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Microtubules and cadherins: a neglected partnership

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1. ABSTRACT

Classical cadherins are fundamental determinants of tissue organization both in health and disease. It has long been recognized that cadherins function in close cooperation with the cytoskeleton, particularly with actin. Less appreciated is the capacity for cadherins to also interact functionally and biochemically with microtubules and their associated proteins. In this review, we aim to highlight the potential for cooperativity between cadherins and microtubules. Cadherins can regulate the organization and dynamics of microtubules through mechanisms such as anchorage of minus ends and cortical capture of plus ends. Such cadherin-induced reorganization of microtubules may then affect cadherin biology by diverse processes that include directed vesicular traffic by microtubule-based motors and regulation of cortical signaling and organization. Ultimately, we hope this will stimulate fresh interest and research to understand a neglected partnership.

2. INTRODUCTION

Classical cadherin adhesion molecules mediate cell-cell recognition and cohesion in all solid tissues of the body (1). They are profound determinants of tissue organization, being essential for morphogenesis and also commonly perturbed in human disease, notably invasive and metastatic cancer (2). Classical cadherins are single-pass transmembrane glycoproteins. Their extracellular domains mediate adhesive binding and recognition whilst their conserved cytoplasmic tails interact, physically and functionally, with many functional effectors in the cell. The latter include molecules that mediate cell signaling and support membrane trafficking, as well as crucial elements of the cytoskeleton (3-5). The morphogenetic impact of cadherins thus arises from cooperation between the adhesive binding ectodomains and the many key intracellular processes that impinge on the cytoplasmic tails.

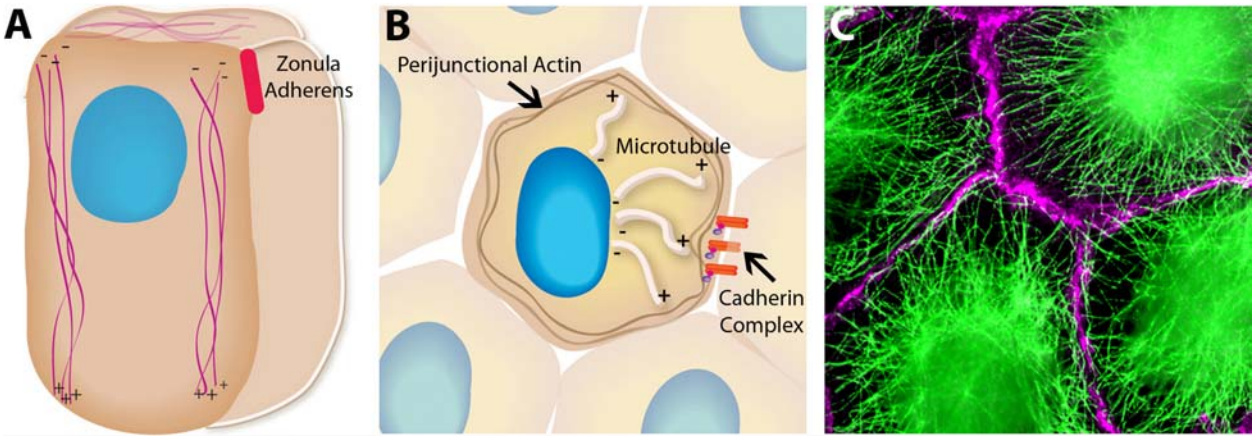

Figure 1. Patterns of microtubule organization at cadherin cell-cell contacts. A. Perijunctional accumulation of microtubules. In polarized epithelia, microtubules commonly accumulate at cell-cell contacts oriented vertically with their minus ends at the apical pole and their plus ends directed to the basal pole, although exceptions exist. B, C. Radial extension of microtubules into cadherin-based cell-cell contacts. Radially-organized microtubules extend with their plus ends directed into cadherin-based cell-cell adhesions, depicted diagrammatically in B. (C) MCF7 mammary epithelial cells were co-stained for E-cadherin (magenta) and β-tubulin (green) to demonstrate the radial extension of microtubules into cadherin adhesions. The location of the minus ends is not known.

Of the core elements of the cytoskeleton, cadherins are best understood to function in close cooperation with actin (3). Actin cytoskeletal integrity and activity is essential for many aspects of cadherin biology, whilst cadherins themselves have the capacity to influence the dynamics and organization of the actin cytoskeleton. In contrast, much less attention has been paid to the potential for cadherins to interact with the microtubule cytoskeleton. In this review, we aim to highlight topical aspects of this neglected partnership. We will discuss what is known about the physical relationship between these two cellular systems and how microtubule organization may be coordinated with cadherin-based cell-cell adhesion. We then focus on how microtubules influence cadherin biology.

3. SPATIAL DISTRIBUTION (S) OF MICROTUBULES AT CADHERIN ADHESIONS

Microtubules are hollow tubes that are extremely dynamic, polarized polymers of α- and β-tubulin dimers. Many different patterns of microtubule organization are found in cells (6). Commonly, microtubules are envisaged to arise with their minus ends anchored to centrosomes, giving rise to outwardly-directed arrays, where the dynamic plus-ends are able to explore cellular space. However, non-centrosomal patterns of organization also exist, with microtubules found in bundles, meshworks or parallel arrays (7-9).

In many epithelial cells microtubules concentrate near cadherin-based cell-cell contacts (Figure 1) (10-12). The organization of these microtubules is complex and varies with cell type. In polarized simple epithelial cells microtubules are commonly observed to run vertically, often apparently orientated with their minus ends directed apically and their plus ends directed towards the basal surface (8, 9, 13). However, exceptions exist where microtubule plus ends extend apically (14). In the stratified epithelium of the skin, microtubules also concentrate at contacts between cells in the suprabasal layer (12). Because cells form layers upon one another in the native tissue, this encompasses the apical and basal surfaces, as well the lateral surfaces of the cells. However, in cultured keratinocyte monolayers microtubules accumulate at the lateral membranes where cell contact occurs (12). Thus microtubules concentrate at cell-cell contacts in a range of epithelia.

Two observations suggest that this perijunctional concentration of microtubules may reflect an influence of cell-cell contact itself. Firstly, microtubule reorganization commonly occurs as cells make contact with one another. In particular, whereas isolated cells often display radial arrays of microtubules that are anchored at their minus ends to perinuclear centrosomes, centrosomal anchorage is lost and apico-basal arrays reorganize to accumulate near junctions as cells grow into monolayers (7, 8, 12, 15). Disassembly of centrosomes and rearrangement of microtubules into non-centrosomal parallel arrays also happens when myoblasts fuse into myotubes, suggesting that this process is not confined to epithelial cells (16). Secondly, and more directly, perijunctional accumulation of microtubules critically depends on the desmosomal linker protein, desmoplakin (12). Interestingly, microtubule accumulation does not require α-catenin, which associates with classical cadherin adhesive complexes, suggesting that cell-cell contacts might recruit perijunctional microtubules via desmosomal adhesion rather than through classical cadherins (12).

In addition to this pattern of perijunctional concentration, subpopulations of microtubules have also

been observed which appear to radiate outwards, coming into close proximity with cadherin-based cell-cell contacts in a variety of cell types (10, 17, 18). Using rhodamine-labelled tubulin to visualize microtubule growth in live newt lung epithelial cells, Waterman-Storer *et al.*, (2000) observed that microtubule ends appeared to terminate within a few micrometers of cell-cell junctions. Likewise, immunofluorescent staining of microtubules in human mammary epithelial cells (10, 19), Hela cells (20) and PtK2 cells (11) revealed patterns of radially-orientated microtubules which extended outwards to cell-cell contacts. Staining for CLIP-170 confirmed that these radial microtubules were oriented with their plus-end directed towards the cadherin adhesions (10). Consistent with this, electron microscopy has identified microtubule ends either very close to adherens junctions (10) or, indeed, extending into cell-cell contacts themselves (21). Thus, although radial arrays are commonly thought to be lost when cells organize into polarized monolayers, this is not universal, and radial microtubules that appear to project into cadherin-based cell-cell adhesions themselves may persist during differentiation.

Several observations further suggest that classical cadherins themselves may influence this radial pattern of microtubule organization. As cells first assemble contacts with one another, radial microtubules are observed to project into newly formed puncta of cadherin (10). One interpretive limitation is that when cadherin adhesion brings cell surfaces together it allows the assembly of other specialized junctions and juxtacrine signaling events that may participate in regulating microtubule behaviour (25). More recently, the availability of recombinant cadherin ligands has made it possible to test a role for cadherin adhesion itself more directly. Two studies showed that when cells were presented with cadherin ligands immobilized on beads or two-dimensional substrata, they extended microtubules into the sites of adhesion with cadherin, but not with non-specific adhesive ligands (10, 20). These reports suggest that the cadherin receptor itself can exert an instructive influence on microtubule orientation, independent of other juxtacrine events that occur when cell surfaces come into contact with one another.

Overall, these observations suggest that different mechanisms for cell-cell interactions can affect microtubule organization in at least two distinct ways. Generation of differentiated epithelia is often accompanied by perijunctional accumulation of microtubule bundles, perhaps in response to desmosomes. Additionally, classical cadherins may be able to generate a second pattern of radial microtubules oriented with their plus ends directed into cadherin adhesions. How commonly these patterns are found, and what contexts might influence their appearance, remains to be thoroughly characterized.

4. HOW CELL-CELL INTERACTIONS AFFECT MICROTUBULE ORGANIZATION

What mechanisms may allow cell-cell interactions to control microtubule organization? Here it is important to emphasize that the steady state organization of dynamic microtubules is controlled by several distinct processes, notably nucleation, and where this occurs; microtubule severing; minus end stabilization and anchoring; and regulation of plus end dynamics, including capture of plus ends. Combinations of these factors are likely to cooperate to generate the distinct patterns of perijunctional accumulation and radial organization that have been described.

Regulation at minus-ends has been most consistently implicated in the pattern of perijunctional microtubule accumulation. Using γ-tubulin as a marker for microtubule nucleation, Reilein *et al.* (2005) identified *de novo* acentrosomal microtubule growth and proposed that these sites formed the base of the vertical bundles that extend parallel to the apico–basal axis of MDCK cells. Additionally, the minus-end anchoring protein ninein relocalizes to the apical regions of cell-cell contact as a variety of cells either grow to confluence and/or differentiate in monolayers (22). In the skin, this occurs in a desmoplakin-dependent fashion, suggesting that redistribution of minus-end anchoring sites may drive the impact of desmosomes on microtubule organization in these cells (12). Apical localization of nucleation and minus-end anchorage, separately or in concert, would be predicted to facilitate both perijunctional accumulation and microtubule orientation with minus ends apical and plus ends directed basally.

It has also been suggested that classical cadherins can affect minus end stability. Chausovsky *et al* (2000) reported that cell-cell contacts affect microtubule density and dynamics in centrosome-free cytoplast preparation. In this assay, removal of the centrosome typically alters the overall pattern of microtubule dynamics, from a pattern of dynamic instability, dominated by the free plus ends, to one of treadmilling with turnover at both plus and minus ends. However, cell-cell contact induced stabilization of minus ends, a process that could be modulated by manipulating N-cadherin (23). To what extent this phenomenon occurs in intact cell populations, and how it may reflect cooperation between classical cadherins and desmosomes, remains to be tested.

How classical cadherin adhesion may recruit radial arrays of microtubules is as yet largely unknown, although several interesting possibilities can be considered. Firstly, if radial microtubules derive from, and are anchored at juxtanuclear MTOCs, then growth from their plus-ends would tend to direct the microtubules outwards. This biasing by perinuclear minus-end anchoring is thought to contribute to the radial pattern of microtubules seen in isolated cells. But to what extent radial microtubules retain perinuclear anchorage when cells form contacts with one another remains an open question. As noted above, sites for minus end nucleation and anchorage are often thought to shift to the periphery when cells form monolayers (9, 12), although exceptions may occur. Thus it is unlikely that simple biasing of microtubule growth by the location of minus-end nucleation can fully explain the radial patterns of microtubules seen at cell-cell contacts.

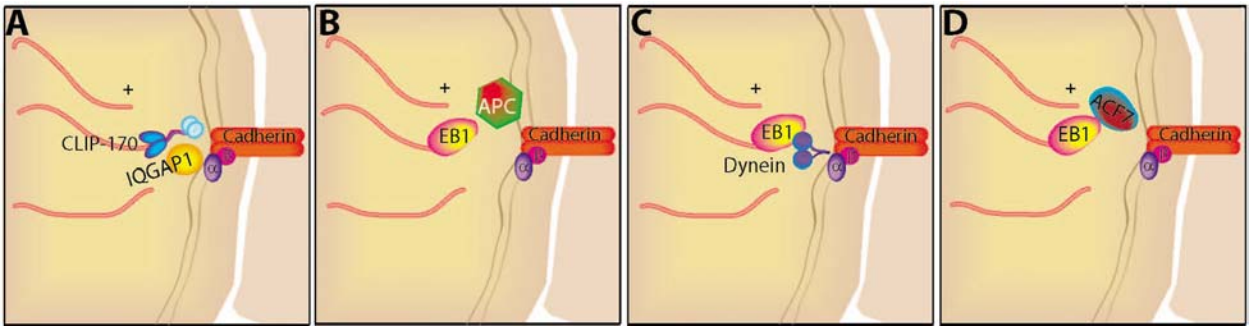

Figure 2. Cortical capture of microtubules at cadherin adhesions. Possible mechanisms include: A) Transient binding of the +TIP, CLIP-170, to IQGAP1 associated with cadherin complexes. B) Interaction between the +TIP, EB1, and cortical APC. The precise mechanism for cortical localization of APC at cell-cell adhesions is not understood. C) Binding of microtubules by dynein associated with β-catenin. D) Capture of microtubules by actin-microtubule cross-linking proteins, such as ACF7, found at cell-cell contacts.

Alternatively, radial organization could occur if interactions with the cell cortex at cadherin adhesions were to affect either the dynamics of plus ends and/or result in capture and anchoring of those plus ends. Cell-cell contact can affect microtubule dynamics in a variety of ways that differ depending on cell type and context (24). Of note for our present discussion, Waterman-Storer *et al.* (2000) demonstrated that in lung epithelial cells microtubules plus-ends become quiescent at the edges of cells that are in contact with adjacent cells, in contrast to the dynamic nature of the microtubules at free cell margins. Such quiescence would be predicted to promote persistence of microtubule plus ends at cell-cell contacts, and hence promote radial organization. How cell-cell contact could control plus-end dynamics is unknown, but is likely to involve cell signaling. Cell-cell adhesions are enriched in many signaling mediators, several of which can be activated by ligation of cadherin adhesion receptors themselves (25). These include signaling by small GTPases, notably Rac and Cdc42 (4, 26). In another context, Rac promotes microtubule plus-end growth into the leading edges of lamellipodia in migrating cells (27); therefore, it is an attractive candidate to stimulate microtubule growth towards cell contacts.

Cell-cell contacts might also promote radial organization of microtubules if the microtubules were physically captured at the cortex of adhesion sites. The orientation of radial microtubules with their plus-ends directed towards cell-cell contacts presents some interesting possible mechanisms. Microtubule plus end are decorated with a specialised subset of microtubule associated proteins (MAPs) termed +TIPs or microtubule plus-end tracking proteins that associate only with growing plus-ends (28). As well as regulating microtubule dynamics, +TIPs are known to mediate the association of microtubule plus-ends with specialised membrane domains at the cell cortex (28, 29).

Interestingly, several +TIPs are reported to be able to interact with proteins found at cadherin adhesions (Figure 2). The +TIP, Cytoplasmic linker protein (CLIP)-170, can bind the cortical protein IQ motif containing GTPase activating protein 1 (IQGAP1) (30). Indeed, IQGAP1 at the leading edges of migrating fibroblasts was reported to act as a cortical targeting site for plus-end-bound CLIP-170, a process that was modulated by the Rho GTPases, Rac and Cdc42 (30). Importantly for this discussion, IQGAP1 can also interact with the cadherin-catenin complex and accumulate at cell-cell contacts (31). This suggests the possibility that cadherin-bound IQGAP1 might capture microtubule plus-ends through CLIP-170. It should be noted, however, that since CLIP-170, as well as most other +TIPs, associates only with growing, but not with pausing or depolymerizing microtubule ends, this capture can only be very transient.

Another potential candidate involves the Adenomatous Polyposis Coli gene product (APC), which can interact directly with microtubules and also indirectly, via the +TIP, EB1 (32-35). APC is found at the cell cortex in many sites, including near adherens junctions (36, 37). In migrating cells, cortical APC is proposed to capture EB1-decorated microtubule plus-ends at free protrusions of cells (32, 34, 38). In other forms of cellular protrusions, association between APC and cell cortices is facilitated by binding IQGAP1 (39) or the Formin, mDia (40), which have also been identified at cadherin-based cell-cell adhesions (19). Whether this, or any of the other potential mechanisms, mediates cortical capture to support radial microtubules at cadherin contacts remains to be determined experimentally.

Other candidates for tethering microtubules to the cell cortex include the microtubule motor, cytoplasmic dynein, is reported to bind β-catenin (11, 17) and actin-microtubule cross-linking proteins, such as ACF7, which may link microtubules to actin filaments (41). Of note, in ACF7 knockout cells, the +TIPs EB1 and CLIP-170 continue to associate with polymerising microtubule plus-ends, however instead of pausing at cortical sites, microtubules continue to grow, eventually bending and tracking parallel to the membrane (42). Additionally, ACF7 re-localises to cell-cell borders in keratinocytes upon calcium-dependent induction of intercellular adhesion (41). Overall, then, several potential mechanisms exist for

cadherin adhesions to direct the orientation of microtubules into adhesive cell-cell contacts. These need not be exclusive of one another, suggesting that such cadherin-directed microtubules may arise through cooperative mechanisms that influence both the growth and cortical capture of microtubule plus ends.

5. CELLULAR FUNCTIONS FOR CADHERIN-DIRECTED MICROTUBULES

What cellular functions might then be served by controlling microtubules at cell-cell contacts? The most obvious possibility is that they influence cadherin biology itself. Indeed, early studies indicated that microtubule integrity could influence cell-cell junctions, albeit with mixed results. Thus, depolymerization of microtubules in primary thyroid epithelial cells with colchicine caused apparent fragmentation of E-cadherin staining between cells (43), while cadherin levels in differentiating murine P19 embryonal stem cells were decreased by colchine, but not by the actin-disrupting drug, cytochalasin B (44). Similarly, Waterman-Storer *et al.* (2000) observed that cell-cell adhesions in newt lung epithelial cells were perturbed following microtubule depolymerisation. In contrast, microtubule depolymerization in other reports increased cellular adhesion, associated with the accumulation of cadherin-catenin complexes at cell-cell contacts (45, 46). These discrepancies remain to be resolved, although differences in the cell types studied and in the assays used to assess adhesion and junctional integrity may have contributed.

Here it is important to note that drugs such as colchicine and nocodazole have significant dose-dependent effects on microtubule integrity and dynamics. In particular, whereas at high concentrations (>10 μM) these agents depolymerize microtubules, at low concentrations (e.g. 100 nM nocodazole) they affect the dynamic behaviour of the plus-ends without causing overall depolymerization of the microtubules (47, 48). Interestingly, the integrity of E-cadherin contacts between MCF7 mammary epithelial cells was perturbed by low doses of nocodazole that did not depolymerize the microtubule cytoskeleton (10). Contacts became fragmented and FRAP studies suggested a failure of cadherin to be actively retained at the cell-cell contacts. Microtubule stabilization by a CLIP-170 fragment caused similar effects, substantiating the notion that it was the dynamic behaviour of the plus-ends that affected the cell-cell contacts, rather than overall integrity of microtubules. This further implies that microtubule integrity and the presence of the dynamic plus-ends can both affect cadherin accumulation, potentially by distinct cellular mechanisms. An important technical distinction in the literature may reflect whether microtubule manipulation (especially with agents such as nocodazole and colchicine) specifically affected plus-end dynamics or depolymerized microtubules more extensively. Any efforts to identify how microtubules affect cadherin distribution must then encompass these distinctions.

5.1. Microtubules, membrane transport and cadherin function

One attractive way in which microtubules may affect cadherin biology is through membrane transport. Microtubule-based motors support diverse forms of vesicular transport within cells. Classical cadherins, such as E-cadherin, engage in a complex itinerary of traffic throughout the cell (5, 49), both after biosynthesis (50) and upon recycling from the cell surface (51, 52). Several lines of evidence indicate that cadherin trafficking to the cell surface depends on microtubule-based transport. Vesicles containing N-cadherin (53) and puncta staining for p120-catenin, some of which are likely to mark cadherin-containing vesicles (54, 55), commonly decorate microtubules and move with velocities (~ 0.5-1 μm/sec) consistent with other forms of microtubule-based transport (54, 55). Microtubule depolymerization reduced directional movement of N-cadherin vesicles (53) while various maneuvers that targeted the plus-end directed motor, kinesin, also disrupted the movement of cadherin-containing vesicles (53, 56).

Consistent with this, kinesins can interact biochemically with cadherins and their associated molecular complex. In embryonic mouse brains and transfected A431 cells, the kinesin-2 (KIF3) motor formed a complex with N-cadherin, β-catenin and p120-catenin (56), while kinesin-1 has been reported to interact with p120-catenin (54, 55). Moreover, deletion of the N-terminus of p120-ctn, which mediates binding to kinesin, but not to cadherin, abolished the ability of exogenous kinesin to induce co-accumulation of cadherin and kinesin in cytoplasmic aggregates (54). This suggests that p120-ctn may be one mechanism to link cadherins to kinesins.

These forms of microtubule-based cadherin transport have demonstrably significant impacts on cadherin function (54, 56). Most strikingly, the Hirokawa group showed that conditional knock-out of KAP3, the cargo-binding unit of kinesin-2, disrupted the accumulation of N-cadherin at cell-cell contacts in neural precursors of the embryonic mouse brain (56). This was accompanied by increased cytoplasmic staining for cadherin, but no changes in its overall expression levels, suggesting a defect in transport of cadherin to the cell surface. This was confirmed in *Kap3*-null mouse embryo fibroblasts, which had normal biosynthetic rates of N-cadherin, but a defect in transport to the cell surface and concomitant reduced cell-cell adhesion. In mouse embryonic brains, the disruption cadherin transport associated with conditional loss of KAP3 was accompanied by a striking overproliferation of neural precursor cells, causing tumor-like nodules. A direct causal link between hyperproliferation and disruption of N-cadherin transport was not made. However, massive hyperplasia is also seen when α-catenin is disrupted in neural progenitor cells (57) and is consistent with evidence that cadherins may affect cell proliferation (58).

Taken with independent evidence that microtubules and kinesin affect N-cadherin transport to the surface in muscle cells (53), these studies clearly establish the capacity for microtubule-based transport to influence

the assembly of cell-cell adhesive interactions. By extension, microtubules that protrude into cadherin contacts may then provide a mechanism to direct newly-synthesized cadherins, or cadherins being recycled after internalisation, efficiently to sites of adhesion.

5.2. Microtubule plus ends, cortical regulation and cadherin biology

Cadherin transport is not the only way in which microtubules can affect cadherin biology. As noted above, we found that nocodazole, used in low concentrations that affect plus-end dynamics without depolymerizing the microtubule cytoskeleton, disrupted the continuity of E-cadherin-based cell-cell contacts and decreased cadherin accumulation at cell-cell contacts (10). This occurred without any change in either the total cellular or surface levels of cadherin, suggesting that substantive changes in cadherin traffic were unlikely to account for this effect. Indeed, no demonstrable changes in cadherin exocytosis were identified. This strongly suggests that the dynamic integrity of microtubule plus ends can affect cadherin biology independently of the role that microtubules play in cadherin transport within cells.

This observation is consistent with increasing evidence that microtubule plus ends can regulate cortical signaling and the actin cytoskeleton (59). Of note, a number of +TIPs associate with guanine nucleotide exchange factors that can influence signaling by small GTPases. These include APC, which can bind the Rac-specific GEF, Asef (APC-stimulated guanine nucleotide exchange factor) (60) and EB1 that interacts with the Rab5-specific GEF, Gapex-5 (61). Interestingly, in Drosophila S2 cells EB1 also binds DRho GEF2 and localizes it to the plus tips of microtubules (62). In Drosophila DRhoGEF2 has been identified in a genetic pathway where it acts downstream of the G-α subunit, *Concertina* (63), to activate Rho signaling. Rogers *et al* (2004) demonstrated that expression of constitutively-active Concertina induced the apparent release of DRhoGEF2 from microtubule ends and proposed that binding to EB1 might allow plus-ends to deliver DRhoGEF 2 to Concertina at the cell cortex where it was then activated to stimulate Rho signaling. In mammalian cells, however, +TIP localization is based on diffusion and transient immobilization at the growing microtubule ends. This process does not, then, help to transport molecules within the cytoplasm, but it can create local accumulations of signaling factors, concentrating proteins at the cortex where plus-ends are localized.

Independent of how EB1 may affect cortical Rho signaling, an important downstream target of Rho is non-muscle Myosin II that was regulated by DRhoGEF2 and also reported to bind EB1 in Drosophila S2 cells (62). In mammalian epithelial cells, Myosin II concentrates around cell-cell junctions and can maintain cadherin adhesion and the integrity of contacts in a ROCK-dependent fashion (64). Interestingly, we found that perijunctional Myosin II was lost when microtubule plus-end dynamics were inhibited (10). This suggests that one way that plus ends may affect cadherin function is by controlling Myosin II localization and/or activation at cell adhesions, perhaps through regulation of cortical signaling.

6. CONCLUSIONS AND FUTURE DIRECTIONS

Overall, then it is likely that microtubules affect cadherin biology through several pathways. In particular, we postulate that a cooperative mechanism exists where cadherin adhesion orientates microtubules into sites of adhesion that, in turn, support adhesive interactions both by vesicular delivery of cadherin and perhaps also controlling cortical signaling and the actin cytoskeleton. That cadherin adhesions require overall microtubule integrity is likely to reflect the impact of microtubule-based cadherin transport, whereas the more subtle impact of dynamic plus ends may arise through cortical regulation. This cooperative association between microtubules and cadherins may be a dynamic process that participates in assembly of definitive cell contacts from nascent adhesions and also when contacts are remodelled during junctional turnover and homeostasis.

While we have concentrated on the capacity for cadherin-regulated microtubules to positively feedback onto cadherin function, this is unlikely to be their only role in the cell. Dynamic microtubules have also been implicated in junctional disassembly (65, 66), by supporting a contractile actomyosin ring that facilitates disassembly and by microtubule-based transport of internalised junctional components (66). Moreover, by virtue of their roles in vesicular transport and cortical signaling, microtubules have the capacity to affect other aspects of membrane biogenesis at cell-cell contacts. For example, it has been recognized for many years that cadherin adhesion can facilitate the assembly of other specialized cell-cell junctions, including tight junctions, desmosomes and gap junctions (67, 68). The recent observation that cadherin-recruited microtubules may facilitate directed transport of gap junction components (20) provides a model for regulation of microtubules to support cross-talk between junctions. Similarly, microtubules may couple adhesion and cell polarization, to support the efficient delivery of basolateral components, such as aquaporin-3 (69) to their definitive location on the cell surface. Recruitment of microtubules may then be a major mechanism for cadherin adhesion to control the biogenesis of specialized surface domains and cell-cell junctions during tissue differentiation.

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Abbreviations: APC: Adenomatous Polyposis Coli; Asef: APC-stimulated guanine nucleotide exchange factor; CLIP-170: cytoplasmic linker protein-170; EB-1: end binding protein-1; E-cadherin: Epithelial Cadherin; GEF: Guanine Exchange Factor; IQGAP1: IQ motif containing GTPase activating protein 1; MAP: Microtubule Associated Protein; MCF7: Mammary breast adenocarcinoma cell line; MDCK: Mardin-Darby Canine Kidney; MTOC: Microtubule organization center

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