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The challenges of abundance: epithelial junctions and small GTPase signalling

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Small GTPases of the Ras superfamily play critical roles in epithelial biogenesis. Many key morphogenetic functions occur when small GTPases act at epithelial junctions, where they mediate an increasingly complex interplay between cell–cell adhesion molecules and fundamental cellular processes, such as cytoskeletal activity, polarity and trafficking. Important recent advances in this field include the role of additional members of the Ras superfamily in cell–cell contact stability and the capacity for polarity determinants to regulate small GTPase signalling. Interestingly, small GTPases may participate in the cross-talk between different adhesive receptors: in tissues classical cadherins can selectively regulate other junctions through cell signalling rather than through a global influence on cell–cell cohesion.

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Introduction

In this review we will examine recent developments that link cellular junctions, GTPase signalling and cellular polarity during epithelial biogenesis. Epithelia form the physical barriers that separate the internal milieu of the body from its external environment, while selectively regulating the passage of fluids, macromolecules and solutes between these compartments. The biogenesis of functional epithelia requires precise coordination of many cellular processes [1]. For example, simple epithelia must polarize to establish a differential distribution of cell surface proteins and lipids, and reorganize the cytoskeleton and vesicular trafficking networks to coordinate polarized cellular transport. They must also assemble a series of specialized cell–cell junctions responsible for cohesion and paracellular permselectivity. This complexity is likely to be amplified in stratified epithelia such as the skin, where multiple layers of cells assemble on top of each other. A fundamental issue, then, is to understand how these diverse cellular processes are coordinated. An important emerging theme is that such coordination is mediated by cell signals activated as cells make contact with one another.

Here we focus on the signalling pathways triggered by assembly of cell–cell adhesion, in particular the activation of small GTPases. We discuss how these signalling pathways are activated and how different pathways must be coordinated spatially and temporally during epithelial biogenesis. It is evident that this is an exceedingly complex area, one where there are few clear or simple answers. Accordingly, we aim to highlight key issues that confront the field, in the hope that this might stimulate further discussion and research.

From contact to epithelialization: cell–cell signals involved in biogenesis

A key event in epithelial polarization and biogenesis is the establishment of E-cadherin-dependent cell–cell contacts [2]. Additional adhesive mechanisms, such as those provided by nectins and junctional adhesion molecules (JAMs), may play a role in facilitating the initial transient cadherin contacts by bringing membranes into apposition and/or forming signalling complexes [3,4,5•,6]. However, cadherin-independent adhesion is usually not sufficient to induce morphological changes or the formation of other adhesive structures (i.e. tight junctions or desmosomes).

A simple and common notion is that cadherin adhesion serves as a master regulator by simply bringing cell surfaces together, thereby allowing other junctions to assemble and polarity complexes to exert their effects. That cadherins may exert a much more selective influence on epithelial biogenesis, however, is suggested by two lines of evidence. First, upon PKC activation, desmosomes and tight junctions can assemble in the absence of cadherin adhesion, suggesting that signalling triggered by cadherins may impinge on the macromolecular assembly of additional adhesive sites [7–10]. Second, mice bearing a conditional knock-out of E-cadherin in the skin died shortly after birth from severe cutaneous water loss because they lacked functioning tight junctions [11••]. Interestingly, the overall integrity of the skin remained intact, probably as a result of compensation by P-cadherin and desmosomes. Therefore, in this stratified epithelium

E-cadherin is not essential for cell–cell cohesion *in vivo*, but rather is specifically required for tight junction assembly, perhaps, as the authors suggest, through cell signalling. These examples highlight the capacity of adhesion receptors to selectively regulate specific processes during epithelial biogenesis.

In the search for signals that allow cell–cell contact to regulate such diverse cellular processes, many studies have focused on the small GTPases of the Ras superfamily. These are particularly good candidates as they regulate many different cellular processes important for epithelial biogenesis, including adhesion, cytoskeleton dynamics, trafficking and differentiation [12]. One attractive paradigm envisages that cell surface receptors activate GTPase signalling when engaged by their ligands at sites of cell–cell contact. Such receptors probably include adhesion molecules, as well as potential juxtacrine signals that require adhesion to bring them into contact with surface-bound ligands. Indeed, cadherins, nectins and JAMs are able to modulate different signalling pathways and recruit polarity determinants.

The 'canonical' Rho-family GTPases, notably Rac and Rho, were the first to be identified as being essential for, and activated by, cadherin adhesion (reviewed in [12,13]). More recently, Rap1, another member of the Ras superfamily, was shown to be activated by homophilic E-cadherin ligation [14••,15•]. These novel results suggest that more GTPases may be involved in cell–cell adhesion signalling than was previously recognized.

Activation of small GTPases by cell–cell adhesion: patterns and paradoxes

Several salient aspects of GTPase activation by cell–cell adhesion receptors are summarized in Table 1. While many subtleties may be glossed over, three major points emerge from this summary that raise important questions for further investigation.

First, a single receptor can activate multiple small GTPases. Are these pathways activated independently or as a cascade of signalling events from a single receptor? Examples of both situations are described in the literature. E-cadherin-dependent adhesion may activate Rac and Rho independently [16,17], whereas nectin adhesion sequentially activates Rap1, Cdc42 and Rac [3,18,19•,20••]. Cdc42 activation also requires prior activation of Rap1 following either E-cadherin or nectin adhesion [3,14••]. The precise upstream activation pathways used by different adhesion

Table 1

Activation of small GTPases by cell–cell adhesion receptors.

Activation	E-cadherin	VE-cadherin	R-cadherin	C-cadherin	N-cadherin	Nectin1-Nectin3	JAM1	Tight junctions
GTP Rho	+	–	–	–	+	ND	ND	ND
	–(MDCKII)	+						
GTP Rac	+	+	+	+	–	+	ND	ND
GTP Cdc42	+(MCF7)	+	–(A431) +(BT-20)	–	–	+	ND	ND
GTP Rap1	+	ND	ND	ND	ND	+	+	ND
Regulators	C3G Tiam-1	Tiam-1		p190RhoGAP	RICS (synapse)	Vav2 FRG C3G		GEF-H1*
References	[13,14••,15•,28,32,38,51,52,53]	[31,54,55]	[30•]	[42,56]	[29,41]	[18,19•,20••]	[57]	[43••,49•]

Activation of different small GTPases by cell–cell adhesion receptors and putative regulators involved. Available data demonstrate that increased levels of active Rho, Rac, Cdc42 and Rap1 (GTP-associated) are induced by different types of cadherins, nectins and JAM1. Modulation of small GTPase activity level is assessed by cell–cell contact formation, receptor clustering or deletion of adhesive receptor by siRNA or knockout cells. Different cadherin family members and cell types show remarkable divergence on the profile of activated small GTPases (E-, VE-, R-, C- and N-cadherins). The transmembrane tight junction protein responsible for activation of small GTPases has not yet been identified (but see review in this issue [58]). Different GTPase activators such as exchange factors (C3G, Tiam-1, FRG and GEF-H1) and GTPase Activating Proteins (p190RhoGAP and RICS) have been implicated in the regulation of small GTPase activity by cell–cell contacts. Counterintuitively, the presence of GEF-H1 at tight junctions leads to Rho inactivation, as GEF-H1 is downmodulated by its interaction with cingulin (*).
ND, not determined; +, activation; –, no activation.