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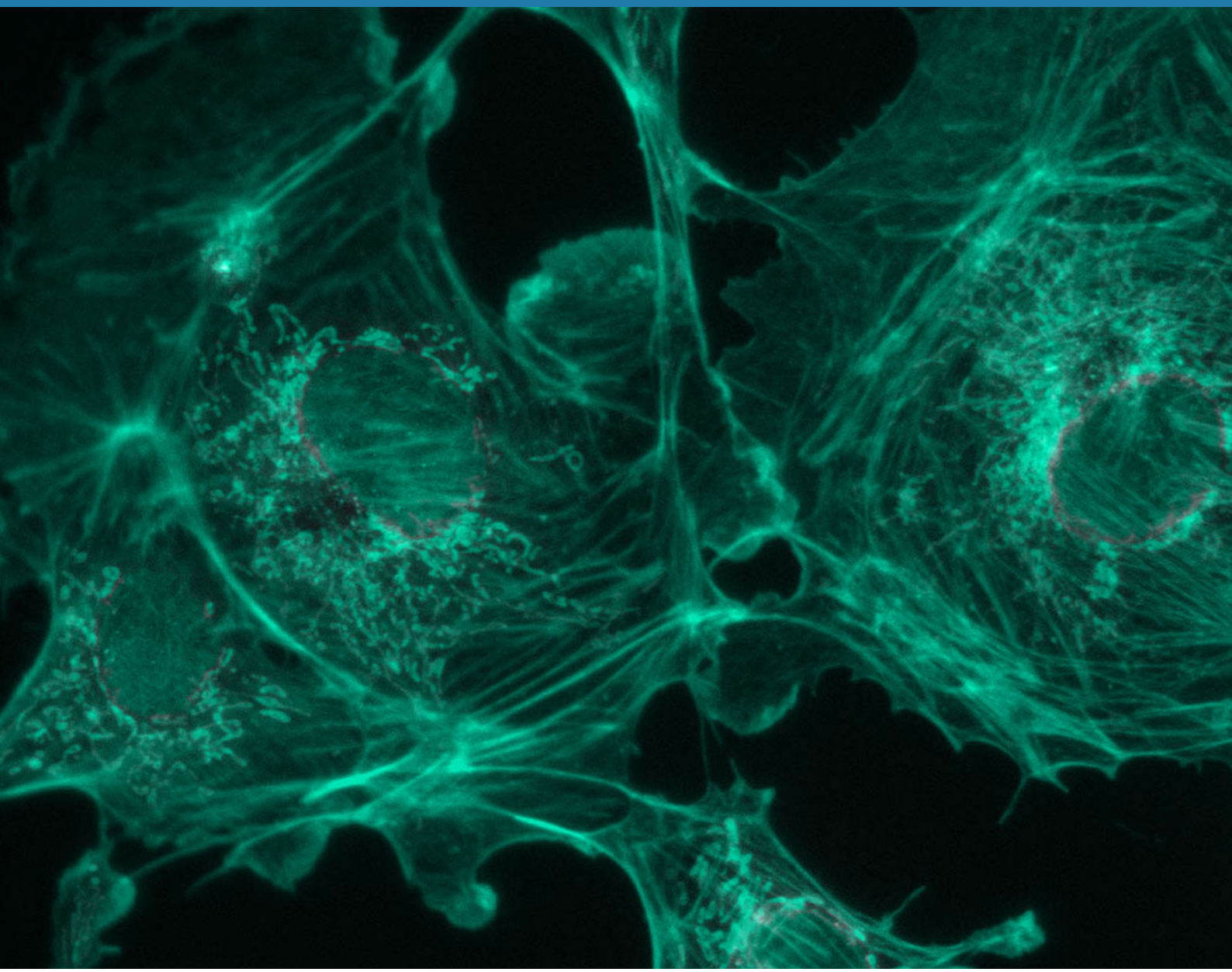

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MORE THAN TRACKS: HOW THE CYTOSKELETON BUILDS EPITHELIA ASYMMETRY

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ABSTRACT
Epithelia are three-dimensional arrangements of cells organized into structures which delimit morphologically and physiologically different compartments. At the cellular level, epithelial cells are characterized by the presence of an apical pole, facing the lumen of an organ or the outer environment, and baso-lateral domains, involved in cell-extracellular matrix and cell-cell interactions. Each pole is characterized by its specific protein and lipid membrane composition, and its distinct assembly of cellular organelles. This polarized organization of cell components ensures the vectorial absorption and secretion of proteins and other molecules, which constitute the essential function of epithelia. Actin and microtubule cytoskeletons are also polarized in epithelial cells. This organization contributes to the *de novo* establishment as well as to the functional maintenance of epithelial polarity. In this article we delineate the central features of epithelial polarity and summarize the current knowledge on how actin and microtubule cytoskeletons contribute to epithelial organization.

Key words: actin, microtubules, epithelial polarity

RESUMEN
Los epitelios son arreglos tridimensionales de células, organizados en estructuras que delimitan compartimientos morfológica y fisiológicamente diferentes. A nivel celular, las células epiteliales se caracterizan por la presencia de un polo apical, en contacto con la luz de un órgano o con el medio extracelular, y los dominios baso-laterales, responsables de las interacciones célula-matriz extracelular y célula-célula. Cada polo se caracteriza por la composición proteica y lipídica específica de sus membranas, así como por el ensamble distintivo de las organelas celulares asociadas. Esta organización polarizada de los componentes celulares garantiza el desarrollo de la función esencial de los epitelios, i.e. la absorción y/o secreción vectorial de proteínas y otras moléculas. Los citoesqueletos de actina y microtúbulos también están polarizados en las células epiteliales, lo que contribuye tanto al establecimiento de novo, como al mantenimiento funcional de la polaridad epitelial. En este artículo delineamos los aspectos centrales de la polaridad epitelial y resumimos el estado de conocimiento actual en relación a la contribución de los citoesqueletos de actina y microtúbulos a la organización epitelial.

Palabras clave: actina, microtúbulos, polaridad epithelial.

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Introduction

An epithelium (the trophectoderm) is the first tissue that differentiates during embryogenesis [1]. The epithelium is also the first tissue that emerged during phylogenesis. In fact, the initial epithelial structures precede metazoan multicellularity: upon starvation, individual Dictyostelium discoideum social amoebas aggregate to form a fruiting body, which includes a tubular epithelial monolayer that surrounds a collection of spores [2]. Both that primitive epithelia and modern epithelia share their function of segregating an internal media from an external environment, what is tightly associated to their coordinated asymmetric distribution of cellular components, and the presence of cell-cell junctions with selective permeability (Figure 1). This organization ensures the vectorial absorption and secretion of proteins and other molecules, which constitute the essential function of epithelia. The establishment and maintenance of that organization imply two types of cell asymmetry: apico-basal polarity, which refers to the distribution of cell components within each individual cell, and planar polarity, which denotes the collective alignment of cell polarity across the tissue plane [3]. Over the last decades, studies performed in *Caenorhabditis elegans*, *Drosophila melanogaster* and mammalian epithelial cells have contributed to the understanding of the network of polarity proteins and lipids that defines the differentiation of distinct cell poles. These investigations identified three basic polarity protein complexes which are conserved throughout evolution: the crumbs (Crumbs/PALS1/PATJ) complex, the PAR (PAR3/PAR6/aPKC/cdc42) and the related PAR4/LKB1 complex, and the Scribble (Scribble/Discs-large (Dlg)/Lethal giant larvae (LGL)) complex. Most of these studies have been nicely reviewed [4-6]. Moreover, several studies have focused on how the interference with the functionality of these networks can lead not only to loss of epithelial function, but also to uncontrolled cell proliferation and development of epithelial derived malignant tumors [7]. An important question to address in order to understand epithelial homeostasis is how polarity signals, trafficking events, and cell cycle issues are integrated to define epithelial architecture. Traditionally, the cytoskeleton and its associated motor proteins were viewed to be passive actors in the construction of cell architecture, functioning downstream regulatory molecules that have already been localized by pre-existing spatial cues. However, results from different model systems have supported the idea that the cytoskeleton also drives the symmetry-breaking process by localizing key regulatory molecules to specific cortical sites [8]. In the present article we delineate the main characteristics of the epithelial polarity and recapitulate the current knowledge on the mechanisms by which the actin and microtubule cytoskeletons contribute to the integration of the different factors that determine epithelial organization.

Epithelial polarity

At the cellular level, epithelial polarity is characterized by the presence of an apical pole, facing the lumen of an organ or the outer environment, a lateral domain, involved in cell-cell interactions, and the basal pole, in contact with the extracellular matrix (ECM). Most epithelial cells develop a columnar type of apico-basal polarity, which implies the organization of a simple apical pole that opposes to the basal domain (Figure 1). Multi-layered epithelia, such as the skin epidermis, develop this type of polarity, but restricted only to the most apical layer of viable cells, whereas the most basal cells are non-differentiated and proliferative [9]. On the other hand, hepatocytes develop a particular type of polarity, where there is more than one apical domain, giving rise to multiple “bile canaliculus” per cell [10]. In any case, the establishment of cell-cell, and cell-ECM interactions constitute the founding events in the acquisition of the epithelial polarity. In vertebrates, cell-cell junctions include tight junctions, adherent junctions, gap junctions and desmosomes. Tight and adherent junctions, also denominated “apical junction complex”, are particularly important for the formation and the

maintenance of apico-basal polarity. Formation of the apical junction complex depends on the polarity complexes Crumbs and PAR. The latter protein complex determines the localization of the apical pole, by mutually excluding the "basolateral" scribble complex [4]. Thereafter, apical cellular junctions contribute to the segregation of the apical and basolateral poles. Whereas apical junctions contribute to the specification of the apical pole location, the cell-ECM interaction determines the polarity axis orientation. An initial event is the interaction of β1-integrins with the extracellular matrix, what leads to the assembly of a protein complex at the cytosolic integrin domain that transduce the signals originated at the ECM to control cytoskeleton dynamics and intracellular signaling [11,12]. The proteins associated to the cellular junctions (Figure 3) as well as β1-integrins are also involved in the specification of epithelial cell division axis by determining the mitotic spindle orientation [13-15]. Both the establishment of cell-cell and cell-ECM interactions and the derived intracellular signaling that ensures the formation of the apico-basal asymmetry depend on the association of those junctions with the microtubule and actin cytoskeleton.

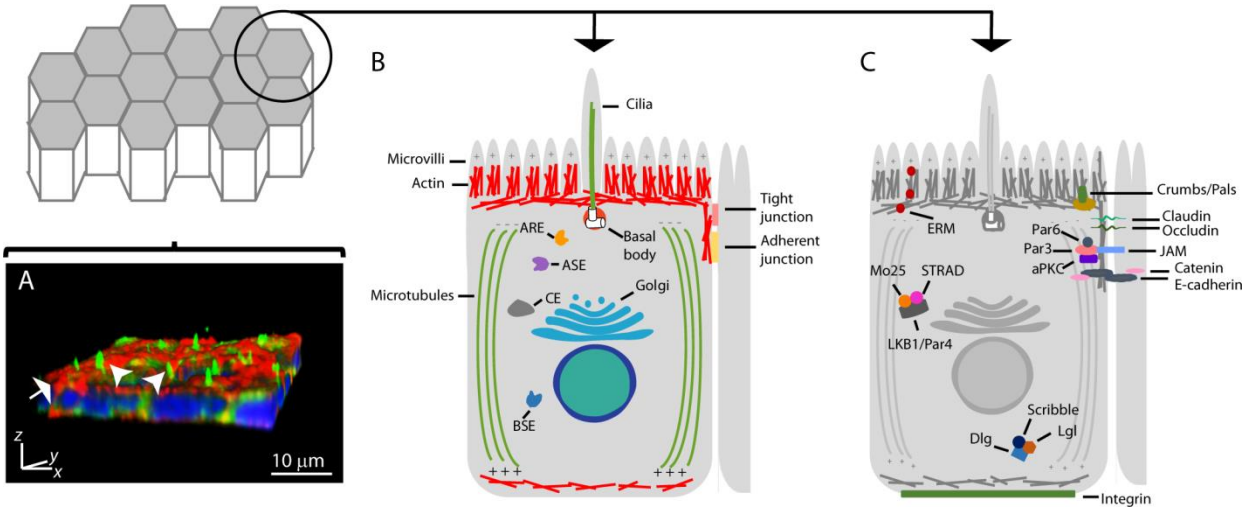

Figure 1. Epithelial organization. The image shows a three dimensional reconstruction of F-actin (red), α-tubulin (microtubules, green) and DAPI (nucleus, blue) staining of the prototype epithelial cells MDCK cultured to polarize on filters (A). Arrowheads indicate microtubule staining at the primary cilia. Arrows specify actin organization at the brush border. The diagram above the image represents the hexagonal prism organization of cells at the epithelial monolayer. The schemas illustrate the polarized distribution of the actin and microtubule cytoskeleton, the Golgi apparatus, the centrosome and the endosomal compartments (B), and the polarity and apical junction protein complexes (C) [4,5]. BSE, basal sorting endosomes; CE, common endosomes; ASE, apical sorting endosomes, ARE, apical recycling endosomes, ERM, ezrin-radixin-moesin.

Acting in the epithelia polarity

Actin filaments are polymers of actin subunits organized in a polarized lattice with two structurally distinct ends: barbed (plus) ends, enriched in GTP bound actin, and pointed (minus) ends, which contain ADP bound actin and exhibits a lower rate of actin incorporation. In differentiated epithelia, these intrinsically polarized filaments organize different arrays at each cell domain (Figure 2), where they participate in processes as diverse as the formation of the apical microvilli, the development of the cellular junctions, the interaction of the basal structures with the ECM, and the regulation of vesicle sorting at the Golgi apparatus and

endosomal compartments. Furthermore, in association with myosin motors, actomyosin contraction drives 3D epithelial tissue organization by generating forces at the cellular level. The latter phenomena have been nicely reviewed elsewhere [16]. In this review we will focus on actin organization and function in apico-basal cell polarity.

Microvilli are cylindrical cell protrusions present at the apical pole of epithelial cells, which increase the cell surface available for apical secretory or absorptive transport. In most epithelial cells which coat hollow organs, these apical protrusions constitute the brush border (Figure 2). A very well-characterized example is the small intestinal cell microvilli. In this case, up to 1000 microvilli protrude from a single apical cell surface. This tight organization of membrane protrusions is enabled by the highly ordered patterning of the actin cytoskeleton, which is organized as parallel bundles of 10-30 filaments situated at the core of each protrusion with the barbed ends facing the plasma membrane at the microvillus tip. These actin bundles are assembled together by crosslinking proteins such as villin, fimbrin or fascin, and interact with the membrane by means of a group of myosin motors, which include myosin-1a, myosin-6 and myosin-7a/b, and proteins of the ezrin/radixin/moesin family [17]. The polymerization and bundling of these actin arrays provide the force to generate microvillar membrane protrusions [18]. Beyond their role in the genesis of the microvilli, actin filaments constitute tracks for myosin dependent trafficking of apical transporters along these structures [19, 20], and participates in apical receptor mediated endocytosis and protein internalization [21].

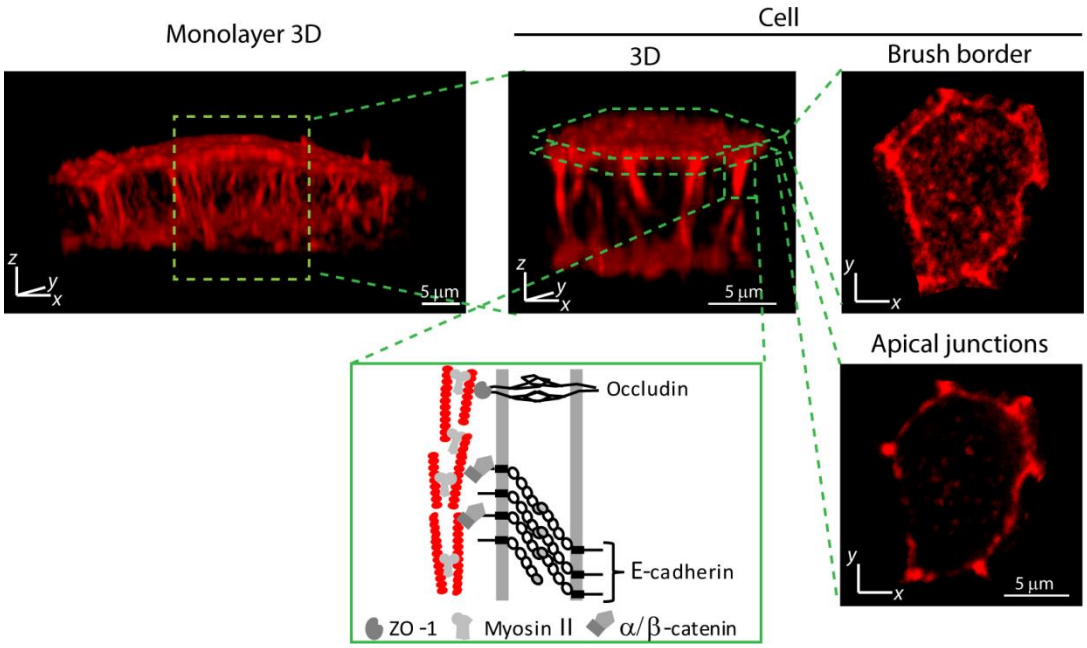

Figure 2. Actin cytoskeleton in epithelial cells. Images show monolayer (A) or single cell (B) 3D reconstructions of F-actin staining of polarized MDCK cells, and single *xy* planes of the same cell at the brush border (C) and apical junction (D) level. The schema illustrates actin (red beads) interaction with apical junction complexes.

The perijunctional actin cytoskeleton comprises actin bundles of filaments which are associated to the apical junctions by directly interacting with E-cadherin associated catenins [22] and occludin associated zonula occludens 1 (ZO-1) [23] (Figure 2). The acto-myosin and junctional complexes form a belt-like ring around the cell perimeter which is responsible for the junctional tension, and can produce contraction of the microvilli. During the initial establishment of apical junctions, actin filaments promote the formation of filopodia which

penetrate into neighboring cells, catalyzing the clustering of apical junction proteins such as E-cadherin and catenins [24]. At this stage, cdc42, Rac1 and Rho GTPases are responsible for recruiting a cohort of proteins to the site of cell-cell contact, which promote actin nucleation and remodeling and acto-myosin contraction, leading to junction maturation [25]. In mature epithelia, the junctional actin cytoskeleton contributes to the regulation of apical junction's stability. On one side, actin nucleation induced by the cdc42/WASP/ARP2/3 complex contributes to E-cadherin endocytosis therefore ensuring adherent junction's remodeling [26]. Additionally, acto-myosin contractility induced by RhoA maintains cell-cell junction tension [27]. Besides its role in promoting apical junctions formation and dynamics, the perijunctional actin cytoskeleton interaction with ZO-1 conditions the formation of organotypic epithelial structures with a single lumen by regulating the symmetric orientation of the mitotic spindle in dividing cells [28].

In the basal surface of epithelial cells cultured on a rigid surface, actin microfilaments organize as stress fibers that reach the plasma membrane through their indirect interactions with integrins. Studies performed in three-dimensional epithelial cells showed that the interaction of β1-integrin with biochemically and biophysically defined ECMs can initiate a signaling pathway that decreases RhoA activation. RhoA inhibition suppresses peripheral actomyosin contraction, leading to downregulation of focal adhesions and centrosome relocation to the opposite pole of the cell, thus ensuring the correct positioning of the apical domain [29-31]. Thus, basal actin cytoskeleton acts as a mechano-transducer which participates in the sensing of the ECM during epithelial cell differentiation.

Besides the distinct arrangements of actin microfilaments associated to plasma membrane, there are also subsets of actin filaments associated to the different cellular organelles. For instance, it has been described the presence of a population of short-branched actin filaments specifically concentrated around the Golgi complex, which associates with budding vesicles [32]. These findings are in agreement with different studies which described that the interference with actin regulatory proteins or with actin dynamics affects the exit of apical and basolateral proteins from the trans-Golgi network in different ways [33, 34]. Even though the mechanisms involved in the organization of these filaments or contributing to the generation of specific cargo routes exiting the Golgi complex are far from being elucidated, Rodriguez Boulan and col. characterized the presence of a dynamic population of actin filaments at the Golgi region that shows remarkable specificity in promoting the dynamin-mediated fission of carrier vesicles for apical but not for basolateral markers [35].

Microtubules in epithelial polarity

Microtubules are protofilaments composed of α- and β-tubulin heterodimers which associate laterally to form hollow tubes. Microtubule filaments are themselves polarized structures that harbor plus and minus ends, characterized by their distinct dynamic instability. Microtubule nucleation occurs at the minus end, and is mostly dependent on γ-tubulin ring complexes, to which they can remain attached. Conversely, microtubule plus ends are subject to continuous polymerization and depolymerization events, regulated by their associated stabilizing and severing proteins [36]. Microtubule stability is also regulated by tubulin post-translational modifications, which condition the affinity and activity of microtubule associated proteins [37].

Whereas in non-differentiated cells microtubule minus ends are generally anchored to a centrally positioned centrosome, thus displaying a radial array, in differentiated epithelial cells microtubules mostly organize in vertical arrays, with their minus ends anchored to alternative microtubule anchoring proteins located at the apical membrane and at apical junctions, and their plus ends oriented towards the basal membrane (Figure 3) [38-40]. In these cells the centrosome also localizes apically, where it constitutes the basal body of the primary cilia

[41], but does not function as a microtubule organizing center [42]. Studies performed in three dimensional cultures of breast epithelial cells showed that β1 integrin interaction with the ECM induces microtubule plus ends polarization towards the basolateral membrane, therefore controlling the orientation of epithelial polarity axis [12]. Besides this main arrangement of microtubules, there are subapical and basal meshes of shorter microtubules with random orientation [43].

Microtubule participation in the maintenance of epithelial polarity has been traditionally attributed to their conventional role as polarized tracks that can direct the traffic of membrane components to the already developed apical and basolateral membranes. Maintenance of distinct apical and basolateral poles requires sorting of newly synthesized as well as endocytosed proteins. Sorting in the biosynthetic pathway occurs either at the trans-Golgi network or, upon endocytosis from the basolateral domain, at the common endosome. Proteins endocytosed at the apical membrane, after an initial sorting at the apical sorting endosome, also converge at that compartment (Figure 1). From the common endosome, proteins can be sorted to the apical pole, via the apical recycling endosome, or towards the basolateral membrane [44]. Exocytic transport from the Golgi, transcytosis from the basolateral to the apical pole and apical recycling, but not basolateral recycling, are microtubule dependent [43]. The asymmetric microtubule distribution facilitates an efficient polarized vesicle targeting. By association with minus-end-directed motors (mostly dynein), post-Golgi exocytic and postendocytic vesicles would be directed towards the MT minus-ends facing the cell apex, while association with plus-end-directed motors (most of kinesins) could target vesicles to the MT plus-ends rich basal surface [43].

Beyond their well characterized role as polarized tracks, microtubules also participate in several events that are crucial for the establishment of epithelial polarity, which are not dependent on microtubule-based vesicle trafficking. As we mentioned above, the organization and stability of adherent junctions is tightly dependent on the actin cytoskeleton and its associated motors. Yap and collaborators showed that microtubule plus ends also participate in the local concentration of E-cadherin, by a mechanism that most probably involves the regulation of myosin II activity at cell-cell contacts [44]. Such a role for dynamic microtubules in the regulation of myosin II activity would also be consistent with increasing evidence that supports the functional interplay between microtubules and the actin cytoskeleton [46]. As a matter of fact, our recent studies showed that centrosomal derived microtubules modulate the "de novo" organization of the brush border and support an essential role for microtubule plus ends in triggering the apical actin organization [47]. Likewise, Golgi derived microtubule plus ends participate in the development of "bile canalicular" structures by facilitating apical actin organization in hepatic cells [48]. Therefore, those studies reveal a novel role of microtubule plus ends as signaling platforms that integrate microtubule with actin dynamics during the initial events that define the epithelial polarity.

The microtubule cytoskeleton also participates in the maintenance of epithelial planar polarity by modulating mitotic spindle orientation. The organization of epithelial cells to form hollow organs with a single lumen entails that each cell divides symmetrically within the epithelial plane, so that both resulting daughter cells remain in the same plane. Hence, the mitotic spindles must orient within the planar axis (Figure 3). Perpendicular divisions, on the other hand, are necessary to create stratified epithelia. Defective spindle orientation flaws the axis of cell division and could eventually disrupt epithelial organization and generate daughter cells unrestrained by contact with neighbors [13]. Mitotic spindle positioning in most epithelia is controlled by astral microtubules that are nucleated at the spindle poles and orient their plus ends towards the cell cortex. There are two primary events that determine spindle orientation: [1] establishment of a polarity axis by the asymmetric distribution of polarity proteins at the

cell cortex; and [2] alignment of the mitotic spindle with respect to this polarity axis by microtubule plus ends "capture" at the cortical sites thus defined.

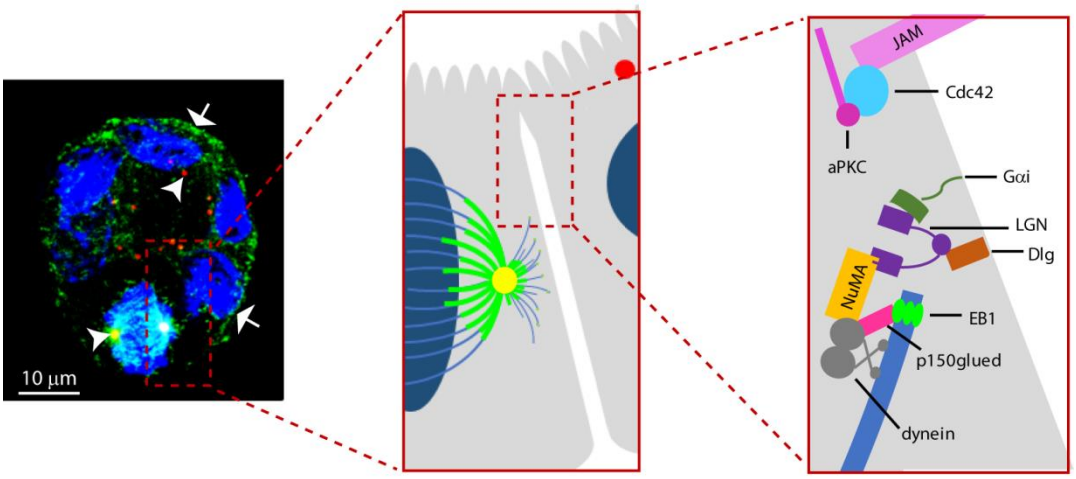

Figure 3. Microtubules role in spindle orientation. The image shows merged EB1 (green), γ-tubulin (red) and DAPI (blue) staining of a central *xy* plane of MDCK organotypic structures. Arrowheads point centrosomes which are located at the apical pole (facing the lumen) in polarized cells, and constitute the spindle poles in mitotic cells (bottom). γ-tubulin/EB1 colocalization at spindle poles is denoted in yellow. Arrows indicate EB1 labeled microtubule plus ends at the basolateral pole. The schema represents the interactions between lateral cortical proteins and microtubules plus ends associated proteins that define the planar axis of epithelial cell division.

The cortical proteins that determine the spindle alignment generate a biased orientation of the spindle by establishing physical connections with astral microtubules plus-ends binding proteins and stabilizing these otherwise highly dynamic structures. The cortical protein complexes that interact with astral microtubule plus ends include the Gαi/LGN complex, which is recruited to the lateral membrane by Dlg and E-cadherin, and excluded from the apical membrane by aPKC phosphorylation. In turn, LGN interacts with "nuclear mitotic apparatus" (NuMA), which binds to dynein/dynactin, the motor protein responsible for the astral-microtubule pulling force that directs the movement of the spindle (Figure 3) [13]. Another cortical protein complex composed by cdc42 and the junctional adhesion molecule-A (JAM-A) that promotes dynein/dynactin gathering at the lateral membrane have also been identified [49]. Far less is known about the molecular actors that regulate spindle orientation at the spindle apparatus. The end-binding protein 1 (EB1) is an autonomously plus end binding protein that regulates microtubule dynamic instability by increasing the periods of microtubule growth, and decreasing those of microtubule severing. In addition to its role in the formation and stabilization of spindle microtubules, studies in drosophila indicated that EB1 is a crucial factor for spindle orientation during symmetric planar division in epithelial cells [50]. Our own studies performed in three dimensional epithelial cell cultures showed that EB1 is loaded on astral microtubules at the spindle poles, and that its presence at these structures is essential for spindle orientation and accurate lumen formation [51]. Although the exact mechanism governing EB1 directed spindle orientation has not been elucidated, EB1 is a scaffold that recruits specific proteins to the microtubule plus ends, including the dynactin subunit p150 glued and the polarity protein Par1, both involved in spindle orientation. P150 glued itself regulates spindle orientation, probably by enhancing dynein processivity [52].

Summary

In the present review we summarized how F-actin and microtubules and their associated motor proteins are common actors in the regulatory events that condition the development of the multicelular apico-basal epithelial organization: first, signaling downstream integrins interaction with the ECM; second, assembly and recycling of apical junctions; third, formation of F-actin sustained apical membrane protrusions; fourth, polarized trafficking of membrane components and fifth, regulation of mitotic spindle orientation. Therefore, the data reviewed support a model where actin and microtubule cytoskeleton are much more than tracks: they are pivots of epithelial homeostasis.

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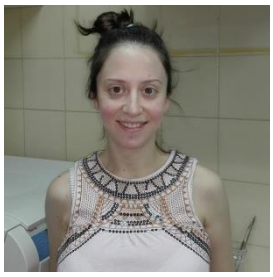

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