Chapter 4

Apical Cytoskeletons Help Define the Barrier Functions of Epithelial Cell Sheets in Biological Systems

Sachiko Tsukita, Tomoki Yano, and Elisa Herawati

4.1  Introduction

Epithelial cells adhere to each other by tight junctions (TJs) to form cell sheets, which is a critical step in epithelial barrier creation and the morphogenesis of vertebrate tissues (Fleming et al. 2000; Tsukita et al. 2001; Anderson et al. 2004; Furuse and Moriwaki 2009; Van Itallie and Anderson 2014; Tanaka et al. 2017). The apical surface of an epithelial cell sheet faces the outer environment, such as the lumen in the intestinal tract or the environment outside the skin surface. In the sheet, the cells’ apical membranes are regarded as a continuous, connected surface, in which the cell-cell adhesion sites are cemented by TJs. Notably, each epithelial cell exhibits basolateral polarity, and therefore the apical surface of an epithelial cell sheet differs from the basolateral one, and possesses specific features that relate to its roles in a particular biological functional system (Nelson 2009; Apodaca 2017). Apical differentiation is a popular topic of study, and includes microvilli, cilia, circumferential rings (Nelson 2009; Apodaca 2017), and ratchet structures, which have been described in Drosophila but not yet in vertebrates (Martin et al. 2009).
Consistent with the critical and varied roles of the apical surface, we recently identified a 3-layered cytoskeletal network of actin filaments, intermediate filaments, and microtubules that exists just below the apical membrane of epithelial cell sheets. The apical cytoskeletons are presumably organized under the control of the TJs and regulate epithelial morphogenesis and barrier functions in conjunction with TJ formation and TJ-based cell signaling. Thus, we propose to define this set of structures as a system called the “TJ-apical complex” (Yano et al. 2017).

A natural question is why this apical cytoskeletal system was not discovered until recently. It is a very thin-layered structure mainly composed of actin filaments, intermediate filaments, and microtubules, which appear as a continuous structure extending horizontally just beneath the apical plasma membrane. We discovered this network using an advanced imaging system, including ultrahigh voltage electron microscopic tomography and confocal super-resolution microscopy, that we developed (Kunimoto et al. 2012; Yano et al. 2013; Tateishi et al. 2017). Although the molecular mechanisms and physiological roles of the “TJ-apical complex” remain to be elucidated, we recently identified four TJMAPs (TJ Microtubule-associated Proteins; previously called J-MAPs), including cingulin which are TJ-associated proteins that bind to the apical cytoskeletons (Yano et al. 2013). During the morphogenesis of epithelial cell sheets, apical constriction and the size of the apical area defined by TJs must be kept in balance. Our recent data suggest that TJMAPs may constitute a platform on which the apical cytoskeletons and TJs associate to organize biological systems, although more study is needed to examine this possibility (Fig. 4.1).

Regarding the functions of the “TJ-apical complex,” multiciliated cells represent a highly specific case in which the apical microtubules are particularly highly developed. In tracheal multiciliated cells (MCCs), the apical microtubules have a specific role in establishing the regular arrangement of basal bodies (BBs), which generate cilia in the apical membranes (Kunimoto et al. 2012; Herawati et al. 2016; Tateishi

---

**Fig. 4.1** Immunofluorescence image (a) and schematic drawing (b) of the apical microtubule network existing beneath the apical membrane of epithelial cells. Microtubules (α-tubulin-staining), green; Tight junctions (ZO1-staining) red; Nuclei (DAPI-staining), blue. Scale, 10 μm. TJ, tight junction. Bar, 10 μm.
et al. 2017). To explore the differentiation mechanism leading to the regular BB alignment, we examined tracheal MCCs expressing GFP-centrin, a BB-associated protein, using our new long-term, high-resolution, live-imaging system. The microtubule-dependent regular arrangement of BBs is critical for the synchronous beating and metachronal waves of hundreds of motile cilia on the apical membrane of MCCs (Guirao and Joanny 2007; Elgeti and Gompper 2013). Thus, the physiological role of the apical cytoskeleton that we revealed in this case is likely to be essential for tissue function (Fig. 4.2).

By comparing the findings for various epithelial cells, both the common and unique characteristics of the apical cytoskeletal structures are being revealed. In the following sections, we discuss the roles of the apical cytoskeletons, especially microtubules, in epithelial cell sheets that have specific functional relevance (Fig. 4.3).

4.2 The Apical Cytoskeletons in General Epithelial Cells

In confluent epithelial cell sheets, each cell is highly polarized in the apico-basal direction, and the cells’ apical membranes are regarded as a continuous surface connected by TJs. Although the mechanism by which TJs are positioned at the most apical part of the lateral membrane is not understood, the TJs determine the edges
of each cell’s apical membrane when the cell sheet is viewed from the top (the apical view). Distinct, specific differentiation mechanisms occur in the apical area. Well-known classical examples of apical differentiation are the circumferential ring at cell-cell boundaries and the terminal web (Leblond et al. 1960; Hull and Staehelin 1979; Owaribe et al. 1981; Burgess 1982; Keller et al. 1985). In *Drosophila* epithelial cells, a “ratchet structure” consisting of actin filaments exists in the medial area of apical membranes (Martin et al. 2009), although no similar structure has been clearly identified in mammalian epithelial cells. By applying our super-resolution microscopy system to cultured epithelial cell sheets, we discovered the detailed structure of the apical cytoskeleton, which was previously unknown and uniquely distributed beneath the apical membrane like a shell (Yano et al. 2013). This location led us to propose that the apical cytoskeletal network is associated with TJs, which are located at the most apical part of the lateral membrane, and that these

---

**Fig. 4.3** Immunofluorescence images, circular diagrams, and schematic drawing of basal bodies (BBs) and basal feet (BF) during BB pattern development of Floret (a), Scatter (b), Partial Alignment (c) and Alignment (d). Orientation of each BB is shown by an arrow connecting the center of a BB marker (green) to the center of a BF marker (red) (insets) and by the circular diagrams of ciliary orientation (direction of white arrows in the immunofluorescence image). (Bottom) Illustration of cilia. Bar, 10 μm
structures form a system called the “TJ-apical complex.” However, to establish this new point of view, we needed to acquire evidence at the molecular level for the association of the apical cytoskeleton with the TJs.

We further examined the apical cytoskeleton in detail, by performing ultra-high voltage electron microscopy experiments in which the microtubules and intermediate filaments were tracked. In general epithelial cells in culture, the apical microtubules and intermediate filaments were distributed in the apical plane in rather uniformly scattered patterns and partly overlapped each other, without any specific patterns in their distribution (Tateishi et al. 2017). In this respect, it is notable that we identified four microtubule-associated proteins, which also associate with TJs, in our recent findings, TJ-associated microtubule-binding proteins. We believe that analyses of the TJ-associated microtubule-binding proteins, which may form the platform for associations among the apical cytoskeletons, TJs, and cell signaling molecules, represent a unique direction for studying epithelial cell sheets and epithelial barriers.

4.3 The Apical Cytoskeletons in Multiciliated Cells, a Possible Extreme Example of a “TJ-Apical Complex” with a Clear Function

MCCs drive fluid transport through coordinated ciliary beating, the direction of which is established by the BB orientation of hundreds of cilia on one cell (Salathe 2007). In airway MCCs, the BBs are uniformly oriented and linearly aligned by an unknown mechanism. To explore the mechanism for BB alignment, we observed GFP-centrin2-labeled BBs in mouse tracheal MCCs in primary culture using our long-term, high-resolution, live-imaging method (Herawati et al. 2016). We found that the differentiating BB arrays sequentially adopted four stereotyped patterns: a clustering “Floret,” “Scatter,” “Partial alignment,” and linear “Alignment” pattern. During this acquisition of regularity, we particularly noted that the patterns and densities of microtubules in the apical plane of the MCCs were well correlated with the BB patterning. In addition, the BB alignment was perturbed by disrupting the apical microtubules with nocodazole or by a basal foot (BF)-depleting Odf2 mutation. Based on these experimental results, we explored the development of BB alignment from random to the final well-ordered pattern biotheoretically. We found that the self-organization could be explained by applying hydrodynamic theories in which the apical cytoskeletons were treated as a two-dimensional viscous fluid that underwent a contractile force mediated by cytoskeletal motors and filament polymerization (Marchetti et al. 2013; Prost et al. 2015; Herawati et al. 2016). These results revealed the functional importance of the cytoskeletal components that exist in the apical plane of the epithelial cell sheet in tracheal MCCs. Although the relationship between the apical cytoskeletons and TJs remains to be elucidated, their
locations and binding molecules suggest that they are closely related both physically and functionally in MCCs. How the apical cytoskeleton is built by the TJ and its related signaling is another critical issue that remains to be explored.

4.4 Perspective

Epithelial cell sheets with a differentiated apical side are formed and organized by mechanisms involving apicobasal polarity, the details of which have been well addressed in other reviews (Shin et al. 2006; Nelson 2009; Rodriguez-Boulan and Macara 2014; Apodaca 2017). In general, to establish the apicobasal polarization in epithelial cell sheets, polarity proteins generate asymmetric membrane domains that form the basis for establishing the cell–cell adhesive TJs and adherens junctions (AJs), which combine to form apical junctional complexes (AJCs). In addition, planar cell polarity (PCP), which forms in the apical plane of epithelial cell sheets, is arranged perpendicular to the basolateral polarity. The actin filaments of the circumferential ring also lie horizontally along the apical membrane at cell boundaries. The “TJ-apical complex” expands horizontally below the apical membrane in an almost evenly scattered pattern. Since it includes TJs, it is likely to play a role in the paracellular barrier. On the other hand, since it is directly or indirectly associated with the apical membranes of epithelial cells, it probably also has a role in the transcellular barrier. Thus, the epithelial barrier is created and regulated by the combination of paracellular and transcellular barriers, which are determined, at least in part, by the “TJ-apical complex.” Our continued investigation of the “TJ-apical complex” in ciliated and non-ciliated epithelial cells is expected to unveil its physiological significance in a variety of biological epithelial barrier systems.

References

Anderson JM, Van Itallie CM, Fanning AS (2004) Setting up a selective barrier at the apical junction complex. Curr Opin Cell Biol 16:140–145. https://doi.org/10.1016/j.ceb.2004.01.005

Apodaca G (2017) Role of polarity proteins in the generation and organization of apical surface protrusions. Cold Spring Harb Perspect Biol:a027813. https://doi.org/10.1101/cshperspect.a027813

Burgess DR (1982) Reactivation of intestinal epithelial cell brush border motility: ATP-dependent contraction via a terminal web contractile ring. J Cell Biol 95:853–863

Elgeti J, Gompper G (2013) Emergence of metachronal waves in cilia arrays. Proc Natl Acad Sci 110:4470–4475. https://doi.org/10.1073/pnas.1218869110/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1218869110

Fleming TP, Papenbrock T, Fesenko I, Hausen P, Sheth B (2000) Assembly of tight junctions during early vertebrate development. Semin Cell Dev Biol 11:291–299. https://doi.org/10.1006/scdb.2000.0179
Furuse M, Moriwaki K (2009) The role of claudin-based tight junctions in morphogenesis. Ann N Y Acad Sci 1165:58–61. https://doi.org/10.1111/j.1749-6632.2009.04441.x

Guirao B, Joanny JF (2007) Spontaneous creation of macroscopic flow and metachronal waves in an array of cilia. Biophys J 92:1900–1917. https://doi.org/10.1529/biophysj.106.084897

Herawati E, Taniguchi D, Kanoh H, Tateishi K, Ishihara S, Tsukita S (2016) Multiciliated cell basal bodies align in stereotypical patterns coordinated by the apical cytoskeleton. J Cell Biol 214:571–586. https://doi.org/10.1083/jcb.201601023

Hull BE, Staehelin LA (1979) The terminal web. A reevaluation of its structure and function. J Cell Biol 81:67–82. https://doi.org/10.1083/jcb.81.1.67

Keller TCS, Conzelman KA, Chasan R, Mooseker MS (1985) Role of myosin in terminal web contraction in isolated intestinal epithelial brush borders. J Cell Biol 100:1647–1655

Kunimoto K, Yamazaki Y, Nishida T, Shinhara K, Ishikawa H, Hasegawa T, Okanoue T, Hamada H, Noda T, Tamura A, Tsukita S, Tsukita S (2012) Coordinated ciliary beating requires Odf2-mediated polarization of basal bodies via basal feet. Cell 148:189–200. https://doi.org/10.1016/j.cell.2011.10.052

Leblond CP, Puchler H, Clermont Y (1960) Structures corresponding to terminal bars and terminal web in many types of cells. Nature 186:784–788

Marchetti MC, Joanny JF, Ramaswamy S, Liverpool TB, Prost J, Rao M, Simha RA (2013) Hydrodynamics of soft active matter. Rev Mod Phys 85:1143–1189. https://doi.org/10.1103/RevModPhys.85.1143

Martin AC, Kaschube M, Wieschaus EF (2009) Pulsed contractions of an actin-myosin network drive apical constriction. Nature 457:495–499. https://doi.org/10.1038/nature07522

Nelson WJ (2009) Remodeling epithelial cell organization: transitions between front-rear and apical-basal polarity. Cold Spring Harb Perspect Biol 1:1–19. https://doi.org/10.1101/cshperspect.a000513

Owaribe K, Kodama R, Eguchi G (1981) Demonstration of contractility of circumferential actin bundles and its morphogenetic significance in pigmented epithelium in vitro and in vivo. J Cell Biol 90:507–514. https://doi.org/10.1083/jcb.90.2.507

Prost J, Jülicher F, Joanny J (2015) Active gel physics. Nat Phys 11:111–117. https://doi.org/10.1038/nphys3224

Rodriguez-Boulan E, Macara IG (2014) Organization and execution of the epithelial polarity programme. Nat Rev Mol Cell Biol 15:225–242. https://doi.org/10.1038/nrm3775

Salathe M (2007) Regulation of mammalian ciliary beating. Annu Rev Physiol 69:401–422. https://doi.org/10.1146/annurev.physiol.69.040705.141253

Shin K, Fogg VC, Margolis B (2006) Tight junctions and cell polarity. Annu Rev Cell Dev Biol 22:207–235. https://doi.org/10.1146/annurev.cellbio.22.010305.104219

Tanaka H, Tamura A, Suzuki K, Tsukita S (2017) Site-specific distribution of claudin-based paracellular channels with roles in biological fluid flow and metabolism. Ann N Y Acad Sci 1405:44–52. https://doi.org/10.1111/nyas.13438

Tateishi K, Nishida T, Inoue K, Tsukita S (2017) Developmental cell three-dimensional organization of layered apical cytoskeletal networks associated with mouse airway tissue development. Sci Rep 7:1–10. https://doi.org/10.1038/srep43783

Tsukita S, Furuse M, Itoh M (2001) Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol 2:285–293. https://doi.org/10.1038/35067088

Van Itallie CM, Anderson JM (2014) Architecture of tight junctions and principles of molecular composition. Semin Cell Dev Biol 36:157–165. https://doi.org/10.1016/j.semcdb.2014.08.011

Yano T, Matsui T, Tamura A, Uji M, Tsukita S (2013) The association of microtubules with tight junctions is promoted by cingulin phosphorylation by AMPK. J Cell Biol 203:605–614. https://doi.org/10.1083/jcb.201304194
Yano T, Kanoh H, Tamura A, Tsukita S (2017) Apical cytoskeletons and junctional complexes as a combined system in epithelial cell sheets. Ann N Y Acad Sci 1405:32–43. https://doi.org/10.1111/nyas.13432

Open Access This chapter is licensed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the chapter’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.