Abstract: The characteristic tall and elongated shape of epithelial cells requires specialized adhesive structures and a distinct organization of cytoskeletal filaments. Cytoskeletal networks coordinate a precise organization of adhesive and signalling complexes along cell-cell contacts and enable exquisite strong cohesion among epithelial cells. E-cadherin, a calcium-dependent adhesion receptor, is an essential adhesive system in epithelia and its dynamic regulation and pathways that stabilize cell-cell adhesion have been extensively studied. This review highlight the less understood mechanisms underlying how cadherin receptor signalling drives cytoskeletal rearrangements which ultimately define the epithelial cell shape. In the past two years, new insights identify specific actin-binding protein and regulators of the epithelial cytoskeleton as a framework to support junction dynamics, plasticity and maintenance.

Suggested Reviewers:
Spatial integration of E-cadherin adhesion, signalling and the epithelial cytoskeleton

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Abstract
The characteristic tall and elongated shape of epithelial cells requires specialized adhesive structures and a distinct organization of cytoskeletal filaments. Cytoskeletal networks coordinate a precise organization of adhesive and signalling complexes along cell-cell contacts and enable exquisite strong cohesion among epithelial cells. E-cadherin, a calcium-dependent adhesion receptor, is an essential adhesive system in epithelia and its dynamic regulation and pathways that stabilize cell-cell adhesion have been extensively studied. This review highlights the less understood mechanisms underlying how cadherin receptor signalling drives cytoskeletal rearrangements which ultimately define the epithelial cell shape. In the past two years, new insights identify specific actin-binding proteins and regulators of the epithelial cytoskeleton as a framework to support junction dynamics, plasticity and maintenance.

Introduction:
E-cadherin adhesion is one the main drivers of epithelial morphology and differentiation at the cellular and tissue level. Dynamic modulation of junctions participates in different processes such as geometric cell shape, planar polarization, invagination and folding of epithelial tissues, cell extrusion from epithelial monolayers or re-establishment of junctions following cell division and cell death [1,2]. Engagement of cadherin receptors as an adhesive unit initiates morphological polarization: the elongation of the lateral domain shared by neighbouring cells, which ultimately increases the overall cell height to generate a columnar epithelial shape (Fig.1a). This process is accompanied by recruitment of polarity and signalling complexes to cell-cell junctions, driving the specialization of membrane domains (for review see [3]).

The filamentous actin (F-actin) organization found in epithelia underpins the regulation of signalling complexes and cell and tissue architecture. F-actin network organization relies on a variety of actin-binding proteins with complementary sets of properties [4]. Properties such as filament elongation, capping, severing or bundling are precisely coordinated to assemble specific structures from building blocks, the actin monomers (G-actin). These diverse properties of cytoskeletal proteins are integrated by a repertoire of regulators, including the Rho family of small GTPases. The latter forms an important class of cytoskeletal modulators [5] and, among them, RhoA, Rac1 and Cdc42 have been studied in detail in epithelia. This review discusses how epithelial F-actin structures provide a scaffolding for small GTPase signalling to support epithelial cell shape and tissue architecture. Excellent reviews can be found elsewhere on the role of GTPase upstream modulators in the function of adhesive structures [6-8].

Form follows function
There are two distinct pools of F-actin at epithelial junctions: an F-actin pool at contact sites (junctional actin) and circumferential thin bundles adjacent to and aligned with cell-cell contacts (Fig.1b,c) (reviewed by [9,10]). Connecting these two F-actin pools are radial fibres that arrive at junctions perpendicularly and may be considered a component of the thin bundles pool (Fig.1c). It is currently unknown how thin bundles and associated radial fibres are connected to E-cadherin complexes and integrated with junctional actin at adhesive contacts. Variations on the morphological appearance and a clear separation between junctional actin and thin bundles are observed in different cell types, junction maturation status or response to specific stimuli (mechanical forces, morphogenesis or destabilization of contacts – see below) [10].

Thin bundles were reported in the early 80’s by electron microscopy as a tightly compacted, contractile filaments parallel to junctions [11,12]. In epithelial tissues in vivo, thin bundles co-localise with and are indistinguishable from junctional actin by immunofluorescence. Yet, their distinct
dynamics [13], regulation and molecular composition indicates their separable identity: junctional actin contains β-actin and myosin IIA, while γ-actin and myosin IIB are found at thin bundles [14,15]. As myosin IIA and myosin IIB can co-assemble on different types of actin filaments [16], it will be interesting to define how wide-spread the restricted distribution of these proteins among different epithelial cell types is.

Junctional actin function on cadherin adhesion assembly and maintenance is well established. However, the specific contribution of thin bundles to cadherin stabilization is less clear, either because there is no clear spatial segregation from junctional actin (i.e. in vivo) or the challenge to disrupt thin bundles selectively, without substantially compromising junctional actin (see below). Nevertheless, coordinated regulation of these two epithelial F-actin pools is likely to control E-cadherin levels, junction strength, configuration, plasticity and ultimately epithelial architecture (see below).

**Junctional actin**

Junctional actin is formed by an F-actin pool at the plane of the contacting membrane, bound to cadherin complexes (i.e. via catenins) and other transmembrane proteins (i.e. nectins [17], growth factors receptors and integrins). In spite of extensive progress in the identification of cytoskeletal proteins found at junctions [18,19], the precise F-actin organization and the specific actin remodelling functions at cadherin contacts lack in-depth understanding. A pool of G-actin at junctions [20] has unknown functions, but may provide a local pool of monomers for filament remodelling. F-actin accumulation at junctions may be a net result of actin polymerization, depolymerisation, stabilization and recruitment of short filaments.

So far, studies have mostly focused on an actin nucleation process at contacts controlled by formins and/or Arp2/3 complexes (for excellent review see [21]). Formins promote F-actin elongation as linear filaments [4]. The family members mDia1 [22,23] and FMNL2 [24] are necessary for E-cadherin stabilization at junctions. Constitutive activation of FMNL2 or mDia1 increases F-actin levels at cell-cell contacts [22,24]; yet they are regulated by different GTPases. FMNL2 recruitment to cadherin receptors is driven by Rac1 activation [24], but mDia1 localization at junctions [23] requires active RhoA [22]. mDia1 also modulates F-actin cables to facilitate the release of exocrine secretory vesicles in pancreatic cells [25] and regulates E-cadherin transport downstream of RhoB [26].

In addition to actin nucleation, Arp2/3 generates branched filaments [4], but these structures are not observed at mature junctions. It is likely that Arp2/3-dependent nucleation is favoured at cell-cell contact via different strategies: (i) distinct regulation by partners such as N-WASP [27] or cortactin, which provides a scaffold for Arp complexes at junctions [28] or (ii) inhibition of branching activity by α-catenin [29-31] or EPLIN, a cross-linking protein [32]. Alternatively, the precise regulation of profilin, a G-actin binding protein, may determine the preferential incorporation of monomers to filaments nucleated by formins instead of Arp2/3 [33].

Conversely, the importance to counteract filament polymerization/elongation for junction stability has recently been demonstrated. First, capping proteins limit the availability of F-actin ends for polymerization [4]. Depletion of CD2AP, a barbed-end capping protein, causes a strong reduction of junctional actin but not E-cadherin levels [34]. Yet, monolayers cannot withstand pathological mechanical stress: they break apart and have increased permeability [34]. Second, filament turnover/size is controlled by de-polymerization, and ADF/cofilin or its co-factor AIP (actin-interacting protein) RNAi reduces E-cadherin levels at junctions [35,36]. Together these results strengthen the appropriate coordination between polymerization, depolymerisation and capping for junctional actin.
Further stabilization of filament arrays at junctions contribute to cadherin contacts maintenance. The F-actin bundling and cross-linking proteins α-actinin 4 [37] and filaminA/B localise at cell-cell contacts are essential for cadherin adhesion in different tissues [38-40]. At junctions, filaminA and filaminB are known to fine tune activation of RhoA and Rac1 via binding to the GEF Trio or the GAP FilGAP, respectively [38,41]. Furthermore, F-actin bundles may be stabilised by a number of different myosins that co-localise with cadherins and are functionally important for junction maintenance [15,42,43]. Yet, in some epithelia, contractile markers such as phosphorylated Myosin Light Chain (pMLC) are not highly enriched at junctional actin, particularly when peripheral thin bundles are clearly separable (Fig.1d). Although long, thick filament bundles are not apparent at junctional actin [44-46], a disordered array of short filaments can be contractile, particularly in the presence of cross-linkers such as α-actinin 4 [47].

Global contractility levels are known to influence actin turnover at junctions [20,48,49]. These data are in line with the paramount influence that a unique architecture of F-actin structures has on the properties of specific cytoskeletal proteins, from regulation of contraction to disassembly [50,51]. Further insights onto the precise F-actin organization at junctional actin are essential to understand the mechanisms of E-cadherin stabilization, turnover and mechanosensitivity [52,53].

Thin bundles and the regulation of lateral height and junction configuration

Circumferential thin bundles are less dynamic than junctional actin and forms the main contractile pool in epithelia, with increased pMLC labelling (Fig.1d) [13]. Thin bundles are already present in cells without junctions as a large band of filaments at the cell periphery (Fig.1b). They become progressively compacted towards newly formed cell-cell contacts until indistinguishable from junctional actin [13]. Alignment of thin bundles in parallel to junctions requires anillin, a bundling protein, and EPLIN, a filament cross-linker [32,54]. Depletion of either protein generates filaments in the cytoplasm. Moreover, following EPLIN RNAi, thin filaments connect to junctions perpendicularly rather than in parallel to cell-cell contacts [54-56].

Circumferential thin bundles participate in morphological polarization and cell-cell contact configuration (shape, linearity and length, i.e. growth or shrinkage) (Fig.2). First, thin bundle stability and contraction correlate spatially and temporally with the vertical elongation of epithelial lateral domains (Fig.1). In some cell types, the microtubule network may also be required to generate a columnar morphology [57]. Conversely, the control of junction lateral height is also important for epithelial cell flattening and invagination [58]. Actin filament stabilisation is partially achieved by a cooperation between tropomodulin (a pointed end capping protein) and tropomyosin (F-actin side-binding protein). In CaCo2 cells, depletion of tropomodulin3 destabilizes the membrane skeleton at junctions, leading to shorter lateral domains with less F-actin and tropomyosin (without changes in E-cadherin localization) [59]. In keratinocytes, regulation of contraction by ROCKI and ROCKII, key Rho effectors [5] is important for thin bundle remodelling: their depletion leads to less compacted bundles and flatter cells, with a 50% reduction of the lateral height [13].

Shorter lateral domains and concomitant expansion of the apical surface are also observed upon depletion of p120CTN in MDCK cells grown on collagen [60]. The mechanisms involved are unclear, but may involve the ability of p120CTN bound to cadherin complexes to localise ROCK at cell-cell contacts [61]. The latter may or may not involve the ROCK partner Shroom3 [62,63], a known regulator of epithelial apical contraction [64]. In endothelial cells and C.elegans, p120CTN may also dynamically modulate contractility at the lateral domains via its interaction with RhoA upstream regulators [65,66]. PAK family members, serine threonine kinases effectors of Rac1 and Cdc42 GTPase known to modulate contraction [67], could also participate: either via transiently activation
by cell-cell contact formation (PAK1) [68] or localization at epithelial junctions (PAK4 and PAK6) [69-72].

Second, thin bundles regulate junction length (corner-to-corner between neighbouring cells). The growth or shrinkage of cell-cell contacts at the plane of the monolayer controls junction size, cell positioning within the monolayer and ultimately cell shape and tissue remodelling (for reviews see [1,10,58]). Junction shrinkage may occur by asymmetric, spatially restricted contraction of thin bundles positioned at different heights along the contacting neighbouring cells. This localized contraction enables apical constriction during invagination [73] or defines the topography of cell extrusion (apical or basal side of the epithelial monolayer). At the molecular level, cell extrusion directionality is driven by specific oncogenes (Ras, APC) [74,75] and the engagement of N-WASP or crosslinking proteins such as filamins or EPLIN [45,76,77].

Third, wavy, curved junctions are found upon dysregulation of GTPase signalling or depletion of cytoskeletal proteins that modulate thin bundles. In different epithelia, inhibition of ROCK [13,15,78] or depletion of myosin VI [42] or tropomyosin5 [79] (a filament side-binding protein) results in the appearance of curved, rather than linear junctions. Without tropomyosin5, junctions are floppier, with reduced recoil upon laser ablation, suggesting that stable thin bundles are required to generate taught, straight junctions [79].

Curved junctions are also found following depletion of tricellulin, a transmembrane protein found at the corners between three epithelial cells (tricellular junctions) [80,81]. These specialised adhesive structures undergo considerable tensile stress [81] and are essential for thin bundle organization and an impermeable epithelial monolayer [80]. It turns out that tricellulin regulates the contractile properties and the attachment of thin bundles to cell vertices, via the interaction with, recruitment and activation of Tuba, a Cdc42 exchange factor. Consistent with the involvement of GTPase signalling, junctions have a wavy appearance after Tuba depletion [82], similar to tricellulin RNAi. Wavy junctions are considered less stable and may contribute to degeneration of cochlear hair cells observed upon tricellulin mutations in autosomal non-syndromic deafness [83].

**Different hues of the same colour:**

Junction remodelling participates in a variety of morphogenetic programs leading to distinct cellular and tissue outcomes [84]. It is feasible that a single regulator participates in distinct junction remodelling and signalling events. By engaging with selected cytoskeletal partners that are spatially- and temporally-restricted along cell-cell contacts, a signalling pathway may be driven towards different cellular outcomes.

For example, Tuba activation at tricellular junctions modulates thin bundle remodelling (see above). However, Tuba can also localise to other sites at junctions via ZO1, where it regulates E-cadherin recruitment to new cell-cell contacts and junctional actin organization around cadherin clusters [81,82]. In addition to how Tuba is recruited (via tricellulin or ZO1), distinct Cdc42 effectors may be involved at different junctional sites. N-WASP is required downstream of ZO1-Tuba-Cdc42 to modulate F-actin co-localization with E-cadherin [82]. The Cdc42 target responsible for thin bundle reorganization via tricellulin-Tuba partnership in epithelia is not known. A likely candidate is MRCK, a kinase effector of Cdc42, that associates with acto-myosin filaments and, similar to ROCKI/II, phosphorylates myosin regulatory chain [85]. In endothelial cells, MRCK is activated upon induction of cell-cell contacts and controls how compact and aligned circumferential bundles are towards junctions [86]. However, in this model, Tuba is not involved, but a different GEF (FGD5) is recruited to activate Cdc42 and MRCK, thereby promoting linear endothelial junctions [86].

Targeting of the exchange factor Tiam1 to sub-domains at junctions has distinct consequences for Rac1 activation. At tight junctions, Par-3 inactivates Tiam1-Rac1 signalling, while β-syntrophin-dependent Tiam1 localization activates Rac1 more basally, thereby generating a gradient of
localized signalling, with different functions for tight junctions or adherens junctions [87]. Similar restricted zone of GTPase activation at zonula adherens is reported for RhoA, where positioning of ROCK1 by myosin II maintains RhoA activation by antagonizing the recruitment of Rnd3/p190RhoGAP, thereby preventing localized RhoA inactivation [88].

A novel concept to fine-tune the extent of GTPase activation at junctions involves cytoskeletal proteins that bind directly and provide an anchor for Rho proteins. Anillin, a bundling protein, interacts with and recruits RhoA to the cytokinesis furrow and cell-cell contacts, where it controls active RhoA localization [89,90]. Upon depletion of anillin, pulses of RhoA activation at discrete spots at junctions of Xenopus cells occur with higher frequency, but reduced life-time [90]. Such altered oscillation of active RhoA severely impairs adherens junctions [54,90]. The direct binding of anillin with active RhoA [89] may be a mechanism to maintain spatial and temporal regulation, albeit this has not yet been formally shown. Another example is the LIM protein Ajuba, an actin bundling protein, which partially localises at adhesion sites and interacts with both GTP- and GDP-bound Rac1 [68]. When phosphorylated, Ajuba stabilizes Rac1·GTP at cell-cell contacts [68]. It is currently unclear the precise contribution of Ajuba bundling function versus GTPase activation for cell-cell contact reinforcement.

**Concluding remarks:**

The lateral domains shared by neighbouring cells are highly dynamic [84]. The extensive plasticity of lateral domains balances regulation of cell-cell adhesion and associated signalling with diversity and variability in morphology, localization and molecular association. This review discusses novel mechanisms that enable such plasticity. First, via the combinatorial action of actin binding proteins to fine tune the activity and localization of core regulators at sub-domains of junctions. Second, the functions of a cytoskeletal protein are strongly modulated by the F-actin structures where it localises and its interacting partners. The latter highlights the importance to dissect the precise organization of epithelial cytoskeleton and a comprehensive understanding of the regulatory network that enables its maintenance and dynamics. Exciting work lies ahead to build this knowledge towards a spatial integration of the actin cytoskeleton with epithelial adhesion, signalling and morphogenesis.

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Figure legends

**Figure 1:** a, Epithelial cells have different morphologies depending on the organ they are found and specific function: squamous (flat cell shape as in lung airway epithelia), cuboidal (i.e. keratinocytes, breast epithelia) or columnar shape (i.e. intestinal epithelia). b, Cells without cell-cell contacts contain a large band of circumferential thin bundles at their periphery. Upon calcium-induced cell-cell adhesion, E-cadherin receptors cluster as adhesive units to: (i) assemble a pool of F-actin at the membrane surrounding the receptors and (ii) induce compaction and contraction of thin bundles until co-localization with junctions. c, Immunofluorescence images of newly-formed junctions of keratinocytes labelled for E-cadherin and F-actin. d, A junction is shown immunostained for F-actin and pMLC. Note the majority of pMLC labelling colocalizes with thin bundles adjacent to junctions. Zooms in b,c show the two F-actin populations: thin bundles (bracket, 1) and junctional actin (square, 2). Arrows point to radial fibres linking these two pools.

**Figure 2:** Diagram representing the remodelling of actin cytoskeleton driven by initiation of E-cadherin-dependent cell-cell contacts. E-cadherin complexes clustering triggers signalling to form junctional actin and remodel pre-existing peripheral thin bundles, which co-localise as junctions mature. Actin remodelling properties involved in epithelial F-actin structures and the identified functions of either actin population in epithelia are listed. The *de novo* formation of junctional actin is better understood and may assemble/recruit a meshwork of short, linear filaments rather than a branched array. Different myosins are found at junctional actin with reduced levels of pMLC relative to thin bundles. The re-organization of thin bundles as a compact and co-aligned array with junctions require contraction. Actin properties that maintain contraction, bundling and stabilization of filaments are relevant for thin bundle reorganization.

**References:**


This paper describes the contraction-dependent recruitment of vinculin and associated Ena/VASP to enable actin assembly at junctions, thereby conditioning actomyosin properties with actin polymerization


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A careful evaluation of the function of the formin FMNL2 on junctional actin and its comparison with the role of mDia2. The article shows FMNL2 contributes via distinct GTPase (Rac1) to the same cellular event as mDia2 (RhoA) during junction stabilization.

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A thorough evaluation of the properties of α-catennin homodimers and monomers, including their impact on actin polymerization and E-cadherin adhesion strength.


This paper dissectes the properties of the actin-binding domain (ABD) of α-catennin and shows that associated E-cadherin clusters are more dynamic than when vinculin is also present in the complex. ABD interacts with actin at the cortex rather than pre-formed bundles drives the continuous formation of transient actin-cadherin complexes.


This paper nicelly demonstrates how localized contraction at the Y-X axis of the epithelial lateral domain drives the extrusion of H-Ras transformed cells and thereby controls epithelial cell integration within the monolayer.

Here the authors define a cadherin nanocluster as a adhesive unit, its characteristics and mechanisms underlying cluster size, the interplay with cytoskeleton meshwork and cluster composition.


This paper interestingly demonstrates that short F-actin filaments can be made contractile by the presence of α-actinin 4.


The authors set up an interesting model of cell doublets containing a single junction to dissect the contribution of myosinII-dependent contraction to actin turnover at E-cadherin clusters. They further dissect the interplay how actin dynamics can modulate mechanosensitive responses of cadherin complexes.


Together with reference 51, these papers demonstrate that the functionality of a specific actin protein is modulated by the distinct architecture of actin filaments at different intracellular sites. This effect seems to be a general property of cytoskeletal proteins driving the activity of depolymerizing factors and contraction.


See above reference 50.


Another function for tricellulins in the recruitment and activation of Tuba, a Cdc42 GEF to control thin bundle contractile properties and the wavy, curved configuration of junctions.


Flares of RhoA activation are shown at the Xenopus epithelial junctions, similar to what demonstrated previously in invertebrates. The authors demonstrate the role of anillin in restricting the localization, frequency and intensity of RhoA activation.
Figure 1

(a) Lateral view

(b) Top view

1- thin bundles
2- junctional actin

E-cadherin
Other receptors
F-actin filaments

(c) Merge
E-cadherin
F-actin

(d) Merge
F-actin
pMLC