Cell–cell adhesion and signalling

Vania MM Braga

Signalling pathways activated by Rho small GTPases have recently been identified that coordinate junction assembly, stability and function, as well as interactions of adhesive complexes with the underlying cortical cytoskeleton. Particularly exciting is the interplay between adherens junctions, activation of Rho proteins and the dynamics of microtubule, actin and intermediate filaments. This interplay has important implications for functional regulation of cell–cell adhesion, and points to a more integrated view of signalling processes.

Addresses
Cell and Molecular Biology Section, Division of Biomedical Sciences, Faculty of Medicine, Imperial College, Sir Alexander Fleming Building, London SW7 2AZ, UK; e-mail: v.braga@ic.ac.uk

Current Opinion in Cell Biology 2002, 14:546–556

0955-0674/02/$ – see front matter

DOI 10.1016/S0955067402003733

Abbreviations

aPKC	atypical PKC
EGF	epidermal growth factor
EGFR	EGF receptor
GAP	GTPase-activating protein
GEF	guanine nucleotide exchange factor
HGF	hepatocyte growth factor
MDCK	Mardin–Darby canine kidney
MTOC	microtubule organising centre
PI3K	phosphatidylinositol 3-kinase
PKC	protein kinase C
VE	vascular endothelial
ZO-1	zonula occludens 1

Introduction

Cell–cell adhesion is an essential component of epithelial morphology and function. Epithelial cells adhere tightly to their neighbours, and several specialised adhesive structures ensure the appropriate integrity and tensile strength of epithelial sheets. These adhesive structures are connected either to intermediate filaments (desmosomes) or to microfilaments (adherens junctions and tight junctions). This association with the cytoskeletal network is necessary for stable cell–cell adhesion and for the integration of cell–cell contacts with the changes in morphology that are characteristic of epithelial cells (e.g. cuboidal cell shape, polarised phenotype).

Over the past few years, many different laboratories have addressed the questions of how cell–cell adhesion is regulated and how the epithelial phenotype is generated. Although the regulatory processes are not fully understood, several signalling pathways that are activated by cell–cell adhesion have been identified. Among these signalling pathways, the ones regulated by Rho small GTPases stand out as the most studied and most interesting. Rho proteins are signalling molecules that regulate a plethora of cellular processes (reviewed in [1,2]). The central core of their activity, however, is directed towards actin filaments and diverse events that require actin reorganisation. In epithelia, small GTPases play a role in the formation and maintenance of adherens and tight junctions (reviewed in [3••]).

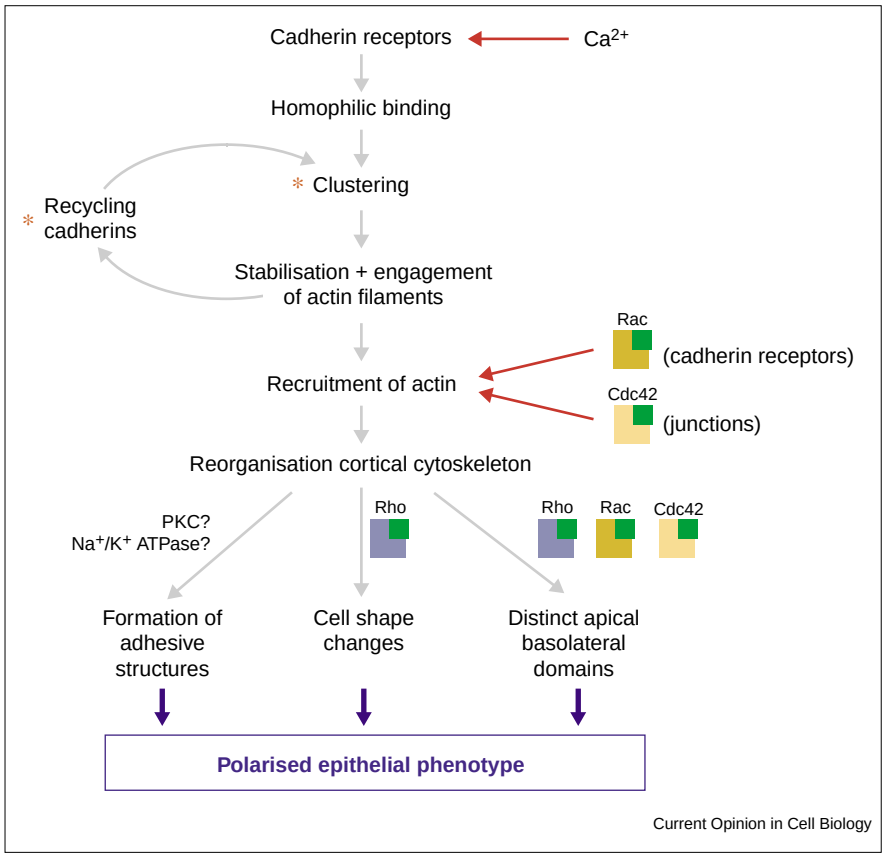
Three members of the Rho small GTPase subfamily have been implicated in the regulation of adherens junctions: Rho, Rac and Cdc42. More recently, other related members of the Ras superfamily (to which Rho proteins belong), such as K-Ras, Rap1 and Arf6, have also been associated with the modulation of cell–cell adhesion. In this review, I focus on recent publications that address the role of small GTPases in adherens and tight junction function, and how the signalling pathways activated by these adhesive structures are involved in the generation of the epithelial phenotype. The role of the Rho family in the disassembly of cell–cell adhesion during tumour progression is reviewed elsewhere [4•], as is the participation of several Rho targets in cadherin-dependent adhesion [3••,5]. These topics are not covered in this review.

Adherens junctions and Rho small GTPases

Adherens junctions are adhesive structures formed by cadherin-dependent adhesion. Cadherins belong to a large family of receptors that require Ca^{2+} for stable cell–cell adhesion (reviewed in [6]). Cadherins associate with the actin cytoskeleton via proteins known as catenins, and this interaction is important for providing strength to cell–cell contacts. The formation of cadherin-dependent cell–cell adhesion and subsequent generation of the epithelial phenotype occurs in a stepwise process with increasing levels of complexity [3••]. Figure 1 highlights the steps in which distinct signalling pathways have been shown to play a role following the establishment of cell–cell adhesion.

It is not clear whether the same regulatory events described in Figure 1 play a role in the maintenance of cell–cell adhesion and epithelial characteristics. For instance, it is known that the maturation of junctions (i.e. how long cells have been in contact) impairs the response of cadherin receptors to inhibition of Rho and Rac [7]. Thus, although perturbation of cadherin-mediated cell–cell adhesion does occur when small GTPases are inhibited in cells containing mature junctions, the mechanisms of junction assembly might be somewhat different. Inhibition of Rac activity in mature junctions results in an increased detergent solubility of cadherin complexes [8]. Interestingly, blocking Rho function in mature junctions affects the composition of cadherin complexes (i.e. the proportion of associated catenins) [9•]. A similar mechanism for junction disassembly has been suggested for IQGAP, a protein that interacts with Rac and Cdc42 [5]. Reduced

Figure 1



A proposed stepwise model for the generation of a polarised, fully differentiated epithelial cell. In the presence of Ca^{2+}, cadherins interact with the same type of molecules on adjacent cells (homophilic binding), and cluster at sites of cell–cell contact. Concomitantly, there is an increased engagement of adhesive receptors with the actin cytoskeleton (via catenins) and recruitment of new actin to the junctions. While reorganisation of the cytoskeleton occurs (involving microfilaments, microtubules and intermediate filaments), three other key cellular processes take place and result in the formation of the epithelial phenotype (cell-shape changes, formation of other adhesive structures and distinct membrane domains). These different steps do not occur in a synchronous manner, and can overlap considerably in time. This working model is based on evidence that allows the dissection of each individual step: the functional dependence of tight junctions and desmosomes on previous cadherin adhesion, genetic evidence, the importance of cadherin interaction with the cytoskeleton (catenin association) and manipulation of distinct signalling pathways. Rho function is important during formation of new cell–cell contacts and for cell compaction and shape changes (generation of tension and contraction; [3••,27,75]). Activated Rac is necessary for actin recruitment to clustered cadherin receptors [7,76], whereas Cdc42 induces actin accumulation at junctions (where many types of other receptors are found) [8,77,78]. All three GTPases are involved in the targeted delivery of membrane proteins to distinct domains [13–15,39•,79]. Na^+/K^+ ATPase and aPKC isoforms may also play a role in the formation of desmosomes and tight junctions (see Table 2; [30•,38••]). The inhibition of small GTPases does not affect the formation of tight junctions (but it does interfere with function). However, activation of Rho, Rac or Cdc42 has deleterious effects on tight junction organisation and function (see Table 2). * denotes a step in which an involvement of small GTPases is predicted from work with other receptors and cell systems, but has not been formally demonstrated with cadherin molecules [3••].

levels of catenins in this complex would result in perturbed cytoskeletal interaction, thereby affecting the stability of the cadherins at junctions. It seems unlikely that the above effects would also occur during the induction of cell–cell adhesion: no differences in cadherin complex composition have been observed in epithelial cells grown in the absence or presence of cell–cell contacts [10,11].

Cadherin recycling is important both during the formation of new contacts and in the maintenance of stable junctions. Adhesive receptors have a lower endocytosis rate and longer half-life when compared with cadherin molecules that are not engaged in adhesion [11,12]. How small GTPase activity interfaces with cadherin recycling, new cell–cell contact formation and cadherin stability is not clear. In epithelia, most studies on the role of Rho GTPases in endocytosis have not addressed cadherin recycling per se, but they have investigated the delivery of other membrane proteins to specific membrane domains [13–15]. The activation of ARF6, a small GTPase involved in membrane trafficking, leads to the perturbation of cadherin adhesion by increasing cadherin endocytosis [16••]. Interestingly, the ARF6 disruptive effect occurs via a pathway distinct from lamellae formation — another process in which ARF6 is involved [16••]. The participation of small GTPases in the destabilisation of junctions has also been studied during oncogenic transformation, tumour progression and growth-factor-induced scattering [4]. The question here is whether similar mechanisms are important for the homeostasis of junctions; that is, in the absence of a destabilising stimuli. However, this possibility warrants further investigation.

Some studies have investigated the interface between endocytosis and cadherins in the disassembly of junctions during tumour progression. More recently, other Ras family members in addition to ARF6 have been implicated in cadherin adhesion. In hepatocytes, K-Ras (but not H-Ras or N-Ras) appears to be important for establishing new cadherin contacts during liver development [17•]. In support of this data is the finding that in keratinocytes, H-Ras activity is not necessary for the formation of cadherin-dependent

Table 1

Activation of specific signalling pathways by Ca^{2+}-induced cell–cell adhesion in different epithelial cells.

					Cadherin function		
Activation of	Assessed by	Observations	Cell type	Time course	Necessary	Sufficient	Refs
Rac	Pull down assay	PI3K-dependent	MDCK	30 min	Y	N/D	[23•]
		EGFR-dependent	Keratinocytes	5 min	Y	Y	M Betson *et al.*, unpublished data
			MDCK	5 min	Y	N/D	[22•]
			CHO (C-cadherin)*	90 min	N/A	N/A	[22•]
Cdc42	Pull down assay	PI3K-dependent	Breast	15 min	Y	N/D	[24•]
		No effect	CHO (C-cadherin)*	N/D	N/D	N/D	[22•]
Rho	Pull down assay	Activation	MDCK	5 min	N/D	N/D	[22•]
		Activation	Keratinocytes	1 h	N/D	N/D	[28]
		No activation	MDCK II	N/D	N/D	N/D	[23•]
		Inhibition	CHO (C-cadherin)*	N/D	N/A	N/A	[22•]
PRK2, PNK	Phosphorylation	Rho targets	Keratinocytes	1 h	N/D	N/D	[28]
MAPK	Phosphorylation	p42/p44	HaCat	5 min	Y	Y	[82]
PI3K	PIP3 production		MDCK	30 min	Y	N/D	[83]
Akt/PKB	Phosphorylation	PI3K target	MDCK	30 min	Y	N/D	[83]
			CaCo2	15 min	Y	N/D	[84]
p38	Phosphorylation		CaCo2	5 min	Y	N/D	[84]
EGF receptor	Phosphorylation		HaCat	5 min	Y	N/D	[82•]
Shc	Phosphorylation		HaCat	5 min	N/D	N/D	[82•]
Fyn	Phosphorylation	Rho dependent	Keratinocytes	6 h	N/D	N/D	[28]
Gab1	Phosphorylation	Interacts with HGF receptor	MDCK	2 h	Y	N/D	[85]

Rho and Rac activity play a role in the regulation of cadherin-dependent adhesion; Cdc42 has partial effects on cadherin localisation at junctions. No evidence is available for the participation of the other signalling pathways in the formation of new cadherin contacts. *, CHO-C-cadherin adhesion was performed by cell spreading onto C-cadherin extracellular domains as substratum. Under these conditions, the observed activation of small GTPases may result from both cadherin engagement and spreading of CHO-C-cadherin cells. The time course of Fyn activation correlates more with keratinocyte differentiation and stratification (movement towards the skin surface) than with cell–cell adhesion formation, which occurs within minutes [49,74]. It is possible that Fyn might participate in the cell–cell adhesion remodelling that occurs during stratification. 'Time course', the earliest time point at which activation was reported; 'Cadherin function': 'Necessary', activation is blocked by incubation with inhibitory anti-cadherin antibodies before and during calcium stimulation; 'Sufficient', clustering of cadherin receptors in low calcium medium can activate the signalling pathway. N/A, not applicable; N/D, not determined; Y, activation observed.

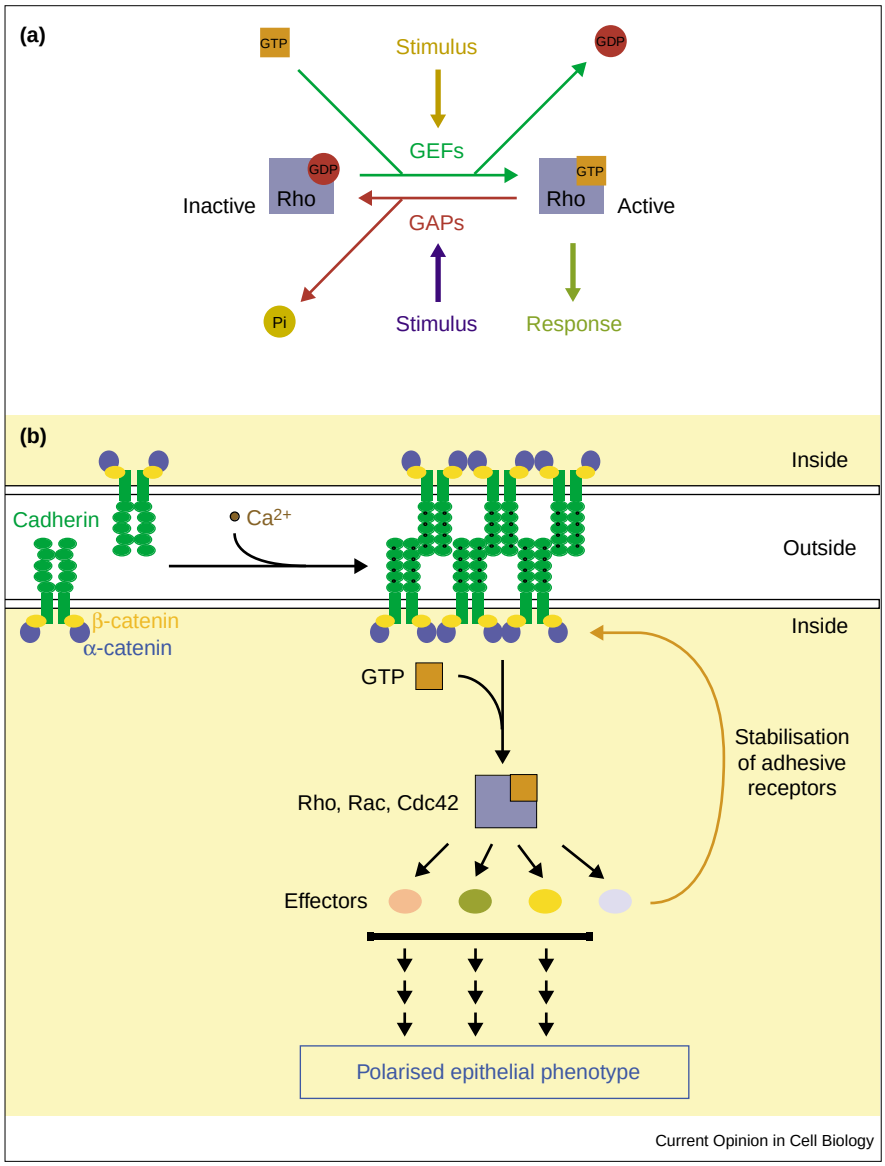
adhesion [18]. An interesting recent observation is that Rap proteins may participate in epithelial morphogenesis [19,20•]. In *Drosophila*, analysis of Rap1 mutants during wing development shows adherens junction misplacement and the dispersal of clonal cells that would otherwise stay in a coherent group. However, the effects of Rap1 on cell–cell adhesion seem specific for the differentiation process during wing development, as it is not essential for the appropriate apical localisation of junctional proteins [20•]. In light of recent publications implicating Rap in integrin activation and function [21], it will be exciting to investigate whether Rap1 is activated following junction assembly and if it is also required for cadherin-mediated adhesion in mammalian cells.

Adhesion-dependent activation of small GTPases and other signalling pathways

The ability of cadherins to participate in different signalling processes such as cell fate, growth control, survival and differentiation is well known. Table 1 summarises the activation of distinct signalling pathways in a cadherin-specific manner following the initiation of new contacts. The activation of these pathways by cadherin-dependent adhesion is clear, as addition of calcium ions per se is not able to induce their activation. However, an important question needs to be addressed. In which cellular process downstream of cell–cell adhesion do these pathways play a role? With the exception of Rho small GTPases, the importance of other signalling pathways for the formation of cadherin-dependent adhesion has not been demonstrated.

The formation of cadherin-mediated cell–cell contacts can activate Rho, Rac and Cdc42 (Table 1; Figure 2b). Confluence of Mardin–Darby canine kidney (MDCK) cells appears to increase the activation of Rac and Cdc42, but inactivates Rho [22•]. Different reports show that Rac and Cdc42 activation occurs very quickly in a cadherin-dependent manner ([23•,24•]; M Betson *et al*,. unpublished data [see Update]). In keratinocytes, the clustering of cadherin receptors is sufficient to activate Rac (M Betson *et al.*,

Figure 2



Rho small GTPases: activation and functional interaction with cadherins. **(a)** Regulation of Rho small GTPase activity. When these proteins associate with GTP, they are activated and competent for binding to different effectors that participate in distinct cellular processes. Inactivation occurs following the intrinsic hydrolysis of GTP, liberating phosphate. Regulatory proteins tightly modulate this cycle: they can facilitate the exchange of nucleotides and thereby activation (e.g. GEF), or accelerate the hydrolysis of GTP, thereby inactivating the GTPase (e.g. GAP). **(b)** Current working model for the functional interaction between cadherin receptors and small GTPases. Dimers of cadherin molecules are found at the cell surface, associated with cytoplasmic proteins known as catenins. Cadherin homophilic binding and clustering at sites of cell–cell adhesion somehow triggers the activation of Rho proteins. The small GTPases then bind to specific targets and activate distinct signalling pathways. These targets participate in processes that either feed back to stabilise cadherin adhesion (e.g. actin polymerisation and cytoskeletal remodelling) or play a role in the development of epithelial phenotype (i.e. the assembly of other adhesive structures, compaction, polarisation, expression of epithelia-specific genes, etc.). It is predicted that each small GTPase activates more than one target, and a cooperation between their different activities is likely to occur during establishment and maintenance of epithelial characteristics. Important questions that are not yet fully understood include the mechanism by which small GTPases are activated, and the steps (Figure 1) in which the different targets play a role.

unpublished data). Interestingly, the initial Rac activation occurs independently of actin polymerisation (M Betson *et al.*, unpublished data). In MDCK cells, adhesion-dependent Rac activation requires phosphatidylinositol 3-kinase (PI3K) function, whereas in keratinocytes it requires epidermal growth factor (EGF) receptor (EGFR) signalling ([23•,24•]; M Betson *et al.*, unpublished data). Unexpectedly, inhibition of either PI3K or EGFR does not block junction assembly, despite preventing Rac activation, as assessed by biochemical pull-down assays ([23•,24•]; M Betson *et al.*, unpublished data [see Update]). Pull-down measures Rac activation using a fusion protein that specifically interacts with and precipitates Rac•GTP present in cell lysates. Perhaps localised Rac activation is still able to occur in the presence of these inhibitors to mediate cell–cell contact formation, and this discrete activation is beyond the sensitivity of pull-down methods. It is also conceivable that alternative Rac-independent pathways exist for actin recruitment to clustered receptors [7,25]. This might compensate for the inhibition of Rac activation and allow stable cadherin adhesion formation. Alternatively, PI3K-dependent and EGFR-dependent Rac activation might be involved in downstream events following induction of new contacts.

It is possible that Cdc42 may act upstream of Rac activation during establishment of adhesive contact in some cell types, as both Rac and Cdc42 activation requires PI3K activity in MDCK cells [23•,24•]. Further experiments are necessary to confirm the hierarchy of these small GTPases during epithelial-junction formation. By contrast, Rho does not appear to be downstream of Rac in the generation

Table 2

Effects of different signalling molecules on tight junction assembly and function.

Activity	Gate function		Fence function: diffusion of		Altered morphology	Perturbed localisation (ZO-1/occludin)	Refs
	Transepithelial resistance	Paracellular flow	Lipids	Proteins			
Activated							
Rho	↓	↑	↑	–	+	+	[45]
Rac	↓	↑	↑	–	+	+	[45]
Cdc42	↓	↑	↑	↑	+	+	[15]
Dominant negative							
Rho	↓	↑	–	–	–	–	[45]
Rac	↓	↑	↑	–	–	–	[45]
Cdc42	↓	↑	–	↑	–	+	[15]
						–	[8,39•,79]
Activated							
PAR6	↓	↑	–	–	+	+	[39•]
Dominant negative							
aPKCλ	↓	↑	↑	↑	+	+	[38••]
Activated							
aPKCζ	ND	ND	ND	ND	ND	+	[39•]

Gate function is evaluated by two different methods: transepithelial resistance (TER, impermeability to ions) and paracellular flow (impermeability to solutes). Fence function is assessed by diffusion of lipids or proteins along the basolateral membrane. Altered tight junction morphology is observed by electron microscopy and the localisation of ZO-1 and occludin by confocal analysis. Arrows (up or down) indicate that there is a change in the parameter after expression of the different molecules. +, altered morphology / staining pattern is observed; –, no alterations seen. ND, not determined. The effects of inhibiting Cdc42 function on ZO-1 and occludin localisation is still unclear. The reasons for these conflicting results are unknown, as the same cell line was used in two of the studies [15,39].

of epithelial morphology in MDCK cells and keratinocytes [26,27]. The activation of Rho following cell–cell contact formation is somewhat controversial. It has been observed in some studies [22•,28] but not in others [22•,23•]. As no investigations using inhibitory antibodies against cadherin have been carried out, it is difficult to distinguish whether cadherin adhesion or Ca^{2+} signalling is necessary for Rho activation. The use of cell lines such as Chinese hamster ovary (CHO) cells and fibroblasts expressing different cadherins [22•] does not address this issue, because the outcome of Rho signalling on junctions differs in distinct cell types [7]. Furthermore, it is not known whether Rho GTPases regulate different cadherin molecules in a similar way. Thus, it is still an open question whether cadherin-mediated adhesion can specifically activate Rho.

How does cadherin-mediated adhesion induce the activation of small GTPases? Experiments performed with different cadherin receptors (vascular endothelial [VE]-cadherin and C-cadherin) suggest that the cadherin cytoplasmic tail is necessary for activation of Rho proteins [22•,29•]. However, no specific domain within the cadherin tail has yet been identified. The function of small GTPases is tightly controlled in a temporal and spatial manner by cytoplasmic proteins (guanine nucleotide exchange factors [GEFs] and GTPase-activating proteins [GAPs]; Figure 2a). After the homophilic binding of cadherins, two possibilities can be envisaged: either the activation of GEFs, or the downregulation of GAPs. Both mechanisms would result in a net increase of GTP-bound proteins (Figure 2a). Alternatively, the two possibilities may occur after cell–cell contact formation. However, the available data are insufficient to support any of these possibilities at the present time.

Cadherin receptors and other adhesive structures

The role of cadherin adhesion is discussed below in relation to the assembly of other junctions and the generation of the epithelial phenotype (Figures 1 and 2b). Early evidence suggests that cadherin-dependent adhesion is essential and a prerequisite for the formation of desmosomes, tight junctions and gap junctions [6]. Three mechanisms have been proposed to explain these observations. First, that the close apposition of membranes when stable cadherin adhesion is induced facilitates the assembly of other junctions. Second, cadherin adhesion provides spatial cues for the correct delivery of junctional components to the membranes. Third, formation of cadherin contacts triggers signalling pathways necessary to promote the assembly of desmosomes and tight junctions. It is likely that all three mechanisms are relevant during formation of epithelial junctions.

Table 3

Effects of cadherin-dependent adhesion and the small GTPases Rho, Rac and Cdc42 on the different cytoskeletal networks.*

Protein / activity	Microfilaments	Refs	Microtubules	Refs	Intermediate filaments	Refs
Cadherin receptors	Reorganisation, actin polymerisation and recruitment to junctions	[6]	Reorganisation, stabilisation of microtubule ends, alteration of microtubule dynamics, localisation and orientation of mitotic spindle	[55••,86–90]	Reorganisation (desmosomes)	[49,50]
Rho	Stress fibres, contraction	[1,2]	Stabilisation, polymerisation orientation	[61•,91]	Collapse (Rho kinase)	[92]
Rac	Lamellae, ruffles, actin polymerisation	[1,2]	?		Collapse	[93]
Cdc42	Filopodia, actin polymerisation	[1,2]	Reorientation of MTOC and mitotic spindle	[41,42,44, 94, 95]	Collapse	[93]

*Please note that not all listed effects were observed in epithelial cells.

The signals that link cadherin adhesion to these cellular processes are not well understood (Figure 2b). It is tempting to speculate that following junction formation, the activation of small GTPases and other signalling pathways plays a role in the targeted delivery of membrane proteins to junctions and also in the polarisation process. In MDCK cells, the Na^+/K^+ATPase pump appears to synergise with cadherin receptors to induce the formation of tight junctions and desmosomes [30•,31]. Interestingly, inhibition of Na^+/K^+ATPase leads to a reduction in Rho•GTP levels. Moreover, Rho activation can rescue the phenotype induced by blocking Na^+/K^+ATPase activity [30]. Published results also suggest the involvement of protein kinase C (PKC) in the assembly of desmosomes and tight junctions, sometimes in the absence of cadherin-dependent adhesion [32–37]. In these studies, however, broad-spectrum PKC inhibitors/activators were used and therefore no specific signalling pathway was identified. Recent data provides supportive evidence, implicating atypical PKC (aPKC) isoforms in the assembly of tight junctions [38•,39•]. Yet, the emerging picture suggests that the coordination between the assembly of adherens junctions, tight junctions and desmosomes may be a more complex system than previously envisaged (see below).

Tight junctions

Tight junctions are formed by the assembly of multi-molecular complexes that form specialised membrane microdomains (reviewed by [40]). Tight junctions have two main functions in epithelial sheets: to render the epithelial sheet impermeable to ions and solutes; and to prevent the mixing between apical and basolateral membrane components (lipids and proteins). Recently, it has been recognised that tight junctions are a hot spot for signalling, with the concerted localisation of Rab small GTPases (which regulate endocytic traffic), PDZ-containing proteins and the Par3–Par6 complex [40]. The latter complex is important as it associates with and recruits to junction signalling molecules, such as aPKC (which binds to Par3) and the small GTPase Cdc42 (which binds to Par6) [40]. The relevance of the Par3–Par6 complex has been shown in single-cell polarisation, apical/basolateral polarity in epithelia and polarity of movement in different organisms [41–44].

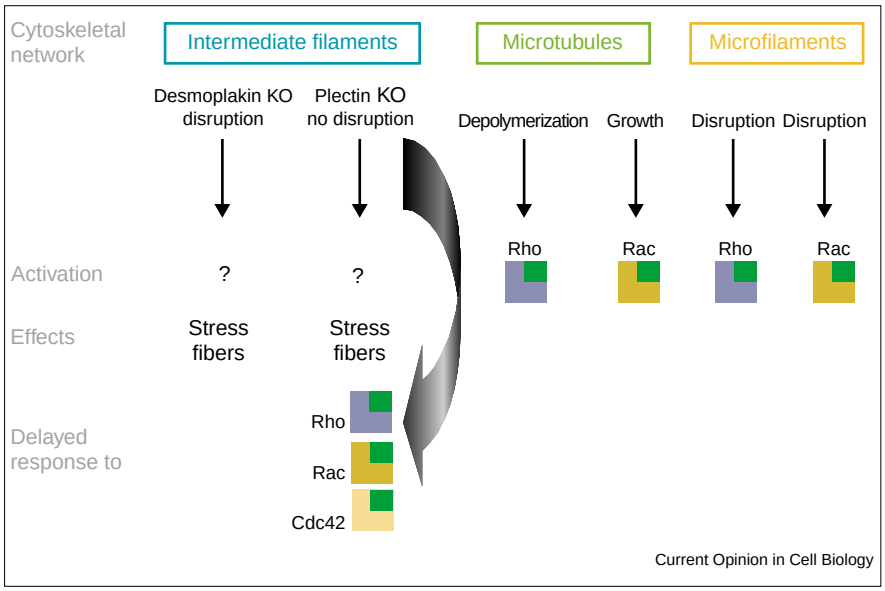
Table 2 summarises the effects of different activities on tight junction assembly and function. Interestingly, these reports suggest that the interference of different signalling pathways with tight junction function occurs primarily during the assembly of tight junctions. One mechanism that regulates tight junction assembly may involve the recruitment of tight junction components to cell–cell adhesive sites. Disruption of the cytoskeleton by activated small GTPases might misplace the spatial cues and perturb correct delivery of the different components. Alternatively, the association of tight junction proteins with the actin cytoskeleton might be regulated; for instance, an increased solubility of ZO-1 is observed after expression of activated RhoA [45]. One interesting possibility is that transient and localised activation of Rho small GTPases, or inactivation of aPKCλ, might regulate the 'leakiness' of tight junctions.

It seems clear that the activation of small GTPases is much more disruptive to tight junction assembly and function than to their inhibition (Table 2). In particular, Cdc42 and Par6 activation appear to negatively regulate tight junction formation and function, although the relevant mechanism is unknown [39]. Thus, it seems contradictory that cadherin-dependent activation of Rho GTPases may play a role in the assembly of tight junctions. However, a detailed analysis of the localised activation of small GTPases following cadherin adhesion, desmosomes and tight junction formation has not yet been performed. The use of different techniques may help to identify the temporal and spatial control of the function of Rho proteins during junction assembly (e.g. fluorescence resonance energy transfer [FRET] and fluorescence recovery after photobleaching [FRAP] [46]). These results may clarify the signalling events that link the formation of the different adhesive structures.

Crosstalk between cytoskeletal networks

Crosstalk between cytoskeletal networks is important for many different cellular processes [47,48•]. Interestingly, the presence of cell–cell contacts induces profound changes in the organisation and dynamics of actin, microtubule and keratin filaments (Table 3). Projections of microtubules and keratin filaments are seen in nascent cell–cell contacts [49–51•],

Figure 3



Crosstalk between small GTPase activity and the organisation of intermediate filaments, microtubules and microfilaments. Disruption of intermediate filaments is observed in desmoplakin knockout cells, but not in plectin knockout cells [52•,59]. In both knockout cells, activation of specific GTPases has not been evaluated, but increased production of stress fibres is observed [52•,59]. In plectin$^{-/-}$ cells, a delayed response to small GTPases is also observed following treatment with growth factors and other stimuli [60]. Disruption of the microfilaments and depolymerisation and growth of the microtubules by specific drugs leads to the activation of Rho and Rac, as assessed by pull-down assays ([80,81]; M Betson *et al.* unpublished data). Activation of Cdc42 has not been assessed following disruption of the different filaments.

and new desmosomes are found interspersed between newly formed adherens junctions [52•,53]. In addition, in polarised MDCK cells, some microtubule filaments are not focused on the centrosome, but run in parallel to junctions, with important consequences for microtubule-dynamics regulation [47,54,55].

What are the mechanisms by which cell–cell adhesion can coordinate all three cytoskeletal networks? Two possibilities can be envisaged. First, there is a growing number of proteins that act as bridges between microtubules and intermediate filaments [56], microtubule and microfilaments [51,57,58], or actin and intermediate filaments [59,60]. The majority of these proteins localise at junctions in epithelia. Second, there is evidence to suggest an involvement of Rho small GTPases and their targets with microtubules and intermediate filaments.

The functional connection between small GTPases and microtubules occurs at two levels that are intrinsically related to each other: binding, and regulation of their activity. Several Rac and Rho targets can associate with microtubules [61–64], as can different GEFs [65–70]. Three mechanisms have been proposed for the regulation of the availability of signalling molecules by microtubules [47]: sequestration and release (direct interaction), delivery via a motor protein, and interaction via a scaffolding protein. In all three scenarios, the release of the associated signalling molecules from the microtubule network would involve modification of the microtubules or the signalling protein. Alternatively, enhanced microtubule depolymerisation [47] may also release associated molecules.

The regulation of small GTPase activity by microtubules occurs in a dual way: Rho GTPase activation interferes with microtubule dynamics and *vice versa* (Table 3, Figure 3). Indeed, the binding of a Rac target to microtubules can stabilise the filaments [63], and the interaction of GEF-H1 (a Rac exchange factor) with microtubules results in its inactivation [70]. Conversely, disruption of either microtubule or microfilament network results in the activation of Rac and Rho ([80,81]; M Betson *et al.*, unpublished data [see Update]). Given these results, one interesting question is whether the cadherin-dependent activation of Rho proteins requires changes in microtubule dynamics. Two possibilities can be envisaged. The formation of new cadherin contacts may lead to the activation of small GTPases, which would then induce changes in microtubule reorganisation and dynamics. Alternatively, cadherin-dependent adhesion could induce changes in the microtubule network, which in turn may release and/or modify associated GEFs, facilitating the activation of small GTPases. Although these two possibilities are not mutually exclusive, future experiments are needed to address the mechanism of interplay between cadherin adhesion, microtubules and Rho GTPases.

So far, circumstantial data suggest that the involvement of small GTPases in the reorganisation of intermediate filaments is likely. In addition to the evidence listed in Table 3 and illustrated in Figure 3, keratins can be phosphorylated by the stress-activated kinase p38 [71,72], which is activated by Rac-dependent signalling pathways. Similarly, Rho kinase, a Rho target, can phosphorylate intermediate filaments in fibroblasts [73]. A functional consequence of the phosphorylation of the intermediate filaments keratin and vimentin is their stabilisation. Thus, small GTPase signalling can potentially influence the organisation and dynamics of this cytoskeletal network. Together, these results suggest that a reciprocal and fine

balance exists between the organisation of cytoskeletal networks and the regulation of Rho GTPase activity. Further experiments will shed light upon the mechanisms of this interesting relationship.

Conclusions

Desmosomes, adherens and tight junctions are now considered highly dynamic macromolecular complexes that integrate signalling processes with the cytoskeletal network. Specific signalling pathways have been identified that coordinate the assembly of the different adhesive structures and regulate their stability and function. The activation of small GTPases by cadherin adhesion and the identification of specific targets of the signalling cascade will open up important avenues for future investigation. Most importantly, further insights into the mechanistic details of how Rho proteins and other signalling molecules cooperate to generate the epithelial phenotype will be welcomed (Figure 1). Particularly exciting is the discovery that small GTPases and cadherin-mediated adhesion can influence the dynamics and organisation of actin, microtubule and intermediate filaments. A considerable amount of crosstalk exists among the different types of filaments, and small GTPases are situated on the crossroads between them. Taken together, these results shift our perception to a more global view, in which the function of adhesive structures and the outcome of specific signalling pathways are integrated with the overall cellular organisation.

Update

In addition to references *22* and *23* and the work by M Betson *et al.* (unpublished data [now in press, see below]), Kovacs *et al.* [96] demonstrated that Rac is activated when E-cadherin-expressing CHO cells spread onto E-cadherin extracellular domains. Interestingly, at early time points, Rac activation occurs independently of PI3K. Expression of PI3K was necessary for Rac activation at later time points, suggesting that different mechanisms might exist to enhance Rac•GTP levels following cadherin homophilic binding. The work by M. Betson *et al.* is now in press [97].

Acknowledgements

I would like to thank M Seabra and M Betson for critically reading the manuscript. Work in my laboratory is funded by Cancer Research UK and the Medical Research Council (MRC Senior Fellowship).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Kjoller L, Hall A: Signalling to Rho GTPases. *Exp Cell Res* 1999, 253:166-179.

2. Van Aelst L, D'Souza-Schorey C: Rho GTPases and signaling networks. *Genes Dev* 1997, 11:2295-2322.

3. •• Braga VMM: Epithelial cell shape: cadherins and small GTPases. *Exp Cell Res* 2000, 261:83-90.
This review discusses the current model of how junctions are formed, focusing in particular on the contribution of small GTPase signalling pathways and their specific targets.

4. • Lozano E, Betson M, Braga VMM: Tumour progression: small GTPases and loss of cell–cell adhesion. *Bioessays* 2002, in press.
A comprehensive review of the mechanisms that lead to the disassembly of cadherin-dependent adhesion during tumourigenesis, from genetic defects to signalling events.

5. Fukata M, Kaibuchi K: Rho-family GTPases in cadherin-mediated cell–cell adhesion. *Nat Rev Mol Cell Biol* 2001, 2:887-897.

6. Yap AS, Brieher WM, Gumbiner BM: Molecular and functional analysis of cadherin-based adherens junctions. *Annu Rev Cell Dev Biol* 1997, 13:119-146.

7. Braga VMM, Del Maschio A, Machesky LM, Dejana E: Regulation of cadherin function by Rho and Rac: modulation by junction maturation and cellular context. *Mol Biol Cell* 1999, 10:9-22.

8. Takaishi K, Sasaki T, Kotani H, Nishioka H, Takai Y: Regulation of cell–cell adhesion by Rac and Rho small G proteins in MDCK cells. *J Cell Biol* 1997, 139:1047-1059.

9. • Sahai E, Marshall CJ: ROCK and Dia have opposing effects on adherens junctions downstream of Rho. *Nat Cell Biol* 2002, 4:408-415.
Inhibition of RhoA results in a reduced association of α-catenin with cadherin, thereby compromising the interaction of the complexes with the actin cytoskeleton. This is the first report to show an effect of Rho inhibition of the composition of the cadherin complex.

10. Braga VMM, Najabagheri N, Watt FM: Calcium-induced intercellular adhesion of keratinocytes does not involve accumulation of β1 integrins at cell-cell contact sites and does not involve changes in the levels or phosphorylation of the catenins. *Cell Ad Com* 1998, 5:137-149.

11. Hinck L, Nathke IS, Papkoff J, Nelson WJ: Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. *J Cell Biol* 1994, 125:1327-1340.

12. Le TL, Yap AS, Stow JL: Recycling of E-cadherin: a potential mechanism for regulating cadherin dynamics. *J Cell Biol* 1999, 146:219-232.

13. Jou T-S, Leung S-M, Fung LM, Ruiz WG, Nelson WJ, Apodaca G: Selective alterations in biosynthetic and endocytic protein traffic in Madin–Darby canine epithelial cells expressing mutants of the small GTPase Rac. *Mol Biol Cell* 2000, 11:287-304.

14. Leung SM, Rojas R, Maples C, Flynn C, Ruiz WG, Jou TS, Apodaca G: Modulation of endocytic traffic in polarized Madin–Darby canine kidney cells by the small GTPase RhoA. *Mol Biol Cell* 1999, 10:4369-4384.

15. Rojas R, Ruiz WG, Leung S-M, Jou T-S, Apodaca G: Cdc42-dependent modulation of tight junctions and membrane protein traffic in polarized Madin-Darby Canine Kidney Cells. *Mol Biol Cell* 2001, 12:2257-2274.

16. •• Palacios F, Price L, Schweitzer J, Collard JG, D'Souza-Schorey C: An essential role for ARF6-regulated membrane traffic in adherens junction turnover and epithelial cell migration. *EMBO J* 2001, 20:4973-4986.
This study reveals the specific effects of ARF6 activation on cadherin receptors. ARF6 activation results in cadherin receptor internalisation and junction disassembly. ARF6 exerts its role downstream of hepatocyte growth factor (HGF) and Src activation. Interestingly, junction destabilisation occurs independently of actin remodelling and lamellipodia formation. These results suggest that ARF6 is most likely to affect cadherin adhesion by interfering with receptor endocytosis.

17. • Matsui T, Kinoshita T, Morikawa Y, Tohya K, Katsuki M, Ito Y, Kamiya A, Miyajima A: K-Ras mediates cytokine-induced formation of E-cadherin-based adherens junctions during liver development. *EMBO J* 2002, 21:1021-1030.
This is the first demonstration that K-Ras, but not H-Ras or N-Ras, has a positive effect on junction formation. This is opposed to H-Ras, whose activation destabilises junctions in epithelia. This report is interesting because of the high homology and similar targets observed for Ras family members, yet the outcome during junction assembly is very distinct.

18. Braga VMM, Betson M, Li X, Lamarche-Vane N: Activation of the small GTPase Rac is sufficient to disrupt cadherin-dependent cell–cell adhesion in normal human keratinocytes. *Mol Biol Cell* 2000, 11:3703-3721.

19. Asha H, de Ruiter ND, Wang M, Hariharan IK: The Rap1 GTPase functions as a regulator of morphogenesis ***in vivo.*** *EMBO J* 1999, 18:605-615.

20. Knox AL, Brown NH: Rap1 GTPase regulation of adherens junction
• positioning and cell adhesion. *Science* 2002, 295:1285-1288.
This is the first demonstration that Rap proteins might play a role in junction organisation, without affecting the localisation of cadherins and catenins.

21. Bos J, de Rooij J, Reedquist K: Rap1 signalling: adhering to new models. *Nat Rev Mol Cell Biol* 2001, 2:369-377.

22. Noren NK, Niessen CM, Gumbiner BM, Burridge K: Cadherin
• engagement regulates Rho family GTPases. *J Biol Chem* 2001, 276:33305-33308.
The effect of cadherin adhesion on the activation of Rho, Rac and Cdc42 are reported in different cell lines. The effects of cell confluence on small GTPase activation are also reported. However, conflicting results arise from the use of different cell lines.

23. Nakagawa M, Fukata M, Yamaga M, Itoh N, Kaibuchi K: Recruitment
• and activation of Rac1 by the formation of E-cadherin-mediated cell–cell adhesion sites. *J Cell Sci* 2001, 114:1829-1838.
This study demonstrates a specific activation of Rac by cadherin-dependent adhesion, in a phosphatidylinositol 3-kinase-dependent manner. No activation of Rho is observed under these experimental conditions.

24. Kim SH, Zhigang L, Sacks DB: E-cadherin-mediated cell–cell
• attachment activates Cdc42. *J Biol Chem* 2000, 275:36999-37005.
Cdc42 activation is observed in a PI3 kinase- and E-cadherin-dependent manner.

25. Hoffmeister KM, Falet H, Toker A, Barkalow KL, Stossel TP, Hartwig JH: Mechanisms of cold-induced platelet actin assembly. *J Biol Chem* 2001, 276:24751-24759.

26. Braga VMM, Machesky LM, Hall A, Hotchin NA: The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts. *J Cell Biol* 1997, 137:1421-1431.

27. Jou TS, Nelson WJ: Effects of regulated expression of mutant RhoA and Rac1 small GTPases on the development of epithelial (MDCK) cell polarity. *J Cell Biol* 1998, 142:85-100.

28. Calautti E, Grossi M, Mammucari C, Aoyama Y, Pirro M, Ono Y, Li J, Dotto GP: Fyn tyrosine kinase is a downstream mediator of Rho/PRK2 function in keratinocyte cell-cell adhesion. *J Cell Biol* 2002, 156:137-148.

29. Lampugnani MG, Zanetti A, Breviario F, Balconi G, Orsenigo F,
•• Corada M, Spagnuolo R, Betson M, Braga V, Dejana E: VE-Cadherin regulates endothelial actin activating Rac and increasing membrane association of Tiam. *Mol Biol Cell* 2002, 13:1175-1189.
The expression of VE-cadherin in VE-cadherin null cells results in Rac activation, and Rho inhibition. Interestingly, there is a remarkable induction of stress-fibres, which is not dependent on Rho function.

30. Rajasekaran SA, Palmer LG, Moon SY, Peralta Soler A, Apodaca GL,
• Harper JF, Zheng Y, Rajasekaran AK: Na^+,K^+-ATPase activity is required for formation of tight junctions, desmosomes, and induction of polarity in epithelial cells. *Mol Biol Cell* 2001, 12:3717-3732.
Blocking Na^+/K^+ ATPase leads to inhibition of Rho activity, and prevents the formation of desmosomes and tight junctions.

31. Rajasekaran SA, Palmer LG, Quan K, Harper JF, Ball WJJ, Bander NH, Soler AP, Rajasekaran AK: Na^+,K^+-ATPase β-subunit is required for epithelial polarization, suppression of invasion, and cell motility. *Mol Biol Cell* 2001, 12:279-295.

32. Balda MS, Gonzalez-Mariscal L, Matter K, Cereijido M, Anderson JM: Assembly of the tight junction: the role of diacylglycerol. *J Cell Biol* 1993, 123:293-302.

33. van Hengel J, Gohon L, Bruyneel E, Vermeulen S, Cornelissen M, Mareel M, van Roy F: Protein kinase C activation upregulates intercellular adhesion of α-catenin-negative human colon cancer variants via induction of desmosomes. *J Cell Biol* 1997, 137:1103-1116.

34. Citi S, Volberg T, Bershadsky AD, Denisenko N, Geiger B: Cytoskeletal involvement in the modulation of cell-cell junctions by the protein kinase inhibitor H-7. *J Cell Sci* 1994, 107:683-692.

35. Lewis JE, Jensen PJ, Johnson KR, Wheelock MJ: E-cadherin mediates adherens junction organization through protein kinase C. *J Cell Sci* 1995, 108:3615-3621.

36. Sheu H-M, Kitajima Y, Yaoita H: Involvement of protein kinase C in translocation of desmoplakins from cytosol to plasma membrane during desmosome formation in human squamous carcinoma cells grown in low to normal calcium medium. *Exp Cell Res* 1989, 185:176-190.

37. Denisenko N, Burighel P, Citi S: Different effects of protein kinase inhibitors on the localization of junctional proteins at cell-cell contact sites. *J Cell Sci* 1994, 107:969-981.

38. Suzuki A, Yamanaka T, Hirose T, Manabe N, Mizuno K, Shimizu M,
•• Akimoto K, Izumi Y, Ohnishi T, Ohno S: Atypical protein kinase C is involved in the evolutionarily conserved PAR protein complex and plays a critical role in establishing epithelia-specific junctional structures. *J Cell Biol* 2001, 152:1183-1196.
An interesting study, in which the role of atypical protein kinase Cλ during tight-junction assembly and function is investigated using a dominant-negative approach. As no drug treatments were used, this is the first report in which a specific role for atypical protein kinase C was clearly observed.

39. Gao L, Joberty G, Macara IG: Assembly of epithelial tight junctions
• is negatively regulated by Par6. *Curr Biol* 2002, 12:221-225.
The authors present the unexpected result that Par6 activation appears to inhibit tight junction morphology and function.

40. Zahraoui A, Louvard D, Galli T: Tight junction, a platform for trafficking and signaling complexes. *J Cell Biol* 2000, 151:F31-F36.

41. Gotta M, Abraham MC, Ahringer J: CDC-42 controls early cell polarity and spindle orientation in ***C. elegans.*** *Curr Biol* 2001, 11:482-488.

42. Kay A, Hunter CP: CDC-42 regulates PAR protein localization and function to control cellular and embryonic polarity in ***C. elegans.*** *Curr Biol* 2001, 11:474-481.

43. Wodarz A, Ramrath A, Grimm A, Knust E: ***Drosophila*** atypical protein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts. *J Cell Biol* 2000, 150:1361-1374.

44. Etienne-Manneville S, Hall A: Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCζ. *Cell* 2001, 106:489-496.

45. Jou TS, Schneeberger EE, Nelson WJ: Structural and functional regulation of tight junctions by RhoA and Rac1 small GTPases. *J Cell Biol* 1998, 142:101-115.

46. Roy P, Rafjur Z, Pomorsli P, Jacobson K: Microscope-based techniques to study cell adhesion and migration. *Nat Cell Biol* 2002, 4:E91-E96.

47. Gundersen GG, Cook TA: Microtubules and signal transduction. *Curr Opin Cell Biol* 1999, 11:81-94.

48. Fuchs E, Karakesisoglou I: Bridging cytoskeletal interactions.
• *Genes Dev* 2001, 15:1-14.
This and the paper by Gundersen and Cook (1999) [47] are excellent reviews on the crosstalk between different cytoskeletal filaments, which focuses on proteins that can interact with more than one type of filament. The reviews also emphasise the cellular processes in which this crosstalk is relevant.

49. Green KJ, Geiger B, Jones JCR, Talian JC, Goldman RD: The relationship between intermediate filaments and microfilaments before and during the formation of desmosomes and adherens-type junctions in mouse epidermal cells. *J Cell Biol* 1987, 104:1389-1402.

50. Zamansky GB, Nguyen U, Chou I-N: An immunofluorescence study of the calcium-induced coordinated reorganization of microfilaments, keratin intermediate filaments, and microtubules in cultured human epidermal keratinocytes. *J Invest Dermatol* 1991, 97:985-994.

51. Ligon LA, Karki S, Tokito M, Holzbaur ELF: Dynein binds to β-catenin
• and may tether microtubules at adherens junctions. *Nat Cell Biol* 2001, 3:913-917.
The authors describe a novel mechanism for the capturing and tethering of microtubules at adhesive cadherin complexes, and provide an example of crosstalk with the actin cytoskeleton.

52. Vasioukhin V, Bowers E, Bauer C, Degenstein L, Fuchs E:
• Desmoplakin is essential in epidermal sheet formation. *Nat Cell Biol* 2001, 3:1076-1085.
This is an interesting paper, in which the inhibition of desmosome formation in desmoplakin knockout epidermis leads to reduced adherens junction assembly and increased stress fibre formation.

53. Vasioukhin V, Bauer C, Yin M, Fuchs E: Directed actin polymerization is the driving force for epithelial cell–cell adhesion. *Cell* 2000, 100:209-219.

54. Bacallao R, Antony C, Dotti C, Karsenti E, Stelzer E, Simons K: The subcellular organization of Madin–Darby canine kidney cells

during the formation of a polarized epithelium. *J Cell Biol* 1989, 109:2817-2832.

55. •• Chausovsky A, Bershadsky AD, Borisy GG: Cadherin-mediated regulation of microtubule dynamics. *Nat Cell Biol* 2000, 2:797-804.
This is an interesting paper, in which N-cadherin cell–cell adhesion is shown to have specific effects on microtubule organisation and dynamics. The authors show that following N-cadherin clustering, microtubules are stabilised.

56. Liao G, Gundersen GG: Kinesin is a candidate for cross-bridging microtubules and intermediate filaments. Selective binding of kinesin to detyrosinated tubulin and vimentin. *J Biol Chem* 1998, 273:9797-9803.

57. Rosin-Arbesfeld R, Ihrke G, Bienz M: Actin-dependent membrane association of the APC tumour suppressor in polarized mammalian epithelial cells. *EMBO J* 2001, 20:5929-5939.

58. Munemitsu S, Souza B, Muller O, Albert I, Rubinfeld B, Polakis P: The APC gene product interacts with microtubules ***in vivo*** and promotes their assembly ***in vitro.*** *Cancer Res* 1994, 54:3676-3681.

59. Andra K, Nikolic B, Stocher M, Drenckhahn D, Wiche G: Not just scaffolding: plectin regulates actin dynamics in cultured cells. *Genes Dev* 1998, 12:3442-3451.

60. Karakesisoglou I, Yang Y, Fuchs E: An epidermal plakin that integrates actin and microtubule networks at cellular junctions. *J Cell Biol* 2000, 149:195-208.

61. • Palazzo AF, Cook TA, Alberts AS, Gundersen GG: mDia mediates Rho-regulated formation and orientation of stable microtubules. *Nat Cell Biol* 2001, 3:723-729.
mDia expression is sufficient to generate and orient stable microtubules, whereas Rok-α expression generates perinuclear microtubules. This mDia role on microtubules is likely to be important during epithelia polarisation.

62. Nagata K-I, Puls A, Futter C, Aspenstrom P, Schaefer E, Nakata T, Hirokawa N, Hall A: The MAP kinase kinase MLK2 co-localizes with activated JNK along microtubules and associates with kinesin superfamily motor KIF3. *EMBO J* 1998, 17:149-158.

63. Cau J, Faure S, Comps M, Delsert C, Morin N: A novel p21-activated kinase binds the actin and microtubule networks and induces microtubule stabilization. *J Cell Biol* 2001, 155:1029-1042.

64. • Daub H, Gevaert K, Vandekerckhove J, Sobel A, Hall A: Rac/Cdc42 and p65PAK regulate the microtubule-destabilizing protein stathmin through phosphorylation at serine 16. *J Biol Chem* 2001, 276:1677-1680.
The authors propose a mechanism for the stabilisation of microtubules that results from Rac/Cdc42 activation.

65. Ren Y, Li R, Zheng Y, Busch H: Cloning and characterization of GEF-H1, a microtubule-associated guanine nucleotide exchange factor for Rac and Rho GTPases. *J Biol Chem* 1998, 273:34954-34960.

66. Glaven JA, Whitehead I, Bagrodia S, Kay R, Cerione RA: The Dbl-related protein, Lfc, localizes to microtubules and mediates the activation of Rac signaling pathways in cells. *J Biol Chem* 1999, 274:2279-2285.

67. van Horck FPG, Ahmadian MR, Haeusler LC, Moolenaar WH, Kranenburg O: Characterization of p190Rho GEF, a RhoA-specific guanine nucleotide exchange factor that interacts with microtubules. *J Biol Chem* 2001, 276:4948-4956.

68. Tatsumoto T, Xie X, Blumenthal R, Okamoto I, Miki T: Human ECT2 is an exchange factor for Rho GTPases, phosphorylated in G2/M phases, and involved in cytokinesis. *J Cell Biol* 1999, 147:921-928.

69. Huby RDJ, Carlile GW, Ley SC: Interactions between the protein tyrosine kinase ZAP-70, the proto-oncoprotein Vav, and tubulin in Jurkat T cells. *J Biol Chem* 1995, 270:30241-30244.

70. •• Krendel M, Zenke FT, Bokosh GM: Nucleotide exchange factor GEF-H1 mediates crosstalk between microtubules and the actin cytoskeleton. *Nat Cell Biol* 2002, 4:294-301.
This study demonstrates that the association of GEF-H1 with microtubules leads to the inhibition of its exchange activity, thereby preventing the activation of Rho and the induction of stress fibre formation.

71. He T, Stepulak A, Holmstrom TH, Omary MB, Eriksson JE: The intermediate filament protein keratin 8 is a novel cytoplasmic substrate for c-Jun N-terminal kinase. *J Biol Chem* 2002, 277:10767-10774.

72. Ku N-O, Azhar S, Omary MB: Keratin 8 phosphorylation by p38 kinase regulates cellular keratin filament reorganization. Modulation by a keratin-1 like disease-causing mutation. *J Biol Chem* 2002, 277:10775-10782.

73. Inada H, Togashi H, Nakamura Y, Kaibuchi K, Nagata K, Inagaki M: Balance between activities of Rho kinase and type 1 protein phosphatase modulates turnover of phosphorylation and dynamics of desmin/vimentin filaments. *J Biol Chem* 1999, 274:34932-34939.

74. Braga VMM, Hodivala KJ, Watt FM: Calcium-induced changes in distribution and solubility of cadherins and their associated cytoplasmic proteins in human keratinocytes. *Cell Adhesion Com* 1995, 3:201-215.

75. Clayton L, Hall A, Johnson MH: A role for the Rho-like GTPases in the polarisation of mouse eight-cell blastomeres. *Dev Biol* 1999, 205:322-331.

76. • Lambert M, Choquet D, Mege R-M: Dynamics of ligand-induced, Rac1-dependent anchoring of cadherins to the actin cytoskeleton. *J Cell Biol* 2002, 157:469-479.
Using optical tweezers and beads coated with N-cadherin extracellular domains, the authors demonstrate that cadherin binding to the actin cytoskeleton is density-dependent and also requires Rac1, but not Cdc42, activity.

77. Kodama A, Takaishi K, Nakano K, Nishioka H, Takai Y: Involvement of Cdc42 small G protein in cell–cell adhesion, migration and morphology of MDCK cells. *Oncogene* 1998, 18:3996-4006.

78. Stoffler HE, Honnert U, Bauer CA, Hofer D, Schwarz H, Muller RT, Dreckhahn D, Bahler M: Targeting of the myosin-I to intercellular adherens type junctions induced by dominant active Cdc42 in Hela cells. *J Cell Sci* 1998, 111:2779-2788.

79. Kroschewski R, Hall A, Mellman I: Cdc42 controls secretory and endocytic transport to the basolateral plasma membrane of MDCK cells. *Nat Cell Biol* 1999, 1:8-13.

80. Waterman-Storer CM, Worthylake RA, Liu BP, Burridge K, Salmon ED: Microtubule growth activates Rac1 to promote lamellipodial protrusion in fibroblasts. *Nat Cell Biol* 1999, 1:45-50.

81. Ren X-D, Kiosses WB, Schwartz MA: Regulation of the small GTPase-binding protein Rho by cell adhesion and the cytoskeleton. *EMBO J* 1999, 18:578-585.

82. • Pece S, Gutkin JS: Signaling from E-cadherins to the MAPK pathway by the recruitment and activation of epidermal growth factor receptors upon cell–cell contact formation. *J Biol Chem* 2000, 275:41227-41233.
This study shows the specific activation of mitogen activated protein kinase (MAPK) and epidermal growth factor receptor by cadherin-dependent adhesion, and points out a possible link between cell–cell contacts and growth control.

83. Pece S, Chiariello M, Murga C, Gutkind JS: Activation of the protein kinase Akt/PKB by the formation of E-cadherin-mediated cell–cell junctions. *J Biol Chem* 1999, 274:19347-19351.

84. Laprise P, Chailler P, Houde M, Beaulieu J-F, Boucher M-J, Rivard N: Phosphatidylinositol 3-kinase controls human intestinal epithelial cell differentiation by promoting adherens junction assembly and p38 MAPK activation. *J Biol Chem* 2002, 277:8226-8234.

85. Shinohara M, Kodama A, Matozaki T, Fukuhara A, Tachibana K, Nakanishi H, Takai Y: Roles of cell–cell adhesion-dependent tyrosine phosphorylation of Gab-1. *J Biol Chem* 2001, 276:18941-18946.

86. Pepperbrock R, Bre MH, Davoust J, Kreis TE: Microtubules are stabilized in confluent epithelial cells but not in fibroblasts. *J Cell Biol* 1990, 111:3003-3012.

87. •• Waterman-Storer C, Salmon WC, Salmon ED: Feedback interactions between cell–cell adherens junctions and cytoskeletal dynamics in newt lung epithelial cells. *Mol Biol Cell* 2000, 11:2471-2483.
The data presented in this paper suggest that a microtubule plus end capping protein is regulated by cell–cell contacts, leading to microtubule stabilisation.

88. Le Borgne R, Bellaiche Y, Schweisguth F: ***Drosophila*** E-cadherin regulates the orientation of asymmetric cell division in the sensory organ lineage. *Curr Biol* 2002, 12:95-104.

89. Lu B, Roegiers F, Jan LY, Jan YN: Adherens junctions inhibit asymmetric division in the ***Drosophila*** epithelium. *Nature* 2001, 409:522-525.

90. Bre M, Pepperkok R, Hill A, Levilliers N, Ansorge W, Stelzer E, Karsenti E: Regulation of microtubule dynamics and nucleation during polarization in MDCK II cells. *J Cell Biol* 1990, 111:3013-3021.

91. Cook TA, Nagasaki T, Gundersen GG: Rho guanosine triphosphatase mediates the selective stabilization of microtubules induced by lysophosphatidic acid. *J Cell Biol* 1998, 141:175-185.

92. Sin W, Chen X, Leung T, Lim L: RhoA-binding kinase translocation is facilitated by the collapse of the vimentin intermediate filament network. *Mol Cell Biol* 1998, 18:6325-6339.

93. Meriane M, Mary S, Comunale F, Vignal E, Fort P, Gauthier-Rouviere C: Cdc42Hs and Rac1 GTPases induce the collapse of the vimentin intermediate filament network. *J Biol Chem* 2000, 275:33046-33052.

94. Nobes CD, Hall A: Rho GTPases control polarity, protrusion, and adhesion during cell movement. *J Cell Biol* 1999, 144:1235-1244.

95. Palazzo AF, Joseph HL, Chen YJ, Dujardin DL, Alberts AS, Pfister KK, Vallee RB, Gundersen GG: Cdc42, dynein, and dynactin regulate MTOC reorientation independent of Rho-regulated microtubule stabilization. *Curr Biol* 2001, 11:1536-1541.

96. Kovacs EM, Ali RG, McCormack AJ, Yap AS: E-cadherin homophilic ligation directly signals through Rac and phosphatidylinositol 3-kinase to regulate adhesive contacts. *J Biol Chem* 2002, 277:6708-6718.

97. Betson M, Lozano E, Zhang J-K, Braga VMM: Rac activation upon cell–cell contact formation is dependent on signaling from the epidermal growth factor receptor. *J Biol Chem* 2002, in press.