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# Adherens Junction Dynamics in the Testis and Spermatogenesis

## Review

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Anchoring or adhering junctions are points at which cells attach to neighboring cells. They play an important role in determining and maintaining tissue organization (for reviews, see Yap et al. 1997; Tsukita et al. 2001; Cheng and Mruk, 2002). In the testis, unique cell-cell actin-based adherens junctions (AJs) between Sertoli cells, as well as between Sertoli and germ cells, and cell-cell intermediate filament-based desmosome-like junctions between Sertoli and germ cells (for reviews, see Russell and Peterson, 1985; Russell et al. 1990; Byers et al. 1993; Russell, 1993) not only provide mechanical adhesion of germ cells onto Sertoli cells, they also play a crucial role in germ cell morphogenesis and differentiation (Ozaki-Kuroda et al. 2002). In addition, the turnover of these junctions in the testis is important for permitting germ cell translocation from the basal compartment to the adluminal compartment of the seminiferous epithelium to complete spermatogenesis. In this review we limit our discussion to the recent and crucial development of the study of AJs instead of desmosome-like anchoring junctions because the latter type has been reviewed elsewhere (Russell and Peterson, 1985; Russell et al. 1990; Byers et al. 1993; Russell, 1993).

### Structure and Molecular Composition

Four structurally and functionally different forms of anchoring junctions exist: 1) AJs between cells, 2) focal contacts between cells and the extracellular matrix (ECM), 3) desmosomes between cells, and 4) hemides-

mosomes between cells and the ECM (for a review, see Alberts et al. 2002). Anchoring junctions are subdivided into two categories based on their connection sites. Both cell-cell AJs and cell-matrix focal contacts are connected to actin filaments, whereas desmosomes and hemidesmosomes are connected to intermediate filaments (for a review, see Alberts et al. 2002). The cell-cell, actin-based AJ is by far the best studied adhering junction type in the testis, and includes ectoplasmic specialization (ES), a testis-specific AJ between Sertoli and germ cells. In the testis, AJs confer adhesion between cells, which is known to be mediated by three AJ-integral membrane protein complexes: namely, cadherin/catenin, nectin/afadin, and integrin/laminin (for reviews, see Taga and Suganami, 1998; Rowlands et al. 2000; Vogl et al. 2000; Cheng and Mruk, 2002) (Figure 1 and Table 1). These three complexes in turn are connected to the actin cytoskeleton (for reviews, see Kemler, 1993; Gumbiner, 1996; Yap et al. 1997; Miyahara et al. 2000; Tachibana et al. 2000) (Figure 1; Table 2). For instance, E- or N-cadherin- $\beta$ -catenin complex interacts with the actin network via  $\alpha$ -catenin, whereas afadin is a putative F-actin-binding protein that also links the nectin/afadin complex to the actin cytoskeleton network (Figure 1). However, recent studies have shown that afadin can also interact with ZO-1, ponsin (an afadin- and vinculin-binding protein), and  $\alpha$ -catenin in the cytoplasm at the site of AJs (Yokoyama et al. 2001), suggesting that the nectin/afadin complex can also link to the cytoskeleton network via  $\alpha$ -catenin, a putative actin-binding protein, in addition to its interaction with actin via afadin. Furthermore,  $\alpha$ -catenin also provides a structural bond that links together the cadherin/catenin and nectin/afadin complexes (Pokutta et al. 2002) indicating that cross-talk exists between the two complexes. In the testis, another AJ complex based on  $\alpha 6 \beta 1$  integrin has been shown to be the major cell adhesion constituent protein complex of the ES (Vogl et al. 2000). Because epithelial cells are not likely to use integrin-integrin interaction to mediate cell adhesion (Weitzman et al. 1995), and because  $\alpha 6 \beta 1$  integrin receptors are largely restricted to Sertoli cells (for a review, see Vogl et al. 2000) (we also failed to detect integrin  $\beta 1$  in germ cells isolated from adult rat testes; Siu et al. 2002), it is apparent that laminin, the binding partner of integrin in other epithelia (for a review, see Dym, 1994), is the putative binding partner of  $\alpha 6 \beta 1$  integrin and should reside in germ cells. Yet this possibility remains to be studied. Re-

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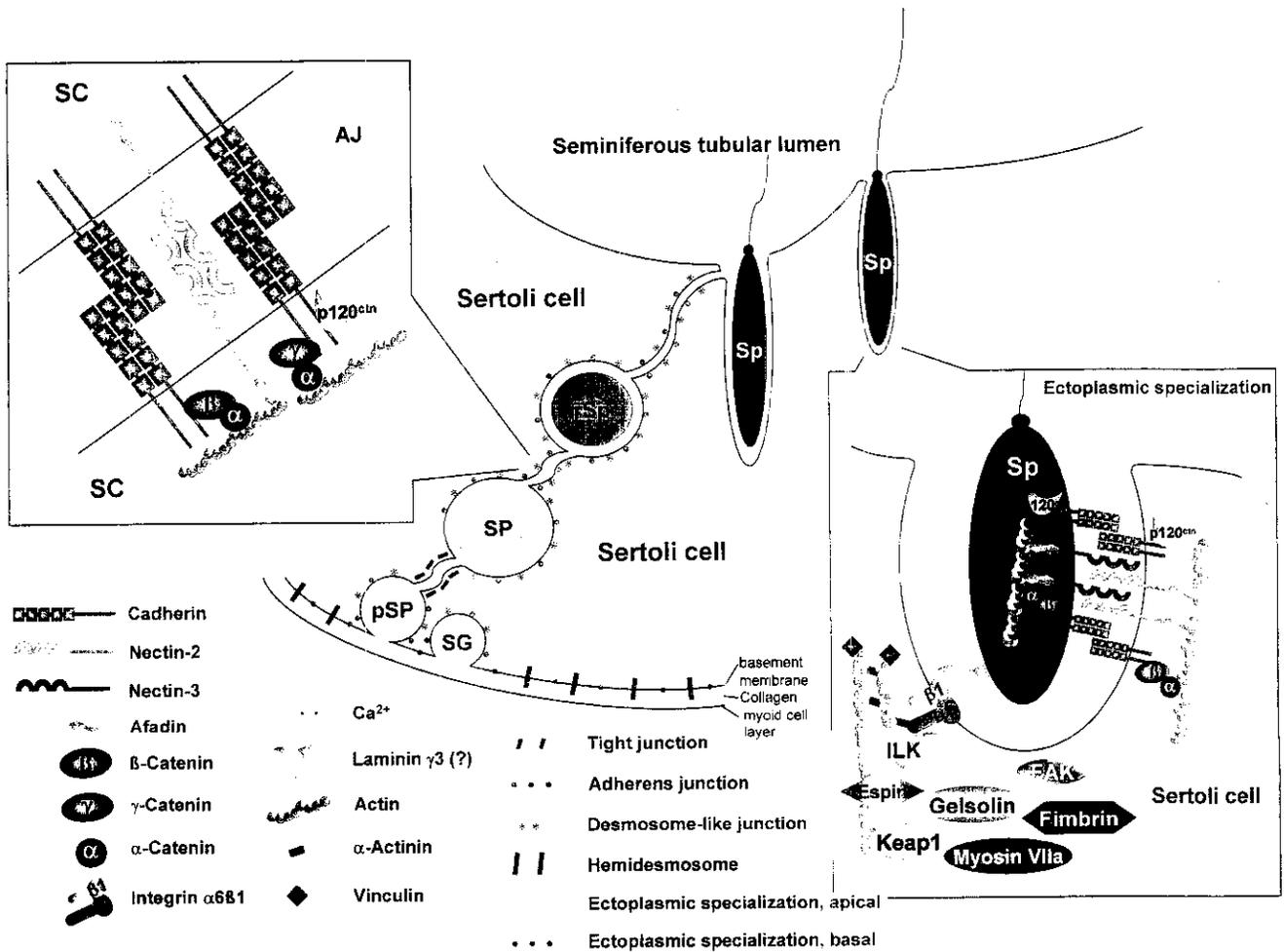


Figure 1. A diagrammatic drawing that illustrates the current structural units of adherens junctions in the testis. Adherens junctions are cell-cell actin-based anchoring junctions and are found between Sertoli cells as well as between Sertoli and germ cells. The cadherin-catenin and nectin-afadin complexes are the basic structural units of adherens junctions between Sertoli cells as well as between Sertoli and germ cells. The integrin-laminin based AJ complex is largely restricted between Sertoli cells and elongated spermatids at the site of ectoplasmic specialization. SG indicates spermatogonia; pSP, preleptotene/leptotene spermatocyte; SP, pachytene spermatocyte; rSp, round spermatid; Sp, elongated spermatid; SC, Sertoli cell; AJ, adherens junction; ILK, integrin-linked kinase; FAK, focal adhesion kinase.

cent studies have shown that laminin  $\gamma$ 3, a potential non-basement membrane binding partner of integrin, is localized to AJ sites between round and elongated spermatids, and Sertoli cells in the testis (Koch et al, 1999). which is consistent with its localization at the apical ES. Its pattern of localization is also in sharp contrast to  $\alpha$ 1/ $\beta$ 1/ $\gamma$ 1 laminin, which is restricted to the basement membrane in the seminiferous epithelium (Koch et al, 1999). These results collectively seem to suggest that the binding partner of  $\alpha$ 6 $\beta$ 1 integrin in the testis at the site of the ES is composed of at least laminin  $\gamma$ 3, and possibly may be laminin 12 (Koch et al, 1999).

*The Cadherin/Catenin Complex*

Cadherin—Classical cadherins, such as epithelial cadherin (E-cadherin), neural cadherin (N-cadherin), and placental cadherin (P-cadherin) are AJ-integral membrane

proteins (for reviews, see Takeichi, 1990; Takeichi et al, 2000). Each cadherin molecule consists of a highly conserved cytoplasmic domain, followed by a single-pass transmembrane region, and one extracellular domain of approximately 550 residues (for reviews see Takeichi, 1990, 1995; Miyatani et al, 1992; Kemler, 1993; Herrenknecht, 1996; Pötter et al, 1999) (Figure 1). Intracellularly, each cadherin molecule interacts with  $\beta$ - or  $\gamma$ -catenin and p120<sup>cas</sup> to form the cadherin/catenin complex, which is the most extensively studied AJ functional unit. p120<sup>cas</sup> binds to the juxtamembrane domain of cadherin (Finemann et al, 1997; Yap et al, 1998), whereas  $\beta$ - or  $\gamma$ -catenins associate with the catenin-binding domain of cadherin (Nagafuchi and Takeichi, 1989; Ozawa et al, 1989; Stappert and Kemler, 1994). Recent studies have confirmed the presence of this complex in the testis (Chung et al, 1998a; Wine and Chapin, 1999; Chapin et

## Rho GTPases and spermatogenesis

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### Abstract

Rho GTPases, such as Rho, Rac and Cdc42, are known to regulate many cellular processes including cell movement and cell adhesion. While the cellular events of germ cell movement are crucial to spermatogenesis since developing germ cells must migrate progressively from the basal to the adluminal compartment but remain attached to the seminiferous epithelium, the physiological significance of Rho GTPases in spermatogenesis remains largely unexplored. This paper reviews some recent findings on Rho GTPases in the field with emphasis on the studies in the testis, upon which future studies can be designed to delineate the role of Rho GTPases in spermatogenesis.

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### 1. Introduction

Throughout spermatogenesis, developing germ cells must translocate across the seminiferous epithelium while differentiating into haploid spermatids. However, how are these intriguing events of cell movement pertinent to spermatogenesis being regulated remain largely unknown (for reviews, see Refs. [1–3]). Recent *in vitro* studies have shown that cytokines, such as TGF- $\beta$ 2 and TGF- $\beta$ 3, can perturb the assembly of the Sertoli cell tight junction (TJ) permeability barrier *in vitro* [4]. Such TGF- $\beta$ -induced effects are possibly mediated by prohibiting the timely expression of TJ-associated molecules such as occludin and ZO-1 [4], which might be needed for assembling TJs [5–8]. A more recent study has shown that TGF- $\beta$ 3 utilizes the MEKs-p38 signal transduction pathway to regulate the Sertoli cell TJ-barrier [9]. Emerging evidence has shown that the effects of growth factors, such as TGF- $\beta$ , and other molecules, such as  $\alpha$ 6 $\beta$ 1 integrins, which constitute the ectoplasmic specializations (a modified testis-specific cell–cell actin-based adherens junction (AJ)) [10] that regulate the cytoskeleton network and junction dynamics are mediated by Rho GTPases (for reviews, see Refs. [1,3,11–13]).

This review summarizes an important and timely development in the field. It also provides some crucial background information on these regulatory molecules in the testis.

### 2. Molecular structure and members of Rho GTPases

Rho GTPases are monomeric G proteins with *M<sub>r</sub>* ranging between 20 and 40 kDa (for reviews, see Refs. [12,14]). Rho GTPases are also members of the Ras superfamily (for reviews, see Ref. [14]). The mammalian Rho GTPase family consists of at least 10 distinctive proteins. These include Rho (A, B and C isoforms), Rac (1 and 2 isoforms), Cdc42 (Cdc42Hs and G25K isoforms), RhoD, Rnd1, Rho6, Rnd2, Rho7, Rnd3, Rho8, RhoG, TC10 and TTF (for reviews, see Refs. [11,12,14]). Like other members of the Ras superfamily, Rho proteins act as molecular switches to control various cellular processes by cycling between GTP-bound (active) and GDP-bound (inactive) states mediated by several regulatory proteins (see Fig. 1) (for reviews, see Refs. [12,14]). Both RhoA [15], RhoB [16], Rac1 [17], Rac2 [9], and Cdc42 [9,17] have been positively identified in the testis by RT-PCR and/or immunohistochemistry. For instance, RhoB, Rac2 and Cdc42 are known to be expressed by Sertoli and germ cells [16,17]. Furthermore, several effector proteins that regulate Rho GTPases have also been identified in the testis by northern and/or RT-PCR (see Table 1). These results thus illustrate that the testis is

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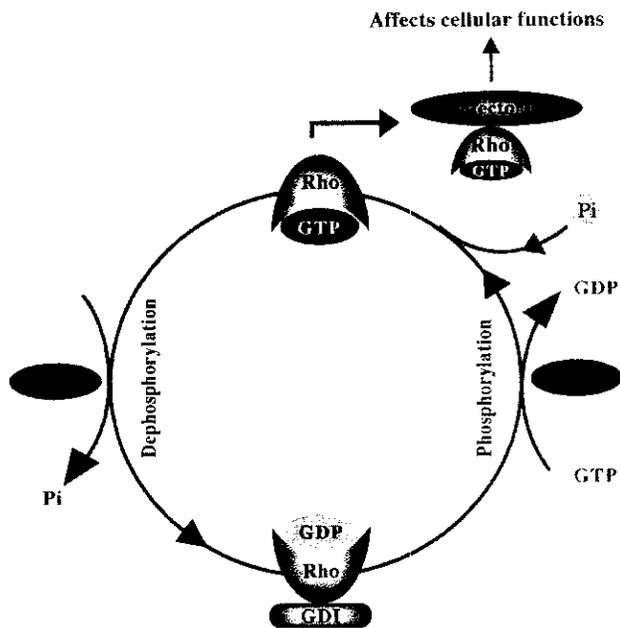


Fig. 1. Cycling of Rho GTPases between GTP- (activated) and GDP- (inactivated) bound state to regulate diversified cellular function. This event is made possible via the interactions of Rho GTPases with different regulators and effector proteins. Rho GDI dissociates from the GDP-bound Rho GTPase, and GEF stimulates the exchange of GDP to GTP facilitating the activation of Rho GTPase. The GTP-bound Rho GTPase interacts with different effectors to affect different cellular functions. Furthermore, GAP interacts with GTP-bound Rho GTPase and catalyzes the hydrolysis of GTP from the complex. GDI, guanine nucleotide dissociation inhibitor; GEF, guanine nucleotide exchange factor; GAP, GTPase activating protein.

equipped with the needed GTPases to regulate different intracellular functions pertinent to junction dynamics and cell movement.

### 3. Functions of Rho GTPases

Studies performed in the past two decades on Rho GTPases using fibroblasts and keratinocytes *in vitro* have shown that Rho GTPases are molecular switches that regulate diversified cellular functions (for reviews, see Refs. [12,14]). These include: (i) actin reorganization, (ii) junction dynamics, (iii) cell movement, (iv) cell cycle, (v) cell transformation, and (vi) gene transcription. Several recent studies have shown that Rho GTPases are crucial molecules that are pertinent to spermatogenesis. These results, in context with the general functional role of Rho GTPases, will be reviewed herein.

### 4. Regulatory proteins of Rho GTPases

The cycling between the GTP- and GDP-bound states in Rho GTPases is regulated by three types of cellular proteins, which include guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and guanine

nucleotide dissociation inhibitors (GDIs) (Fig. 1). GEFs facilitate the exchange of GDP for GTP, which promotes the binding of GTP to Rho GTPases activating the Rho protein [11,18,19]. GAPs stimulate the intrinsic rate of GTP hydrolysis of Rho GTPases converting their GTP-bound state to GDP-bound state, inactivating Rho GTPases [20,21]. GDIs appear to sequester GDP-bound Rho GTPases in the cytoplasm and inhibit the dissociation of GDP from Rho GTPases [22–24]. Ironically, the interplay of these three regulatory proteins determines the level of activated Rho GTPases within a cell, which in turn affects the cytoskeleton network. A number of GEFs and GAPs that regulate Rho GTPases have been identified, whereas only three Rho GDIs are known, which include Rho GDI $\alpha$ , Rho GDI $\beta$ , and Rho GDI $\gamma$  (for reviews, see Ref. [11]) [24–27]. These regulators work synergistically, spatially and temporally to determine the level of GTP-bound Rho GTPases at specific sites within a cell to regulate cellular function [11]. To date, at least one GDI called GDI- $\alpha$  [27] and five GAPs [28–35] have been identified and characterized in the testis (see Table 2). Most of these regulatory proteins, such as  $\beta$ -chimaerin, are largely associated with germ cells. These observations are somewhat intriguing since earlier studies have implicated Sertoli cells are largely responsible for the events of junction dynamics pertinent to cell movement in the seminiferous epithelium (for reviews, see Refs. [1,2]). The exclusive presence of these GTPases in germ cells seemingly signifies that germ cells play a more active role than originally anticipated to regulate their movement in the seminiferous epithelium (see Tables 1 and 2). For instance,  $\beta$ -chimaerin, a GAP, is a stage-specific protein largely restricted to developing spermatids in the rat testis [31]. However, its precise role in spermatogenesis is not entirely known since inhibitors specific to these GAPs are not yet available. Furthermore, how these GAPs interact with Rho GTPase to affect cellular function between Sertoli and germ cells remains obscure.

### 5. Rho GTPase effector proteins

Using yeast two-hybrid system, affinity chromatography, and ligand overlay assays, a number of Rho-interacting proteins have been identified; their interactions with Rho GTPases in turn regulate the functional status of the Rho proteins [11]. To date, at least 15 Rho effector proteins have been identified, such as ROCK1, ROCK2, Rhotekin, Rho-kinase, and PIP 5-kinase (see Table 1). Recent studies have identified both ROCK and p140mDia in the rat testis [16]. Once a Rho GTPase interacts with its corresponding effector, this complex can induce changes in actin polymerization, focal adhesion, stress fiber formation, smooth muscle contraction, neurite retraction, cytokinesis and other cellular processes (see Table 1). Amongst these effector proteins, at least seven have been positively identified in the testis, some of which are putative protein kinases and lipid

Table 1  
Effector proteins for Rho

Effector protein	$M_r$ (kDa)	Interacting Rho protein	Type of protein	Physiological function	GTPase-binding motif	Found in the testis	References
ROCK1, ROCK2	160	RhoA RhoB	Ser/Thr kinase	(i) actin organization; (ii) stress fiber formation; (iii) cell motility; (iv) cytokinesis; (v) focal adhesion formation; (vi) neurite retraction	RKH	n.k.	[68,70,96,107]
P140mDia	140	RhoA	scaffold	actin organization	-	-	[106,108,109]
PKN	120	RhoA	Ser/Thr kinase	n.k.	REM	-	[91,92,110,111]
PRK1, PRK2	120	RhoA RhoB	Ser/Thr kinase	n.k.	REM	n.k.	[112,113]
Citron kinase	183	n.k.	Ser/Thr kinase	cytokinesis	-	n.k.	[114,115]
MBS	n.k.	RhoA	phosphatase	MLC inactivation	-	n.k.	[96]
Rhophilin	71	RhoA	scaffold	n.k.	REM	-	[88,92]
Rhotekin	61	RhoA	scaffold	n.k.	REM	n.k.	[116,117]
Kinectin	160	RhoA	scaffold	kinesin binding	RKH	-	[118,120]
PLD	25	RhoA	lipase	regulate PA levels	-	-	[121,127]
DAG kinase	78	RhoA	lipid kinase	regulate PA levels	-	-	[128,133]
PI 4,5-kinase	n.k.	n.k.	lipid kinase	(i) actin organization; (ii) regulate $PIP_2$ level	-	n.k.	[78,129,132,135]
PI 3-kinase	n.k.	RhoA	lipid kinase	n.k.	-	-	[134,137]

ROCK, Rho-associated protein kinase; p140mDia, mammalian homolog of diaphanous in *Drosophila*; PKN, protein kinase N; PRK1, PRK2, protein kinase C-related kinase 1, 2; MBS, myosin-binding subunit; PLD, phospholipase D; DAG kinase, diacylglycerol kinase; PI 4,5-kinase, phosphatidylinositol-4-phosphate 5-kinase; PI 3-kinase, phosphatidylinositol-3-kinase; MLC, myosin light chain; PA, phosphatidic acid;  $PIP_2$ , phosphatidylinositol 4,5-bisphosphate; RKH, ROK-kinectin homology (also known as class-I Rho-binding motif); REM, Rho effector homology (also known as class-II Rho-binding motif); n.k., not known; -, has been positively identified in the testis.

kinases (Table 1). Recent findings that demonstrate the crucial roles of kinases and phosphatases in the regulation of Sertoli cell TJ dynamics [36] further strengthen the notion that these molecules are essential for cell movement.

## 6. Regulation of junction dynamics by Rho GTPases

### 6.1. Effects on tight junction (TJ) dynamics

Numerous studies have implicated the significance of Rho GTPases in the regulation of TJ dynamics in different

cell types. For instance, ADP-ribosylation and inactivation of Rho by C3 exoenzyme causes functional disruption of TJs in T84 epithelial cells via ZO-1 redistribution, which induces ZO-1 to move from the membrane at the sites of TJs to cytosol [37]. Furthermore, constitutively active RhoA can induce an increase in the tightness of TJs in MDCK cells, which is mediated by Rho-associated kinase and a yet-to-be identified Rho effector [38,39]. These two studies suggest the crucial role of Rho in TJ dynamics in epithelial cells.

Apart from their direct effects on the TJ-integral membrane proteins, there is increasing evidence demonstrating that Rho GTPases regulate TJ integrity by altering actin

Table 2  
GTPase activating proteins in the testis

GTPase activating protein (GAP)	$M_r$ (kDa)	Target GTPases	Species	Localization and Function	References
Rotund RacGAP (RnRacGAP)	n.k.	Rac, Cdc42	<i>Drosophila melanogaster</i>	deletion of <i>rn</i> locus affects spermatid differentiation, males are sterile	[28,29]
$\alpha$ 2-Chimaerin	45	Rac	rat	mRNA found in the testis particularly in pachytene spermatocytes	[30]
$\beta$ -Chimaerin	34	Rac	rat	expresses exclusively in the testis at sexual maturation, stage-specific expression parallel to acrosomal assembly at late stage of spermatogenesis	[31]
Male germ cell RacGAP (MgcRacGAP)	58	Rac1, Cdc42	human	specific expression of <i>mgcRacGAP</i> mRNA in spermatocytes; interacts with <i>Tar1</i> , a sulfate transporter, found in spermatocytes and spermatids	[32,33]
Myr5	n.k.	Rho	rat	found in lung, spleen, liver and testis	[34,35]

organization, thereby affecting TJ permeability. Nonetheless, the mechanism by which Rho GTPases regulate actin organization is not fully characterized [40,41]. Yet, it is not of interest to note that F-actin staining was shown to be weak at the site of cell–cell contacts in MDCK cells expressing dominant negative Rac1N17, but it was strong in constitutively active Rac1V12 cells [42], whereas F-actin staining was more intense at cell–cell contacts of overexpressed Rac1V12 and RacN17 cells versus control cells [40]. These studies thus implicate the role of Rho GTPases in actin reorganization in TJs. Cdc42 was also shown to play an important role in the regulation of actin organization and TJ function. For instance, MDCK cells expressing dominant negative Cdc42N17 and dominant active Cdc42V12 are having significantly lower resistance across the TJ-barrier, suggesting Cdc42 regulates the gate function of TJ [43]. However, Rho GTPases, such as RhoB, Rac2 and Cdc42, apparently are not involved in the assembly of Sertoli cell TJ-barrier *in vitro* since an induction of their expression was not detected during TJ-barrier assembly [9]. Yet, these proteins may still be crucial to TJ dynamics in the testis by affecting the actin cytoskeleton network.

## 6.2. Effects on cell–cell actin-based adherens junction (AJ) dynamics

### 6.2.1. Rho GTPases found in AJs can affect cell adhesiveness

Small Rho GTPases including Rac and Rho have been found at the site of cell–cell contacts [40,42,44–46] and are known to be involved in the regulation of cell adhesion [47,48]. In keratinocytes, Rho and Rac are required for the establishment and stabilization of cadherin-dependent cell–cell adhesion and actin reorganization. Stable cadherin adhesion failed to form when confluent patches of keratinocytes microinjected with C3 exoenzyme or dominant negative Rac (N17Rac) causing E-cadherin to move away from the site of cell–cell contacts [47]. However, microinjection of constitutively active Rho (L63Rho) and Rac (L61Rac) into confluent keratinocytes after AJs were assembled did not perturb cadherins and failed to induce cadherin recruitment to the site of cell–cell contacts. These results thus suggest that the activation of Rho and Rac is required for the establishment, but possibly not the maintenance, of stable cadherin-mediated cell adhesion [47]. It was reported that the inhibition of endogenous Rho and Rac can effectively remove P-cadherin from keratinocytes, however, it took a considerably longer incubation to remove E-cadherin from the site of AJs [48]. These results seemingly suggest that the ability of Rho and Rac in regulating cell adhesiveness is dependent on the maturation status of the AJ and the cell, as well as the class of cadherins [48]. Furthermore, studies using human epidermal keratinocytes have shown that cells having constitutively active Rac1 can disrupt cell–cell contacts via clathrin-independent endocytosis of E-cadherin and the ability of Rac1 to regulate AJ

disassembly is dependent on colony size [49,50]. Recent studies have shown that AF-2364 [1-(2,4-dichlorobenzyl)-indazole-3-carbohydrazide]-induced AJ disruption in the testis, which causes premature release of germ cells from the seminiferous epithelium [51,52], mediates its effect via  $\beta$ 1-,  $\beta$ 2-integrin and  $\beta$ 4-integrin [16,53] and testin [51,52] (note: integrins were shown to be the putative cell adhesion molecules in apical ectoplasmic specializations, a modified testis-specific cell–cell actin-based AJ [10] that functionally links to testin, an AJ-signaling molecule (for review, see Ref. [3]) through a yet-to-be defined pathway). These observations are crucial to the potential use of AF-2364 for male contraception because they explain why would AF-2364 limit its effects in the testis inducing germ cell from the seminiferous epithelium without damaging AJ structure in other organs, such as liver and kidney [51,52] since the  $\alpha$ 6 $\beta$ 1 integrin/testin complex is unique to the ectoplasmic specialization in the testis (for review, see Ref. [3]) and is likely the target structure of AF-2364. Nonetheless, the downstream signaling pathway mediated by integrin was shown to be RhoB/ROCK/ LIMK [16] (Fig. 3). Taking these results collectively, they clearly illustrate the pivotal role of GTPases in AJ function in the testis.

### 6.2.2. Effects of Rho GTPases on AJ dynamics are cell type-specific

The effect of GTPases on cell adhesion is also cell type-specific. For instance, the activation of Rac can increase the level of immunoreactive cadherin,  $\beta$ -catenin, and actin at cell–cell borders in MDCK cells, suggesting that Rac may strengthen cadherin-based adhesion [42,54,55]. However, Rac activation failed to induce recruitment of cadherin to AJs in human keratinocytes and HaCat cells (a keratinocyte cell line) in contrast to MDCK cells [56]. More intriguingly, sustained Rac activation in MDCK cells can specifically remove cadherin from newly formed and stable cell–cell contacts dose- and time-dependently [56]. Numerous studies have revealed the complex roles of Rac1 in regulating AJ dynamics, possibly mediated by two distinct regulatory mechanisms [42,55,56]. Such a postulate is supported by the observation that dominant active Rac expressed in MDCK cells can either enhance or prevent E-cadherin-mediated cell adhesion [57]. More research is needed to resolve some of these apparently conflicting results that the effects of Rho GTPases on AJ-protein recruitment are cell-specific. Using Sertoli-germ cell cocultures *in vitro* to study the events of Sertoli-germ cell AJ assembly, we have demonstrated an induction of RhoB, Rac2 and Cdc42 mRNA levels when germ cells attach onto the Sertoli cell epithelium, suggesting the potential role of Rho GTPases in Sertoli-germ cell AJ assembly in the testis [16] (Lui and Cheng, unpublished observations).

### 6.2.3. Mechanism of action

Regulation of AJ dynamics can be achieved through the direct interaction of Rho GTPase and its effector proteins.

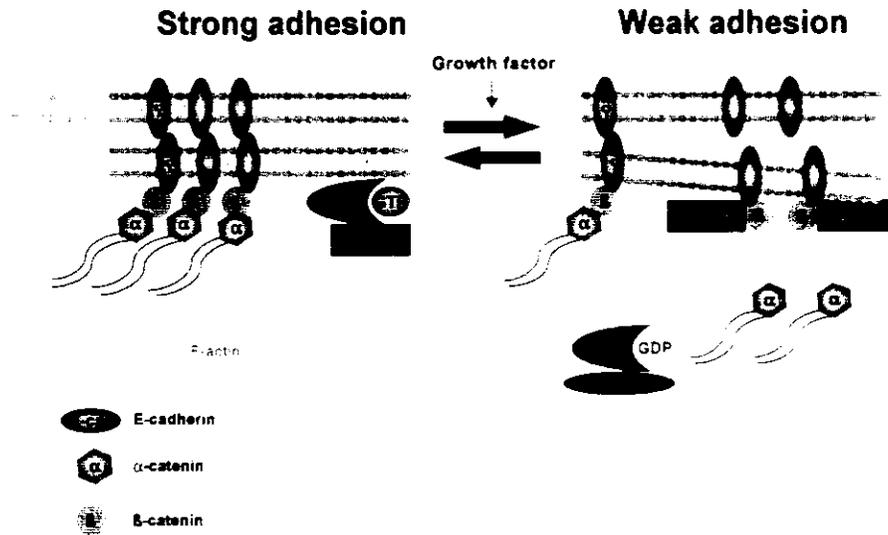


Fig. 2. Regulation of E-cadherin-mediated cell–cell adhesion by IQGAP. Changes in cell adhesiveness is being regulated by altering the interactions between IQGAP,  $\beta$ -catenin, and Rac and Cdc42. When IQGAP dissociates from Rac/Cdc42, it binds to the  $\beta$ -catenin of the cadherin–catenin complex, this in turn destabilizes the cadherin–catenin complex, disrupting cell adhesion. Furthermore, this event is regulated in part by growth factors. This diagram was prepared based on Refs. [11,58,95].

For instance, IQGAP, is an effector protein that is involved in regulating E-cadherin activity (for review, see Ref. [58]). IQGAP is localized at the site of cell–cell contact, which exerts negative regulatory effect on cadherin-mediated cell–cell adhesion [59,60] (Fig. 2). This is achieved by direct

interaction of IQGAP with  $\beta$ -catenin causing the dissociation of  $\alpha$ -catenin from the cadherin–catenin complexes [60]. It was shown that Cdc42 and Rac1 are responsible for regulating the IQGAP function by inhibiting the interaction between IQGAP and  $\beta$ -catenin, resulting in stabiliz-

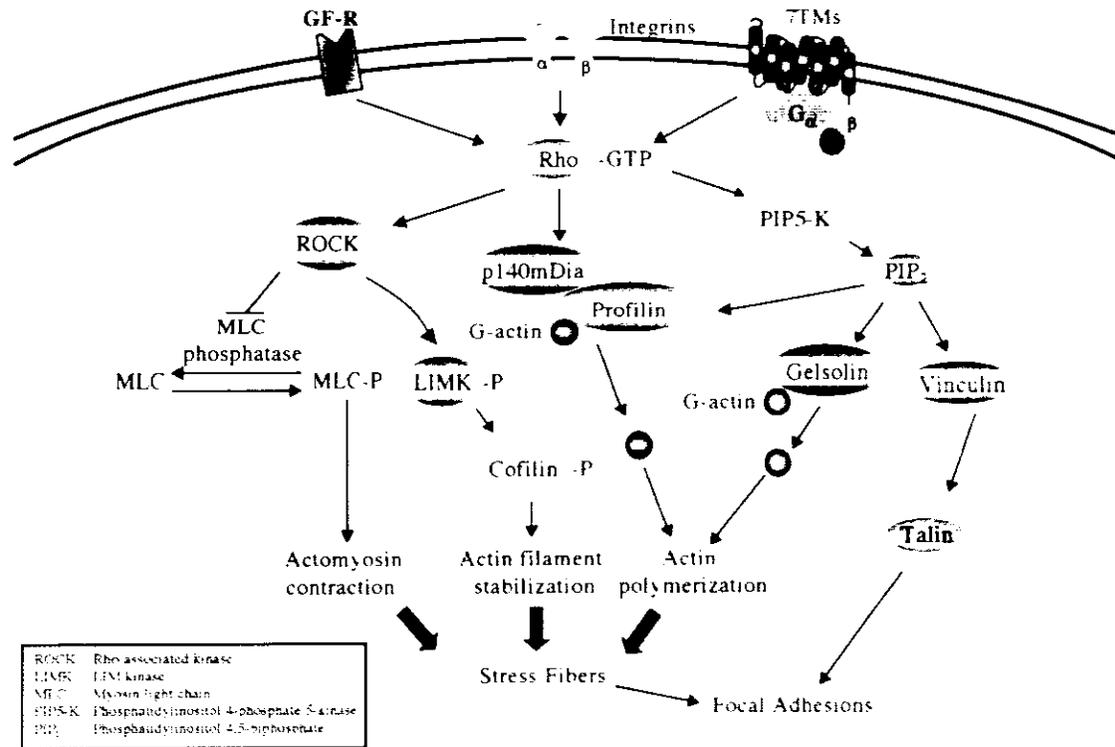


Fig. 3. A schematic drawing of different signaling pathways by which Rho GTPases regulate actin cytoskeleton network. The GTP-bound Rho is able to activate different downstream effectors including ROCK, p140mDia, and PIP5-K. These effectors further regulate the corresponding downstream target proteins, such as LIMK by ROCK, profilin by p140mDia, and PIP2 by PIP5-K via phosphorylation or direct protein–protein interactions. This in turn leads to changes in actin filament organization. This figure was prepared based on Refs. [11,12,24].

ing the cadherin–catenin complex [59]. Taking these results collectively, it is clear that Rac and Cdc42 are important regulators of cell adhesion. Using an *in vivo* model and Sertoli-germ cell AJ disruption induced by AF-2364 [1-(2,4-dichlorobenzyl)-indazole-3-carbohydrazide] [51,52], it was shown that the cascade of events leading to germ cell loss from the epithelium involves an initial activation of integrins and RhoB GTPase, which is followed by the downstream activation of ROCK and LIMK [16]. This in turn perturbs the actin cytoskeleton network that disrupts the Sertoli-germ cell AJs.

## 7. Regulation of cell movement by Rho GTPases

Several studies have shown that Rho GTPases can be regulated by growth factors and neurotransmitters [61–64]. This also provides the link between growth factor signaling and the events of cell adhesion and cell movement via reorganization of the actin filament network (for reviews, see Refs. [65,66]).

Reorganization of actin filament, cell–substratum contacts, and cell–cell adhesion are involved in cell movement. Accumulating evidence has indicated that the Rho GTPase family is involved in the reorganization of actin filament, which in turn regulates cell movement. The functional roles of Rho, Rac, and Cdc42, however, are different. For instance, Rho regulates stress fiber formation [62]. Lamellipodia extension is regulated by Rac [61], and the activity of filopodia is controlled by Cdc42 [63,67].

To understand how Rho GTPases regulate actin reorganization, intensive investigations were conducted to identify specific effectors for Rho GTPases (Fig. 3). ROCKs, mDia and PIP5-K are three crucial effectors that regulate Rho-induced stress fiber assembly (for reviews, see Refs. [11,24]). And both ROCKs and mDia are found in the rat testis [16]. ROCKs (ROCK1 and ROCK2) are putative Ser/Thr protein kinases, which contain a coiled–coiled region, a ROK-kinectin homology (RKH) Rho-binding domain, a PH domain, and a Cys-rich region [68,69]. ROCK has been shown to phosphorylate the myosin-binding subunit (MBS) of the myosin light chain (MLC) phosphatase [69,70]. LIMK, is another ROCK target protein. When LIMKs are phosphorylated by ROCKs, the activated LIMK can inhibit cofilin by phosphorylation, which in turn stabilizes the filamentous actin structures [24,71,72].

mDia is the mammalian homologue of diaphanous in *Drosophila* and a member of the formin-homology (FH) family of proteins. It consists of three FH domains and a small GTPase-binding domain (GBD) [73]. Multiple proline-rich motifs in the FH1 domain bind to the G-actin-binding protein known as profilin, their interactions lead to the dissociation of actin from profilin that promotes actin polymerization (for review, see Ref. [74]).

Rho also stimulates PIP 5-kinase and causes an elevation of PIP<sub>2</sub> levels [75]. High level of PIP<sub>2</sub> dissociates gelsolin

and profilin from actin that contributes to actin reorganization [76,77]. Besides, elevated PIP<sub>2</sub> causes conformational changes of vinculin, this enhances its binding ability to actin and talin [78].

Apparently, these Rho downstream effectors are important in actin reorganization, which in turn regulate cell movement. However, very few studies have been done to investigate the role of these effectors in the testis. Understanding the mechanism by which Rho GTPases regulate the cellular cytoskeleton network will provide a useful guideline of how to study the role of Rho GTPase effectors and their functions in the testis.

## 8. Other biological functions of Rho GTPases

It is apparent that Rho GTPases also play a crucial role in regulating other cellular processes that are dependent on the actin cytoskeleton, such as phagocytosis [79,80], pinocytosis [61], morphogenesis [81], cytokinesis [82,83], G<sub>1</sub> cell cycle progression [84] and cell transformation [18] (for review, see Ref. [24]). Nonetheless, very few studies were performed to explore their physiological functions in the testis. For instance, Sertoli cells are the phagocytotic cells in the testis that engulf residue bodies cast away from spermatids during spermiation, and that eliminate apoptotic germ cells during spermatogenesis [85,86]. Needless to say, Rho GTPases may play a critical role in these events because these processes are tightly integrated to the reorganization of the cytoskeleton network. It is obvious that a thorough understanding of Rho GTPases in these cellular events will yield new insights in understanding their role(s) in spermatogenesis.

## 9. Current status of research in the testis

Studies by immunohistochemistry analysis have shown that there is a strong staining of Rac1 at the basal compartment of the seminiferous epithelium and its localization appears to be predominant in stage VIII [17], Cdc42, however, is largely associated with spermatocytes and Sertoli cells, and the most intense staining was detected surrounding the heads of the elongated spermatids, still, it is not known if Cdc42 is stage-specific [17]. These results seemingly suggest that Rho GTPases are possibly involved in spermatogenesis, yet, the mechanism or pathway by which they mediate their effects are not known. An early study using C3 exoenzyme has identified RhoA in the membrane of bovine sperm tail, interestingly, its inactivation can lead to a decrease in sperm motility [15]. To elucidate the function of Rho in the testis, studies were performed to identify the downstream effector(s) for Rho in the testis. Toshima et al. [87] reported the identification of a novel protein kinase in the testis, TESK1, which shares 50% homology with LIMK, is largely expressed by round spermatids. TESK1 was shown to stimulate the

formation of integrin-mediated actin stress fibers and focal adhesions via phosphorylation of cofilin [88]. Rho GTPase is an effector protein found in the testis associated with germ cells undergoing meiosis, but not with somatic cells [89]. Rho effectors, such as citron kinase and PKN, are also found in testis, but their role(s), if any, is not known [90,91]. Other studies were also performed to investigate the function of Rho GTPase regulators, such as GAPs and GDIs. For instance,  $\alpha_2$ -chimaerin,  $\beta$ -chimaerin, and MgcRac are three testis-specific GAPs largely restricted to germ cells and are responsible for cytoskeleton reorganization [31,33,92] (Table 2). The significance of Rho GDI $\alpha$  in the testis has been demonstrated in Rho GDI $\alpha^{-/-}$  mice since  $-/-$  male mice were infertile with impaired spermatogenesis, the seminiferous epithelium was devoid of spermatids and spermatocytes, and vacuoles were found in the epithelium representing degenerating germ cells [93]. Also, *Limk2*-deficient mice were having impaired spermatogenesis and the *Limk2^{-/-}* testes were smaller in size compared to normal mice [94]. The seminiferous epithelium of *Limk2^{-/-}* testis contained few spermatocytes in their early stage of development and the number of germ cells beyond the pachytene stage were greatly reduced versus normal mice [94].

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