibly via its effects on intracellular protein trafficking and cell-polarity proteins. This review provides a summary on the latest findings regarding the role of 14-3-3 family of proteins and their potential implications on spermatogenesis. We also highlight research areas that deserve attentions by investigators.

Introduction

During spermatogenesis, in addition to self-renewal of spermatogonia via i) mitosis, type B spermatogonia will differentiate into primary spermatocytes, which eventually enter ii) meiosis to give rise to haploid spermatids. Spermatids begin their maturation in a process known as iii) spermiogenesis behind the blood–testis barrier (BTB) that transforms round spermatids into elongated spermatids (from step 1 to step 19 spermatids in rats) with profound changes in the spermatid head (condensation of the chromatin materials and formation of the acrosome) and elongation of the tail until iv) spermiation. At spermiation, fully developed spermatids (i.e. spermatozoa) will leave the seminiferous epithelium, entering the tubule lumen to undergo

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Declaration of interest
The authors herein declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research/work reported.

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maturation in the epididymis (for a review, see Hess & Franca 2008). During these four distinctive phases, extensive restructuring occurs at the Sertoli–Sertoli and Sertoli–germ cell interface across the entire seminiferous epithelium in both the basal and apical compartments as well as in the BTB in adult mammalian testes (for reviews, see Cheng & Mruk 2002, Mruk & Cheng 2004). Recent studies have shown that efficient restructuring at the cell–cell interface facilitates both the migration of developing spermatids in the seminiferous epithelium during spermiogenesis and spermiation, and the transit of primary preleptotene spermatocytes across the BTB. Such dynamics are mediated, at least in part, via changes in the protein–protein interactions between integral membrane proteins and their adaptors (e.g. α-, β-, γ-catenins, and ZO-1 that tether integral membrane proteins, such as *N*-cadherin, occludin, JAMs, and nectins, to the cytoskeletal proteins; catenins and ZO-1 also recruit signaling molecules, such as protein kinases and/or phosphatases, to the same site; for reviews, see Lee & Cheng 2004, Mruk & Cheng 2004, Zhang *et al*. 2005). The net results of these interactions determine whether the integral membrane proteins are structurally linked to the cytoskeletal network, thus affecting the status of cell adhesion in the epithelium. Consequently, it is of interest to identify and investigate proteins that are crucial to protein–protein interactions at the cell–cell interface in the seminiferous epithelium. In this minireview, we focus on a protein family named 14-3-3, which has recently been shown to be a crucial regulator of protein–protein interactions in various epithelial and endothelial cells in mammals. While many of the studies were done in organs other than the testes, except for a few reports (Wine & Chapin 1999, Chapin *et al*. 2001, W P Wong & C Y Cheng, unpublished observations), we thought it pertinent to critically evaluate recent data in the field to provide a framework with which functional studies can be performed to tackle the role of 14-3-3 in spermatogenesis.

Background and physico-chemical properties of 14-3-3

14-3-3 proteins comprise a family of small acidic proteins (~30 kDa) found in virtually all tissues of both invertebrates and vertebrates. They were discovered in 1967 during a systemic classification of brain proteins, and named by their specific location following diethyl aminoethyl (DEAE)-cellulose chromatography and starch gel electrophoresis (Moore & Perez 1967). The 14-3-3 family members are highly conserved, with seven isoforms identified in mammals, at least 12 isoforms in plants, two isoforms in *Drosophila*, and two isoforms in yeast (Wang & Shakes 1996, Rosenquist *et al*. 2000, 2001). In mammals, despite their highest expression in the central nervous system, 14-3-3 protein family exists ubiquitously in almost all other tissues, especially in the intestines and testis (Boston *et al*. 1982). Different 14-3-3 isoforms display a certain degree of tissue specificity, and they are present at different concentrations in tissues (Perego & Berruti 1997).

The seven isoforms identified in mammals (β-beta, γ-gamma, ε-epsilon, ζ-zeta, η-eta, θ-theta, and σ-sigma; Table 1) share about 50% amino acid identity and, consequently, highly similar protein conformations to form either homodimers or heterodimers that serve as the functional protein units (Jones *et al*. 1995,Chaudhri *et al*. 2003). Each 14-3-3 monomer is composed of nine α-helixes and relatively unconserved carboxyl-terminal and amino-terminal regions (Aitken 2006). Theoretically, the rigid helical structure may provide the functional basis for target binding, whereas the flexible carboxyl terminal allows for alternative binding activities (Bridges & Moorhead 2004). The amino-terminal region forms the interface for dimerization, thus determining the specific combinations of isoforms (Bridges & Moorhead 2004,Aitken 2006).

Dimeric 14-3-3 proteins have a cup-shaped conformation, with two highly conserved amphipathic grooves as target-binding pockets (Liu *et al*. 1995, Xiao *et al*. 1995, Rittinger *et al*. 1999). From the peptide library, two high-affinity phosphorylation-dependent motifs were identified among a variety of 14-3-3 binding partners, which are RSXpSXP and RXXXpSXP

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(X, any amino acid; pS, phosphoserine, which can be substituted by phosphothreo-nine, pT; R, Arg, S, Ser and P, Pro; Yaffe *et al*. 1997, Rittinger *et al*. 1999, Bridges & Moorhead 2004). Moreover, although constituting only a small proportion, a third phosphorylation-independent binding motif, which still binds to the same binding pocket on 14-3-3, has been reported (Petosa *et al*. 1998, Masters *et al*. 1999).

To date, more than 200 binding partners of 14-3-3 have been reported, most of which were identified based on proteomic studies. These 14-3-3 binding proteins are involved in a wide range of cellular activities, such as transcription, protein synthesis, metabolic pathways, cell cycle, cell signaling, cytoskeletal organization, and cellular trafficking (Dougherty & Morrison 2004, Jin *et al*. 2004, Kjarland *et al*. 2006, Shikano *et al*. 2006). Notably, such 14-3-3 interactome studies were performed in different cell types, with different methodologies (such as co-immuno-precipitation or affinity chromatography), and with the interacting proteins targeting different 14-3-3 isoforms (Kjarland *et al*. 2006). Thus, upon comparison, only a small proportion of these parameters (e.g. binding partners of 14-3-3) overlap between different 14-3-3 isoforms (Bridges & Moorhead 2005). However, in a study utilizing the same method but targeting different isoforms, a small degree of overlapping was still reported, which seems to suggest isoform specificity even though there is functional redundancy between different 14-3-3 isoforms (Benzinger *et al*. 2005, Bridges & Moorhead 2005, Kjarland *et al*. 2006). This isoform specificity has also been substantiated in some functional studies, which are summarized in Table 1. These observations thus illustrate that 14-3-3 is a crucial molecule in conferring protein–protein interactions in different cells. Since it is found abundantly in the testes (Table 1), it is not surprising that this protein may be crucial to Sertoli–Sertoli and/or Sertoli–germ cell interactions during spermatogenesis. In fact, recent studies have demonstrated the presence of several 14-3-3 members in the testis, such as 14-3-3θ, 14-3-3β, and 14-3-3ζ; and 14-3-3θ was found in both Sertoli and germ cells (W P Wong & C Y Cheng, unpublished observations). In a study using immunohistochemistry and fluorescent microscopy (Fig. 1), it was shown that 14-3-3θ was localized prominently at the elongating spermatid–Sertoli cell interface, which is consistent with its localization at the apical ectoplasmic specialization (apical ES, a testis-specific atypical adherens junction, AJ, type (for review, see Wong *et al*. 2008*b*)). Meanwhile, some 14-3-3θ staining was detected in the basal compartment, consistent with its presence at the BTB (Fig. 1; W P Wong & C Y Cheng, unpublished observations).

Unique features of 14-3-3

The molecular mechanisms utilized by 14-3-3 proteins to regulate cellular functions can be classified as clamping, masking, or scaffolding, all of which are mediated via protein–protein interactions between 14-3-3 and its target proteins. No clear evidence exists for any enzymatic activities of 14-3-3 (Fu *et al*. 2000, Bridges & Moorhead 2004, Mrowiec & Schwappach 2006, Shikano *et al*. 2006).

Clamping describes the process through which the conformation of the target protein is modified by the binding of 14-3-3. This action is facilitated by the rigid helical structure of 14-3-3, which shows little change after being bound with a ligand (Liu *et al*. 1995, Yaffe 2002). Thus, 14-3-3 binding helps to reshape a target protein and, consequently, alters its properties. An example of such a clamping target is serotonin-*N*-acetyltransferase, which is stabilized and kept in an active conformation by 14-3-3 binding (Obsil *et al*. 2001). Notably, the binding between serotonin-*N*-acetyltransferase and 14-3-3 is mediated by two phosphorylation sites, which bind to the two separate subunits of a 14-3-3 dimer (Ganguly *et al*. 2005). Along with the activation of enzymes, 14-3-3 clamping may also inactivate catalytic activities or alter the distal conformation of the target proteins (Moorhead *et al*. 1996, Athwal *et al*. 1998, McKinsey *et al*. 2001, Bridges & Moorhead 2004).

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The masking of a specific region in a target protein by 14-3-3 may alter a sequence-specific property of the target protein, such that access by other possible interacting proteins is blocked. This process can be viewed as a competition between 14-3-3 and any other interacting proteins for the target protein (Mackintosh 2004). For instance, 14-3-3 binding masks the nuclear localization signal of Cdc25C protein (a cell-cycle regulator) and sequesters the protein in the cytoplasm (Peng *et al*. 1997, Kumagai & Dunphy 1999).

14-3-3 also employs a scaffolding mechanism by bringing two target proteins into proximity and co-localizing them at the same cellular site to carry out their cellular effects. This mechanism is plausibly based on the dimeric structure of 14-3-3, which has two independent binding pockets for target proteins. For instance, if a protein kinase and its substrate are bound in the two binding pockets on 14-3-3 simultaneously, the catalytic activity of the kinase is promoted, given the proximity of the enzyme and its substrate. Moreover, the catalytic activity may be tightly regulated owing to the restricted spatial orientation of the two 14-3-3 binding proteins (Bridges & Moorhead 2004). On the other hand, regulation may be carried out to allow for scaffolding, because both the enzyme and the substrate need to be partially phosphorylated before they bind to 14-3-3 (Bridges & Moorhead 2004). Scaffolding is possibly an important function for cellular signaling processes, which involve various regulated kinase activities.

Functions of 14-3-3

14-3-3 appears to be a crucial cellular regulator via its widespread interactions with hundreds of proteins that are known to modulate an array of cellular events in multiple epithelia/endothelia (Table 2). The numerous binding partners of 14-3-3 can be categorized into proteins related to i) intracellular trafficking, ii) cell-junction dynamics, and iii) cell polarity, all of them suggesting the involvement of 14-3-3 in corresponding physiological events (Fig. 2). Such involvement is likely important for spermatogenesis because some of these 14-3-3 binding partners are implicated in germ cell movement and cell polarity during spermiogenesis and spermiation (Wong *et al*. 2008*a*).

Intracellular trafficking

As discussed above, Cdc25C protein can be docked in the cytoplasm via its binding to 14-3-3. This is one method by which 14-3-3 regulates protein localization. 14-3-3 also regulates cellular protein localization by modulating endoplasmic reticulum (ER) transportation and, thereby, the subsequent cell-surface expression of its binding partners. The membrane-trafficking mechanism involves retrograde transport mediated by coat protein complex I (COPI) and anterograde transport mediated by coat protein complex II (COPII). If a cargo protein has a significant forward trafficking signal, it will interact with COPII and proceed to the Golgi bodies and other intracellular destinations (Barlowe 2003, Bonifacino & Glick 2004). However, if this cargo protein expresses an ER localization signal, it will be recognized by COPI and transported back to the ER (Ma *et al*. 2001).

14-3-3 has also been found to bind to a number of membrane proteins and modify their cell-surface expression levels (Mrowiec & Schwappach 2006). The first evidence of this important function came from studies of the potassium channel protein KCNK3 (TASK-1). KCNK3 is a multimeric protein with an N-terminal ER localization signal (i.e. COPI interacting motif) and a C-terminal 14-3-3 binding site (O'Kelly *et al*. 2002, Rajan *et al*. 2002). Truncation of the C-terminal 14-3-3 binding site of KCNK3 was shown to alter its cell-surface expression (Rajan *et al*. 2002), thereby modifying the K^+-channel function. Moreover, cell-surface expression of TASK-1 was shown to be abolished with the mutation of serine at the -1 site at the N-terminus (Rajan *et al*. 2002). In another report, the interaction between KCNK3 and 14-3-3 or COPI was found to be mutually exclusive (O'Kelly *et al*. 2002). All this evidence suggests that unphosphorylated KCNK3 is recognized by COPI and retained on the ER,

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whereas 14-3-3 binding with phosphorylated KCNK3 can prevent COPI recognition and allow its cell-surface expression (Mrowiec & Schwappach 2006, Shikano *et al*. 2006). This thus provides a unique mechanism to regulate the K^+-channel function by regulating the steady-state level of KCNK3 protein. Several other membrane proteins, such as the MHC II, K_{ATP}-channel subunit, and kainate receptor subunit, have been reported to be subjected to similar regulation in their cell-surface expression (Anderson *et al*. 1999, Yuan *et al*. 2003, Vivithanaporn *et al*. 2006). Furthermore, some evidence suggests that 14-3-3 may also regulate the interaction between COPII and its cargo (Shikano *et al*. 2006; Fig. 2A). Considering the numerous binding partners of 14-3-3, we suggest that 14-3-3 may function in the seminiferous epithelium during spermatogenesis by regulating protein localization.

Cell-junction dynamics

14-3-3 has been reported to be involved in regulating cell-junction dynamics. One example of such regulation is its involvement in integrin-mediated cell adhesion (Fig. 2B). $p130^{Cas}$ is a docking protein that can form intracellular signaling complexes through integrin-mediated cell adhesion. In both yeast and mammalian cells, 14-3-3 has been found to be associated with $p130^{Cas}$ in a phosphoserine-dependent manner (Garcia-Guzman *et al*. 1999). Induced by the attachment of cells to the extracellular matrix, $p130^{Cas}$ and 14-3-3 were found to be significantly co-localized upon integrin-ligand binding (Garcia-Guzman *et al*. 1999). Thus, 14-3-3 seems to participate in the integrin-activated signaling pathways via $p130^{Cas}$ and to further regulate subsequent cell adhesion responses (Fig. 2B). In the testis, the α6β1-integrin/laminin-333 adhesion complex, wherein α6β1-integrin residing in Sertoli cells and laminin-333 (laminin α3/β3/γ3) restricted to elongating spermatids (Yan & Cheng 2006), is one of the best studied adhesion complexes at the apical ES (Cheng & Mruk 2002,Mruk *et al*. 2008,Vogl *et al*. 2008). Recent studies have shown that the anchoring junction restructuring in the seminiferous epithelium mediated by the integrin/laminin protein complex involves the activation of the integrin downstream adaptors, such as $p130^{Cas}$, which recruit ERK to the apical ES to elicit junction restructuring (Siu *et al*. 2003,2005). It was not known at the time how $p130^{Cas}$ can be docked at the apical ES to carry out its function. Since 14-3-3 has been shown to be abundant at the apical ES (Fig. 1), it is likely that 14-3-3 plays a major role in recruiting $p130^{Cas}$ and other regulatory proteins (e.g. ERK MAP kinase) to the apical ES to facilitate its restructuring during spermatid movement in spermiogenesis and spermiation. This possibility should be carefully evaluated in future studies.

Cell polarity

Cell-polarity proteins, such as Par1, Par3, Par6, and Par5 (i.e. members of 14-3-3 in *Caenorhabditis elegans*), play critical roles in cell polarization. Notably, the Par3/Par6/aPKC complex is a known binding partner of 14-3-3. During cell–cell contact assembly, components of the Par3/Par6/aPKC complex are brought sequentially into contact with the adhesion junction-associated molecules (Assemat *et al*. 2007, Ebnet *et al*. 2008; Fig. 2C). In the testis, Par3, Par6, and aPKC have been identified by corresponding specific antibodies in Sertoli and germ cells in the seminiferous epithelium of adult rats (Wong *et al*. 2008*a*). The consequent Par3/Par6/aPKC complex has been shown to serve as a crucial polarity complex at the apical ES to confer spermatid orientation, so that the heads of the developing spermatids in the seminiferous epithelium are properly oriented towards the basement membrane (Wong *et al*. 2008*a*). More important, it was shown that this Par3/Par6-based polarity complex is also working in concert with proteins at the apical ES (e.g. JAM-C) and proteins at the BTB (e.g. JAM-A and *N*-cadherin); as a result, the cellular events of spermiation and BTB restructuring that occur simultaneously at the opposite ends of the Sertoli cell epithelium at stage VIII of the seminiferous epithelial cycle can be precisely coordinated (Wong *et al*. 2008*a*). While the precise downstream mechanism(s) that coordinates these two concurrent cellular events involving the Par-based polarity complex remains to be elucidated, it is likely that 14-3-3 and

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two non-receptor protein tyrosine kinases – c-Src and FAK and their corresponding activated forms p-cSrc and p-FAK –are involved. For instance, it is known that c-Src and FAK are components of the BTB (Siu *et al*. 2003, Lee & Cheng 2005), whereas their activated p-cSrc and p-FAK forms are found mostly at the apical ES (Siu *et al*. 2003, Wong *et al*. 2005, Beardsley *et al*. 2006). It is possible that 14-3-3 serves as the docking platform for protein kinases (e.g. c-Src and FAK) and their activated forms to mediate the junction restructuring events that facilitate spermiation at the apical ES and the transit of preleptotene spermatocytes at the BTB at stage VIII of the cycle. This concept should be vigorously tested in future experiments.

Par3 can be phosphorylated by Par1 on the Ser-151 and Ser-1085 residues. These two phosphorylated Ser residues are the putative binding sites for Par5/14-3-3 (Benton & Johnston 2003, Hurd *et al*. 2003), allowing these proteins to form a protein complex (Fig. 2C). The binding of 14-3-3 with the Ser-151 residue blocks the oligomerization and functioning of Par3, and the masking of the Ser-1085 residue inhibits the binding of aPKC (Nagai-Tamai *et al*. 2002, Benton & Johnston 2003, Brajenovic *et al*. 2004; Fig. 2C). Consequently, if the cell membrane domain has active Par1 proteins associated, the phosphorylated Par3 will be incapable of forming a functional Par3/Par6/aPKC complex at the cell membrane (Ebnet *et al*. 2008; Fig. 2C), leading to changes in polarity.

Interestingly, Par1 itself can also be phosphorylated by aPKC at Thr-595 and bind to 14-3-3. The binding inhibits the kinase activity of Par1 and releases it from cell membrane (Hurov *et al*. 2004, Suzuki *et al*. 2004). Using this mechanism, the functional Par3/Par6/aPKC complex can be localized at the desired cellular site (Fig. 2C). Another study has shown several other phosphorylation sites on Par1 to be important for 14-3-3 binding, and the simultaneous mutation of those phosphorylation sites restricted Par1 at the plasma membrane (Goransson *et al*. 2006). These results suggest that complex interactions exist among polarity proteins, facilitating the formation and/or dissolution of the polarity protein complex during the epithelial cycle of spermatogenesis, and that 14-3-3 is a crucial regulator mediating protein–protein interactions. For instance, a study has reported that Par6, Pals1, and JAM-C interact at the apical ES in normal rat testes (Wong *et al*. 2008*a*). However, at the time of germ cell depletion from the epithelium induced by adjudin, Par6, Src, and Pals1 formed a tighter protein complex, which, in turn, weakened the association of Pals1 and Par6 with JAM-C, destabilizing the JAM-C-based adhesion, and leading to germ cell loss from the epithelium (Wong *et al*. 2008*a*). It is plausible that 14-3-3 plays a crucial role in the mediation of protein–protein interactions at the apical ES involving Pals1, Par6, Src, and JAM-C.

In addition to its likely involvement in the apical ES restructuring, 14-3-3 was also implicated in the regulation of BTB restructuring during spermatogenesis (Wong *et al*. 2008*a*; Fig. 3). For instance, it has been shown that Par3, Par6, aPKC and JAM-A interact at the BTB to maintain cell adhesion at the site (Wong *et al*. 2008*a*). At stage VIII of the epithelial cycle, when primary preleptotene spermatocytes are in transit at the BTB, Par3/Par6/aPKC complexes disassemble, which thus destabilizes JAM-A-based cell adhesion, facilitating the transit of primary spermatocytes at the BTB (Fig. 3). It is likely that 14-3-3 plays a role at the BTB in mediating protein–protein interactions involving Par3, Par6, aPKC, and/or JAM-A (Fig. 3).

Regulation

The regulation of 14-3-3 activity can occur either on the binding partners of 14-3-3 or on the 14-3-3 protein *per se*, both forms of which are carried out through changes in the phosphorylation status of specific amino acid residues.

Two important regulators involved in 14-3-3 binding are the protein phosphatases PP1 and PP2, which dephosphorylate the desired Ser or Tyr residue(s) at the 14-3-3 binding sites. In

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the binding between phosphorylated Par3 and 14-3-3 discussed above, PP1 plays an important regulatory role by dephosphorylating multiple sites on Par3, thus controls the binding of 14-3-3 and aPKC to Par3 (Traweger *et al*. 2008). A similar regulatory pathway exists for Cdc25C, which can be released from 14-3-3 by PP1 and subsequently translocated to the nucleus (Margolis *et al*. 2003).

The activity of 14-3-3 proteins can also be regulated by phosphorylation. Several protein kinases, such as protein kinase B, protein kinase C, and casein kinase 1, have been shown to be capable of phosphorylating 14-3-3. Moreover, these proteins have been positively identified in the testis and are known to be expressed by Sertoli and/or germ cells (Lee & Cheng 2005, Siu *et al*. 2005). For instance, since p-PKB–Thr^{308} and p-PKB–Ser^{473} were both shown to be induced during anchoring junction disruption at the Sertoli cell–germ cell interface, the phosphorylation of 14-3-3 is likely to occur at the apical ES. It has been reported that 14-3-3 phosphorylation always results in decreased target binding (van Heusden 2005). For 14-3-3ζ, its dimerization and consequent activity can also be regulated by phosphorylation, in which the phosphorylated Ser58 disrupts the dimeric conformation (Woodcock *et al*. 2003). In short, these findings have demonstrated that the effects of 14-3-3 on protein–protein interactions at the apical ES and BTB in the seminiferous epithelium are regulated by its interactions with many of the recently reported adaptors (e.g. p130Cas), protein kinases (e.g. PKB and Src), polarity proteins (e.g. Par6 and Pals1), and integral membrane proteins (e.g. JAM-A, JAM-C, and integrins).

Concluding remarks and future perspectives

Herein, we summarize and discuss some of the latest findings regarding 14-3-3 (also known as Par5) in the epithelium including the seminiferous epithelium. Earlier studies have shown that it is a component of the polarity protein complex. However, in light of the great number of protein–protein interactions that it is involved in, 14-3-3, along with components of the polarity protein complex, which clearly have physiological functions other than conferring cell polarity. For instance, recent findings have illustrated that Par6 and Par3, the close ‘cousins’ of 14-3-3, are involved in spermatid release/adhesion as well as BTB dynamics, possibly coordinating the cellular events of spermiation and BTB restructuring, which occur at the opposite ends of the Sertoli cell epithelium in the testis at stage VIII of the epithelial cycle (Wong *et al*. 2008*a*). Since 14-3-3 interacts with Par3 and Par6 in the testis. This 14-3-3-based polarity complex is likely to serve as a crucial regulatory platform in the seminiferous epithelium to affect and/or coordinate many cellular events during spermatogenesis. We suggest that future studies should be directed to delineate the role of 14-3-3 in post-meiotic germ cell development, in particular spermiogenesis, since there is considerable turnover of proteins in the developing spermatids as well as at the Sertoli cell–spermatid interface.

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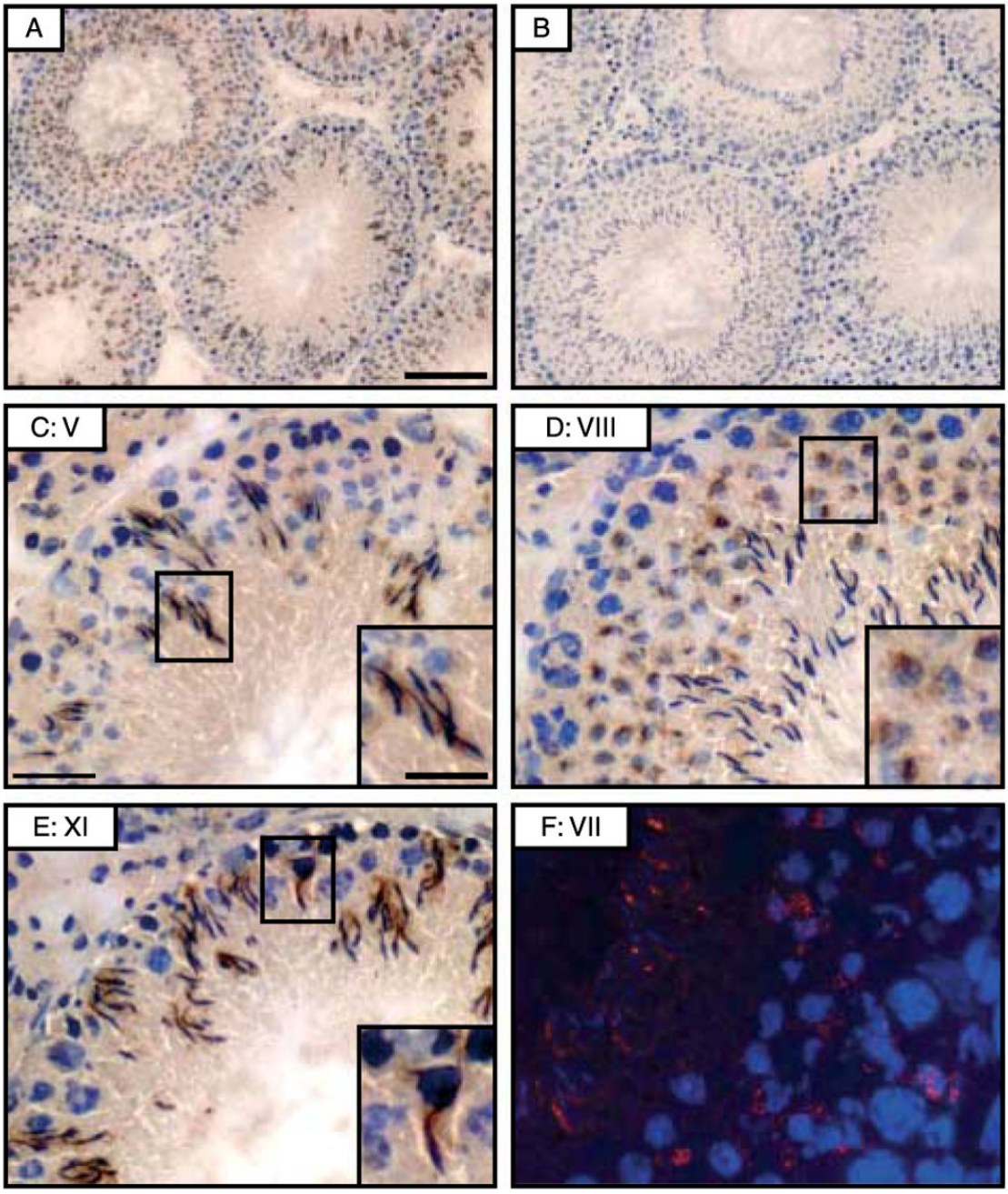

Figure 1.

A study to assess the cellular localization of 14-3-3 in the seminiferous epithelium of adult rat testes. (A–E) Immunohisto-chemical localization of 14-3-3θ in normal adult rat testes, in which frozen cross sections (~7 μm thick) were immunostained using an anti-14-3-3θ IgG (Santa Cruz Biotechnology, Cat. Sc-732; Lot. A2908) (A and C–E) or normal rabbit IgG (B). Low magnification of testes stained with anti-14-3-3θ polyclonal antibody (A) or normal rabbit IgG (B) illustrating the presence of 14-3-3θ in the seminiferous epithelium. In higher magnification, intense staining is obvious surrounding the heads of elongating spermatids at the apical ES site (C and E) as well as round spermatids (D) and at the basal compartment consistent with its presence at the BTB. Bar in (A)=100 μm, which applies to (B), bar in (C)=30 μm, which applies

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to (D and E), bar in inset in (C)=15 μm, which applies to insets in (D and E) and (F). A study by immunofluorescence microscopy further illustrates the localization of 14-3-3θ in seminiferous epithelium of normal adult rat testes, by using frozen testis cross sections (~7 μm thick) stained with anti-14-3-3θ IgG. CY3-conjugated donkey anti-rabbit secondary antibody (red fluorescence) was used for visualizing 14-3-3θ, confirming the existence of 14-3-3θ at the apical ES in the seminiferous epithelium in a stage VII tubule. Cell nuclei were stained by DAPI. Bar in (F)=12 μm. The Roman numeral after C, D, E and F represents the seminiferous epithelial stage of the tubule.

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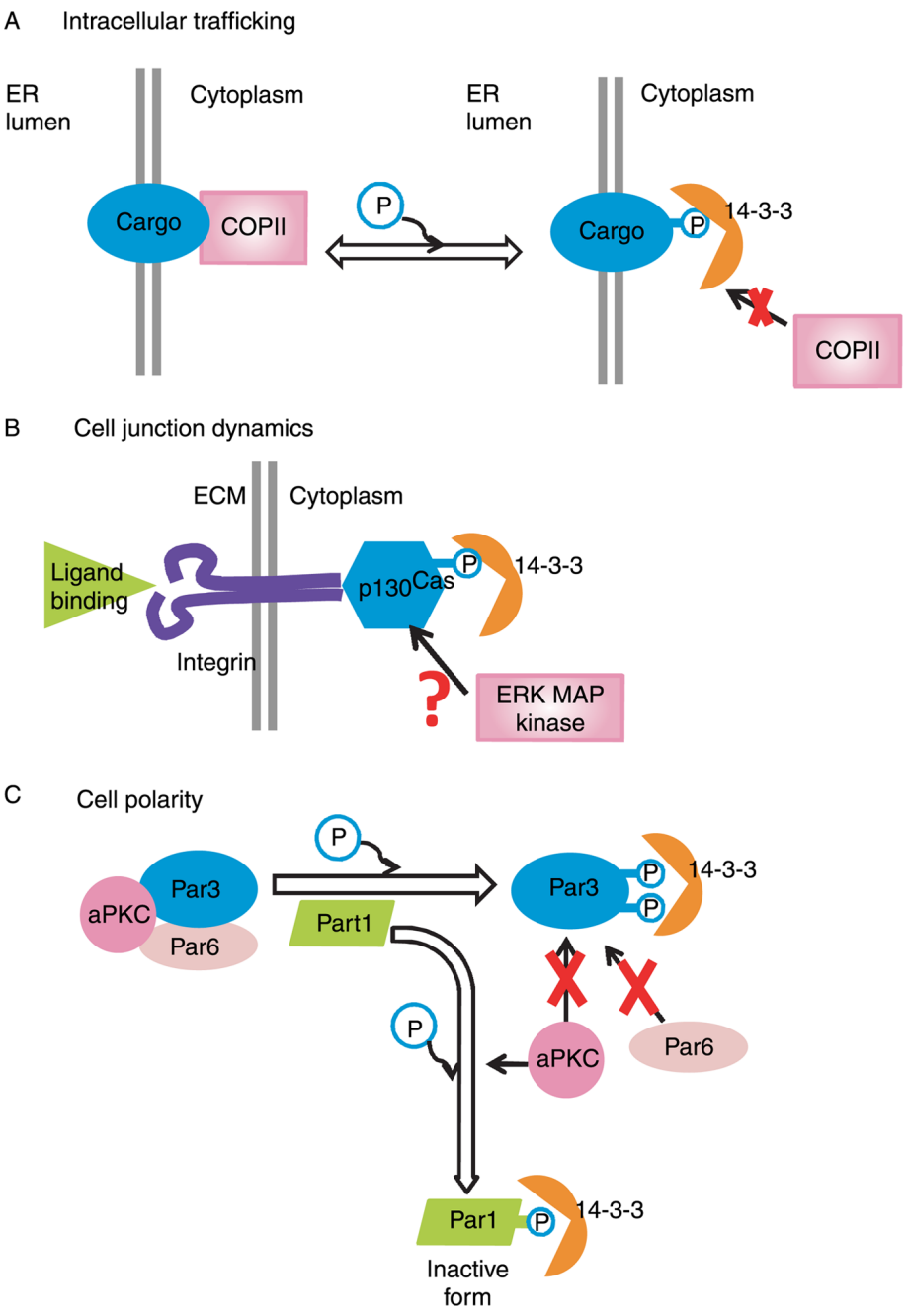

Figure 2.

The three possible regulatory functions of 14-3-3 in the testis. (A) Effects on intracellular trafficking. 14-3-3 binding modulates endoplasmic reticulum (ER) transportation by masking the COPII (or COPI) binding sites on cargo proteins. (B) Effects on cell-junction dynamics. 14-3-3 participates in the integrin-activated signaling pathways via $p130^{Cas}$. (C) Effects on cell polarity. Par1 catalyzes the phosphorylation of Par3, leading to 14-3-3 binding that inhibits the formation of Par3/Par6/aPKC complex. In turn, Par1 can be phosphorylated by aPKC (atypical protein kinase C) and bound with 14-3-3 as inactive form.

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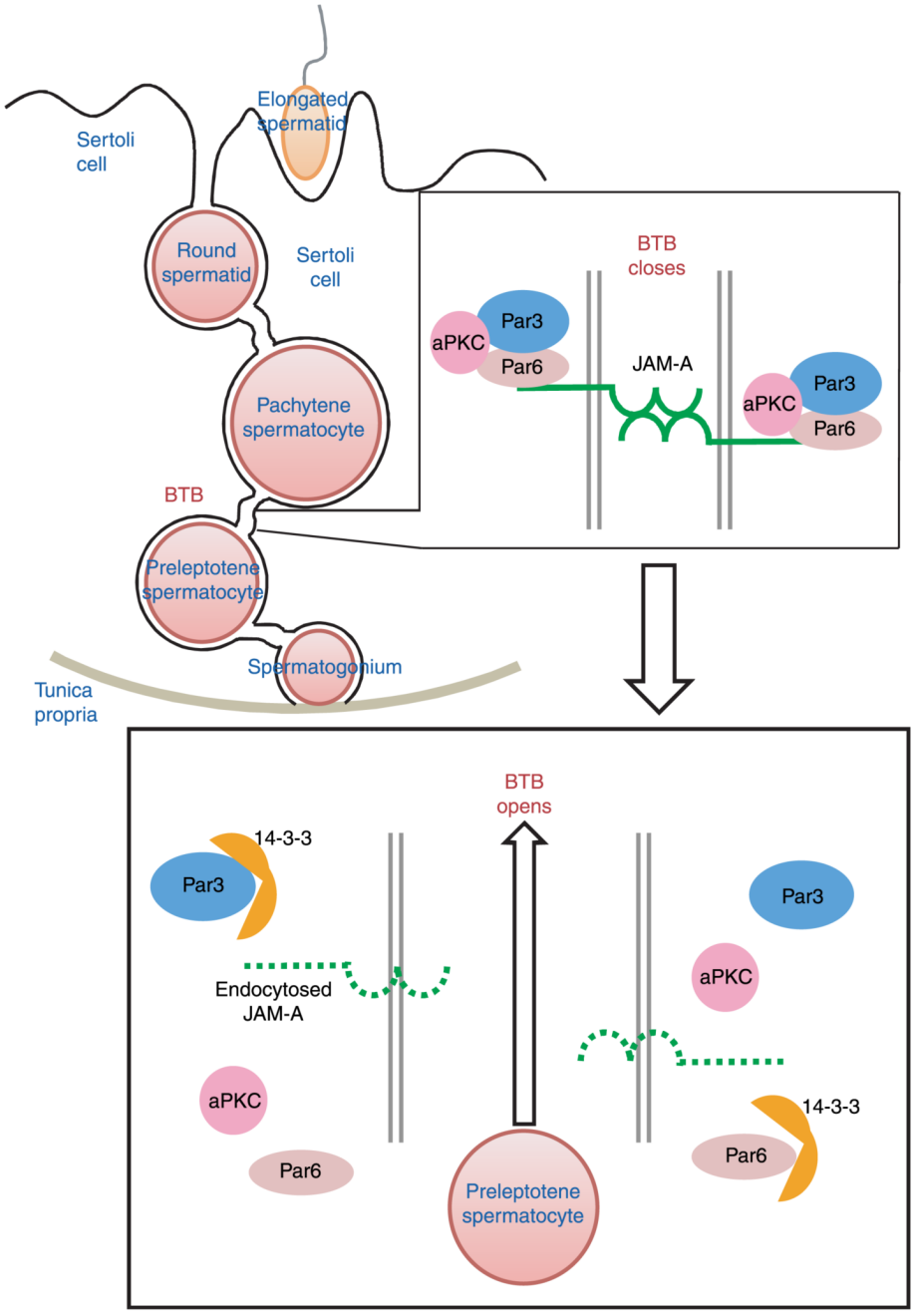

Figure 3.

A hypothetical model illustrating the possible regulatory role of 14-3-3 during BTB restructuring at stage VIII of the seminiferous epithelial cycle of spermatogenesis. The direct contact between the Par3/Par6/aPKC complex and JAM-A stabilizes the BTB, conferring an intact barrier. At stage VIII of the epithelial cycle, the degradation or endocytosis of JAM-A (dashed line) induces the opening of BTB. This diminishing level of JAM-A at the BTB may be contributed by the disassembly of the Par3/Par6/aPKC protein complex, because of the masking of Par3 and/or Par6 by 14-3-3 protein.

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Table 1

Different isoforms of 14-3-3 and their physiological function

		Physico-chemical characteristics and functions		
	Alternative name	**Physico-chemical features**	**Functional characteristics**	**Changes in phenotype after knockdown**
Isoforms[a]				
β	α (Kjarland et al. 2006)	–	Phosphorylated by protein kinase C (van Heusden 2005) May have oncogenic properties (Niemantsverdriet et al. 2008)	–
γ	–	–	An upstream regulator of p53, may serve as a tumor suppressor (Niemantsverdriet et al. 2008)	14-3-3γ knock-out mice: survival not affected, although some protein level changed (van Heusden 2005)
ε	–	Only form heterodimer (Kjarland et al. 2006)	Related to neuronal migration (Kjarland et al. 2006)	–
ζ	δ (Kjarland et al. 2006)	After being phosphorylated on Ser58, dimer disrupted (Woodcock et al. 2003)	Related to cellular metabolism (Kjarland et al. 2006) Can bind phosphorylated PAR3 (Kjarland et al. 2006) Can be phosphorylated by Bcr, leading to change in activity (Aitken 2006) Cannot be phosphorylated by protein kinase C (van Heusden 2005) Likely to behave as an oncogene (Niemantsverdriet et al. 2008)	14-3-3ζ knockdown in human epithelial cell line: sensitized stress-induced apoptosis and induced expression of adhesion proteins (Niemantsverdriet et al. 2008) Myc–14-3-3ζ overexpression disrupts polarity in MDCK cells, while it is associated with Par3 and aPKC (Hurd et al. 2003)
η	–	–	May serve as a tumor repressor (Niemantsverdriet et al. 2008)	–
θ	τ (Aitken 2006)		Present in male germ cells (Berruti 2000) Can be phosphorylated by Bcr (van Heusden 2005) and protein kinase C (Aitken 2006) Stimulates apoptosis (Niemantsverdriet et al. 2008)	–
σ	–	Only form homodimers (Bridges & Moorhead 2005, Kjarland et al. 2006); least conserved, derived from a retrotransposon event (Bridges & Moorhead 2005)	Related to cell-cycle regulation (Bridges & Moorhead 2005, Kjarland et al. 2006) Expression induced after DNA damage (van Heusden 2005) Highly expressed in epithelial cells (Aitken 2006) May serve as a tumor repressor (van Heusden 2005)	–

[a] All members of the 14-3-3 family share similar Mr as of 30 kDa.

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Table 2

Different classes of binding partners of 14-3-3 illustrating its potential role in regulating various cellular functions[a]

Binding partner categories	Reported binding partners
Polarity proteins	Par1/MARK kinase protein families (Brajenovic et al. 2004, Jin et al. 2004, Goransson et al. 2006) Par3/Par6/aPKC complex (Brajenovic et al. 2004, Jin et al. 2004)
Cell-junction proteins	p120 catenin (Jin et al. 2004) Plakophilin (Jin et al. 2004) AF6/afadin (Jin et al. 2004)
Cytoskeleton	Actin and tubulin (Jin et al. 2004, Meek et al. 2004)
Cytoskeleton-regulating proteins	Kinesin and profilin (Jin et al. 2004, Meek et al. 2004)
Signaling proteins	PI-3 kinase (Meek et al. 2004) $p130^{Cas}$ (Garcia-Guzman et al. 1999) Raf kinase (Berruti 2000)
ER localization	COP1 and COP2 (Shikano et al. 2006)

[a]This table is not intended to be exhaustive since more than a hundred binding partners for 14-3-3 have been identified in different epithelia (Jin *et al.* 2004, Meek *et al*. 2004) based on studies using gene-profiling techniques, among others. Only selected binding partners are listed here which are known to regulate spermatogenesis based on recent studies in the field.