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Polarity and Stratification of the Epidermis

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Abstract

Polarity is a fundamental property of epithelial cells. In this review, we discuss our current knowledge of the polarity of a stratified epithelium, the epidermis, focusing on similarities and differences with simple epithelial models. We highlight how the differences in tissue architecture and physiology result in alterations in some aspects of cell polarity. In addition, we discuss one of the most prominent uses for cell polarity in the epidermis – orienting the mitotic spindle to drive the stratification and differentiation of this tissue during development.

1. Introduction

While polarity of some simple epithelial cells has been studied extensively, our knowledge of how cell polarity is generated, maintained, and utilized in stratified epithelia is just beginning to emerge. In contrast to simple epithelium, the stratified epithelium of the skin is composed of numerous cell layers. The basal keratinocytes of the epidermis have many of the same polarity components as simple epithelial cells; however, owing to the unique architecture of the skin, there are important differences in both the establishment and roles of polarized proteins in these two types of epithelia.

Simple epithelia have lateral domains in contact with other cells, an apical domain exposed to a lumen or the environment, and often a basal domain that interacts with the underlying extracellular matrix (Fig. 1). Stratified epithelia maintain the basal domain, but all other surfaces are in contact with other cells. Because there is no exposed apical domain, we will refer to the apical surface as the side opposite the basement membrane for the remainder of the review. Because of this architectural difference, it was unclear 1) whether basal keratinocytes were polarized and 2) what functions this polarity imparts to the tissue. Here we discuss data demonstrating that these cells are polarized in some respects and that this polarity is important for generating proper tissue architecture through directing mitotic spindle orientation.

2. Polarity

Polarity is a general term that can be used in reference to various cellular components. Below, we discuss the polarization state of transmembrane proteins, peripheral membrane proteins, cell junctions, the cytoskeleton, organelles, and lipids. We do not yet fully

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understand the interdependencies between the polarization of these components; therefore, we attempt to distinguish between these types of polarity when discussing molecular mechanisms.

2.1 Polarity of transmembrane proteins

In basal keratinocytes many basolateral markers, including the epidermal growth factor receptor (EGFR) and E-cadherin, are found all around the surface of the cell. In contrast, data on the localization of many transmembrane markers that are apically localized in simple epithelia, such as the hydrogen potassium ATPase and Crumbs3, are lacking. There are a number of possible explanations for this lack of demonstrable polarity of an apical domain separate from the lateral membranes. First, apical membranes may indeed be polarized; however, as keratinocytes have no microvilli or other apical elaborations, the decreased surface area may make it harder to detect the polarity markers by immunofluorescence. Consistent with this, Par3 (section 2.3) shows much stronger apical enrichment in enterocytes than in basal keratinocytes (our unpublished data). Second, stratified and simple epithelia have different physiologies and serve different purposes. As some commonly used markers of apical polarity have physiological roles that are not important for epidermal function, the relevant polarized transmembrane proteins in basal cells may have yet to be identified. Third, there may be no functional distinction between apical and lateral membranes in keratinocytes; rather, they form one continuous structure.

2.2 Polarization of cell-cell junctions

One of the hallmarks of polarized simple epithelia is asymmetric localization of cell-cell junctions and cell-substratum adhesions [1, 2]. Though epidermal cells contain these same junctional components, the organization of some of them is quite distinct from those in simple epithelia.

2.2.1 Polarization of cell-ECM adhesion proteins—The basal domain of keratinocytes is adjacent to the basement membrane, an extracellular matrix (ECM) that is secreted by both the basal keratinocytes and underlying fibroblasts [3, 4]. Integrin are polarized to the basal side of basal keratinocytes, where they facilitate attachment to the basement membrane. The roles of integrins and their associated proteins in generating polarity in migrating cells has been studied extensively and has influenced our thinking about their roles in apical-basal polarity. Due to space constraints, we are unable to cover this literature, but it has been extensively reviewed in a number of recent articles [5, 6].

The two most extensively studied integrin-based structures that are polarized to the basal domain in both keratinocytes and simple epithelial cells are “focal adhesion”-type integrins (mostly prominently α/β1 integrin pairs in the epidermis) and the hemidesmosomes. Hemidesmosomes are constructed around α6/β4 dimers exclusively on the basal side of the cell to bridge the intracellular keratin network and the ECM [7]. The tight localization of β4 integrin is likely due in large part to the presence of its ligand, laminin, only in the ECM [8]. However, there may also be additional vesicular pathways that target β4 integrin and ECM components to the basal membrane [9]. At the basal surface of the cells, polarized β1 integrins contact the underlying ECM and activate signaling and F-actin attachment within the cytoplasm. The polarized localizations of the integrins therefore generate polarized cytoskeletal attachments and organizations [10]. While the attachment of keratins to hemidesmosomes has been visualized by electron microscopy, an in vivo description of the organization and dynamics of integrin-actin contacts has not yet been achieved.

β1 integrin is required for the maintenace of cell polarity in the epidermis [11]. Loss of this protein results in a severely compromised basement membrane and mislocalization of

integrins around the cell cortex. In addition, the polarization of peripheral membrane proteins was lost and cell division orientation was randomized [12]. How β1 integrin regulates cell polarity in keratinocytes is not yet understood mechanistically. However, in cultured MDCK cells, β1 integrin promotes polarization by signaling through the small GTPase, Rac1, and phosphatidylinositol 3-kinase [13-15].

2.2.2 Cell-cell junction polarity—Simple epithelial cells exhibit pronounced polarization of cell-cell junctions. Their tight junctions and zonula adherens are localized apically, and adherens junctions (AJs) are present on the basolateral membrane. Basal keratinocytes lack tight junctions and zonula adherens, and E-cadherin is found all around the cell cortex. This observation raises intriguing questions about whether AJs, thought to act as signaling centers to generate polarity in simple epithelia, play a similar role in keratinocytes despite being unpolarized. In order to address this question, a number of studies have generated conditional knockouts of components of the AJ in the epidermis. Mice lacking both E- and P-cadherin in the epidermis (the two classical cadherins expressed in this tissue) exhibited tissue integrity and adhesion defects but did not have polarity problems [16]. Similarly, conditional deletion of p120-catenin in the epidermis resulted in hyperplasia, though no problems in polarity were described [17, 18]. However, conditional ablation of α-catenin in the epidermis induced hyperplasia and loss of polarity, along with disruption of a number of signaling pathways [12, 19-21]. Therefore, α-catenin's function in cell polarity may be independent of its adherens junction role.

2.2.3 The special case of tight junctions—Tight junctions are not present in the basal cells of the epidermis, forming only in the differentiated granular layer [22, 23]. There, similar to the apically localized tight junctions in simple epithelia, they restrict the flow of small molecules, generating a barrier between the living inner cells and the dying and dead surface cells. When considering polarity across the whole epidermis, rather than across a single cell, tight junctions are polarized. The transcriptional control and functional assembly of tight junction proteins in granular cells have not been extensively examined.

It is tempting to speculate that the lack of tight junctions explains the apparent absence of polarization of membranes and membrane proteins in basal cells. While plausible, there are several lines of evidence against this idea. First, distinct membrane domains have been observed in the absence of cell-cell contacts [24]. Second, loss of tight junction components, while resulting in defects in transepithelial resistance, did not cause loss of membrane protein polarity [25, 26]. These data demonstrate that some aspects of polarity do not require tight junctions.

2.3 Peripheral membrane-associated protein polarity

Even though basal keratinocytes lack a demonstrable apical domain characterized by distinct lipid composition and transmembrane proteins, they robustly polarize several peripheral membrane associated proteins to the apical side of the cell. The conserved polarity protein Par3 is apically localized in keratinocytes [12]. At the apical cortex, Par3 forms a complex with atypical Protein Kinase C (aPKC) and Par6, which, based on work in other model organisms and tissues, is thought to be crucial for proper cellular polarity [1, 2]. Studies addressing whether and how these proteins generate polarity in the epidermis have not yet been reported. In simple mammalian epithelia, investigations into the function of these proteins have focused primarily on their roles in tight junction formation at the apical surface [27-31]. Clearly, further investigation is required to elucidate how Par3 and its partners can themselves polarize to the apical surface of basal keratinocytes and how they polarize the cytoplasm.

Another peripheral membrane protein, the NF2 tumor suppressor Merlin, is required for apicobasal polarity in basal keratinocytes by linking the AJ to Par3, suggesting a connection between cell-cell junctions and polarity in the epidermis [32]. It remains unclear, however, how adherens junctions, present along both the apical and lateral membranes of basal keratinocytes *in vivo*, can induce apicobasal polarity.

A number of cytoskeletal regulators and receptors that have been shown to be crucial polarity regulators in simple epithelia do not appear to have conserved functions in the epidermis. Cdc42, a member of the actin-regulating Rho family of GTPases, is required for proper polarization in a wide variety of model organisms and tissue types [33]. When Cdc42 was conditionally ablated in the mouse epidermis, polarity was not dramatically affected, though late-developing defects in the basement membrane were described [34, 35]. Similarly, Rac1, another GTPase that is known to regulate polarity in simple epithelia, had no reported polarity defects when conditionally deleted in the mouse epidermis (although it was profoundly required for epidermal maintenance) [36, 37]. Whether redundancy exists in the epidermis that masks polarity defects when individual Rho GTPases are conditionally deleted has yet to be explored and should be addressed by further genetic studies.

2.4 Membrane polarity

The apical domain of some simple epithelia contain higher levels of phosphatidylinositol 4,5 bisphosphate (PI-4,5-P2), while lateral membranes are enriched in phosphatidylinositol 3, 4, 5 trisphosphate (PI-3,4,5-P3) [38]. Functional significance of segregation of these lipids has been found in cultured MDCK cells. Ectopic addition of PI-4,5-P2 to basal membranes or addition of PI-3,4,5-P3 to apical membranes is sufficient to at least partially transform these membrane domains (basal to apical and vice versa) [38, 39]. PTEN, a lipid phosphatase, is required to form these lipid domains [38, 40].

Currently, there is no direct evidence for or against lipid asymmetry in basal keratinocytes in the epidermis. Conditional ablation of PTEN in the epidermis resulted in hyperplasia, but polarity defects were not examined or reported [41]. This is perhaps not surprising, as even simple epithelia seem to differ in their compartmentalization of these lipids. In *Drosophila* photoreceptor epithelial cells, PI-3,4,5-P3 has been reported to accumulate in the apical domain, and in rat pancreatic acinar cells, no enrichment of PI-4,5-P2 was detected on apical verses basolateral domains [42, 43].

2.5 Organelle Polarity

Cytoplasmic organelles also show polarization in both simple epithelial cells and basal keratinocytes. The centrosome, which acts as the primary microtubule-organizing center in basal keratinocytes, is found just apical to the nucleus in interphase [12]. In agreement with the centrosome's known roles in organization of the Golgi Apparatus, the Golgi is preferentially enriched apical to the nucleus (unpublished observations). Whether the apical localization of the centrosome or the Golgi is functionally significant in basal keratinocytes is still unknown, as it has not yet been possible to specifically disrupt these localizations without causing other pleiotropic effects. Primary cilia are enriched at the apical surface of the basal keratinocytes, consistent with the position of centrosomal proteins [44, 45]. As the primary cilia is an important signaling center, its apical localization puts it in close contact with differentiated cells of the epidermis and somewhat removed from signals coming from beneath the cell. Whether this physical location is important for controlling signaling events has not been addressed.

3. Stratification of the Epidermis

Perhaps the clearest role for cell polarity in basal keratinocytes is its requirement for spindle orientation and asymmetric cell divisions, which drive the stratification of the epidermis (Fig. 2). Embryonic stratification of the epidermis is associated with a change in spindle orientation [12, 46]. Before e13.5, a large majority of cell divisions in the epidermis occur with the spindle oriented parallel to the underlying basement membrane. The cleavage furrow is thus perpendicular to the basement membrane, resulting in the generation of two basal daughter cells. For the purpose of this review, these divisions will be termed symmetric because they produce two daughters in the same cell layer. However, it is important to note that it has not been possible to follow the fates of these daughter cells independently over time during embryogenesis. If there is heterogeneity within the basal layer, a point of some uncertainty still, it is possible that there are functionally asymmetric divisions within the plane of the epithelium. Beginning around e13.5, there is an increase in mitoses in which the spindle is oriented perpendicular to the basement membrane [12, 46]. These divisions are termed asymmetric, as they give rise to one basal and one suprabasal cell. While live-imaging of cell fate determinants during an asymmetric division has not yet been accomplished, short-term lineage tracing of cells supports the conclusion that divisions with perpendicular spindle orientations give rise to cells with distinct differentiation markers [12]. Thus, changes in spindle orientation are correlated with changes in both the architecture of the epidermis (stratification) as well as its differentiation.

3.1 Cell Biology of Spindle Orientation

The identification and characterization of the molecular machinery responsible for spindle orientation was possible due to pioneering work on asymmetric cell divisions in model organisms – particularly *Drosophila melanogaster* and *Caenorhabditis elegans* [47]. While there are some differences between species and/or cell types, the machinery for spindle orientation is at least partially conserved in mammals.

As discussed previously, the polarity protein, Par3, marks the apical region of basal epidermal cells both during interphase and mitosis [12]. Though not as mechanistically well defined in the epidermis as in *Drosophila* neuroblasts, Par3 forms the polarity landmark that directs the local assembly of machinery that orients the spindle [48]. Par3 recruits Inscuteable and partner of Inscuteable (Pins), whose mammalian homolog is LGN [48, 49]. In embryonic epidermis, these three proteins are found in a complex that localizes at the apical region of asymmetrically dividing cells [12]. While the functional role of mInscuteable has not yet been tested in the epidermis, its disruption in *Drosophila* neuroblasts and in the mammalian neocortex results in defects in spindle orientation [50, 51]. Loss of LGN or Pins in mammalian epidermis or fly neuroblasts also resulted in defects in spindle orientation [49, 52].

Several mutants have been reported that result in loss of cell polarity and apical accumulation of Par3 or aPKC in the basal cells of the epidermis. These include the adherens junction protein α-catenin, β1 integrin, and the tumor suppressor Merlin [12, 53]. In all of these cases, randomized spindle orientation resulted, consistent with an integral role for cell polarity in spindle orientation. However, these mutants have pleiotropic effects, and the direct effect of loss of polarity regulators on spindle orientation in the epidermis has not yet been reported.

In addition to proper polarity, the actin cytoskeleton and cell shape may be required for efficient spindle orientation. Studies in other cell types have revealed a strong correlation between cell shape and spindle orientation [47, 54]. In embryonic epidermis devoid of the SRF transcription factor, levels of cortical myosin were decreased and cell rounding at

mitosis was inhibited [55]. These cells exhibited spindle orientation defects without immediate loss of cell polarity. However, it is not clear whether it was the changes in cell shape or the loss of cortical actin per se that caused the defects.

One important function for the Par3/Insc/LGN complex is to recruit the effectors of spindle orientation – molecules that can interact with and generate force on the mitotic spindle. These effector proteins include NuMA and dynein/dynactin. NuMA is a microtubule-binding protein that localizes to both the spindle poles and the apical cell cortex in asymmetrically dividing cells [12]. Dynein/dynactin is a minus-end directed microtubule motor protein complex with a similar localization as NuMA. Loss of these proteins results in alterations in spindle orientation in the developing epidermis [52].

While we now have a framework of machinery for asymmetric cell divisions, we know surprisingly little about how symmetric divisions are controlled. Knockdown of LGN, NuMA, and dynactin in embryonic epidermis was reported to result in a loss of asymmetric divisions and an accompanying increase in symmetric ones [52]. Therefore, division orientation was not randomized, and instead, asymmetric divisions were selectively lost, suggesting that LGN and NuMA are not necessary for symmetric division orientation. This is in contrast to findings in simple epithelial cells in culture which require LGN and NuMA for correct spindle positioning [56-58]. In addition, dynein/dyanctin is required for spindle positioning in many contexts, including in yeast [59]. How a symmetric spindle could be oriented without dynein-dynactin is unclear. One possibility is that asymmetric divisions are more sensitive to loss of dynactin (or LGN and NuMA) and that low levels of these proteins after knockdown are sufficient for some level of symmetric division orientation. An alternative mechanism for symmetric positioning independent of the above-mentioned machinery has been proposed in *Xenopus* epiboly-stage epithelia. Instead of using cortical force generators, these cells generate opposing pushing forces from their apical and basal edges that balance to maintain the spindle in its correct position [60].

3.2 Asymmetric Cell Division and Cell Fate Choices

Asymmetric divisions are coupled to cell fate changes in the epidermis. The transition from a proliferative basal cell to a suprabasal cell committed to differentiation is highly regulated. A number of structural changes and signaling pathways play roles in this decision. Among these, three are implicated in cell fate decisions controlled by asymmetric cell divisions: integrin/ECM attachment and signaling, Notch signaling, and EGFR signaling.

Currently the most compelling data regards the connection between asymmetric divisions and Notch signaling. Notch signaling drives differentiation of keratin-5/14 positive basal cells into keratin 1/10-positive post-mitotic suprabasal cells [4, 61, 62]. Active Notch induces cell cycle arrest and differentiation when expressed in keratinocytes [62]. Inappropriate activation of Notch in the basal layer induced differentiation; loss of function resulted in defects in commitment to differentiation [61]. Therefore, during normal development, Notch is necessary and sufficient to induce many aspects of differentiation. In *Drosophila*, there are clear examples of how Notch signaling controls cell fate during asymmetric divisions. In these cases, the Notch antagonist, Numb, is polarized so that one daughter cell selectively inherits it and adopts a distinct fate [63]. In epidermis, loss of Numb does not result in detectable differences in differentiation [52]. However, when asymmetric divisions are lost in the epidermis, as occurs upon loss of LGN or NuMA, differentiation is significantly blocked. Expression of active Notch can drive differentiation under these conditions suggesting that Notch acts downstream of the asymmetric division [52]. To date, no molecular connection between division orientation and Notch signaling has been reported.

EGFR is another candidate that has been implicated in cell fate decisions following asymmetric cell divisions. In both neuronal progenitors and epidermal cells in culture, EGFR can be asymmetrically inherited and has been suggested to control cell fates [64, 65]. We have also observed asymmetric EGFR localization in mitotic cells in developing epidermis (unpublished data). Active EGFR signals promote proliferation in the epidermis. In addition, EGFR can block Notch expression to inhibit differentiation in cultured keratinocytes [66]. Whether this pathway is physiologically important and acts in concert with asymmetric cell divisions has not been tested.

Finally, there are extrinsic factors that can control the proliferation/differentiation decision, such as integrin/ECM adhesion [3, 4]. Integrin signaling provides a potent mitogenic signal. Misexpression of integrins can drive suprabasal proliferation, while loss of integrin β1 results in hypoproliferation [11, 67]. Basal cells are exposed to the extracellular matrix while suprabasal cells are not. Therefore, extrinsic environmental cues from the ECM likely collaborate with intrinsically polarized cell fate determinants to control differentiation upon asymmetric cell division.

3.3 Balancing Growth and Stratification

As the embryo grows, the epidermis must balance an increase in surface area with an increase in cell layers to form a tissue with the correct surface area and thickness. The model cell types used to understand the mechanism of asymmetric cell divisions (*Drosophila* neuroblasts, *C. elegans* zygote) divide exclusively asymmetrically. In contrast, basal epidermal cells divide both symmetrically and asymmetrically [68]. Current evidence suggests that epidermal cells have the capacity to divide in either orientation and thus must integrate various environmental stimuli to determine which way to divide [68]. We have very little understanding about what factors influence this choice in basal cells or how they impact spindle orientation. Currently, there is evidence for both transcriptional and post-transcriptional control of this decision [68]. The expression of some components of the asymmetric cell division machinery, especially mInscuteable, appear to be highly regulated. mInscuteable shows tissue and cell type specificity and temporal control both during development and the cell cycle [12, 68]. However, cells can divide symmetrically even in the presence of mInscuteable, suggesting a post-transcriptional mode of regulation [68]. Thus, the localization and/or activity of this complex must be regulated. One example of how this may occur comes from analysis of the tyrosine kinase Abl1. Abl1 regulates the localization of NuMA at the cell cortex, as well as, potentially, cell polarity and/or LGN localization, thus controlling spindle orientation [69]. Determining how extracellular signals impinge upon signal transduction pathways (like Abl1 and others) to affect spindle orientation should be an exciting and rich area for future investigation.

3.4 Delamination and Stratification during Homeostasis and Cancer

Whether spindle orientation and asymmetric cell divisions are important in adult epidermis is still under debate [70]. The adult epidermis is rather squamous and many cells have an oblique spindle orientation. Determining whether these oblique division are functionally asymmetric and whether they are important in homeostasis is still under investigation. In recent lineage-tracing studies of adult epidermis, evidence for at least two distinct populations of cells, stem and progenitor, was reported [71]. Modeling the activities of these populations suggested that both can divide in a symmetric or asymmetric manner, suggesting that the control of this ratio will also be important in homeostasis. An alternative method to stratify or to maintain stratified is through delamination of basal cells. Basal cells could either differentiate and lose their adhesion, thus being forced out of the basal layer, or they could actively migrate out and then differentiate. The presence of occasional basal cells

that express differentiation markers and a decrease in the number of asymmetric divisions suggests that delamination may also happen in adult skin [70].

Recent work has also exposed possible roles for division orientation in cancer development in the epidermis. Increased vascular endothelial growth factor (VEGF) signaling induced an increase in the number of symmetric divisions in the adult epidermis [72]. This pathway could be important for increasing the pool of proliferative cells and driving hyperproliferation in cancerous tissue. Accordingly, blocking the VEGF pathway had the opposite effect [72]. These data suggest that division orientation regulates proliferative state during homeostasis as well as development. The idea that loss of asymmetric cell divisions would promote tumor formation has been tested and confirmed in some invertebrate systems, but there has not yet been a direct test of this hypothesis in mammals.

4. Summary

Significant advances have been made in our understanding of cell polarity in model organisms and cultured cells. The challenge now is to translate this into intact mammalian tissues to determine how conserved machinery is used by different epithelia to meet their physiological roles. We are at early stages in understanding both how polarity is generated within the epidermis and what functions polarity provides in addition to regulating spindle orientation.

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Highlights

- basal keratinocytes are polarized
- basal keratinocytes have distinct polarity organization from simple epithelia
- polarity is used for spindle orientation, stratificaion and cell fate determination in the epidermis.

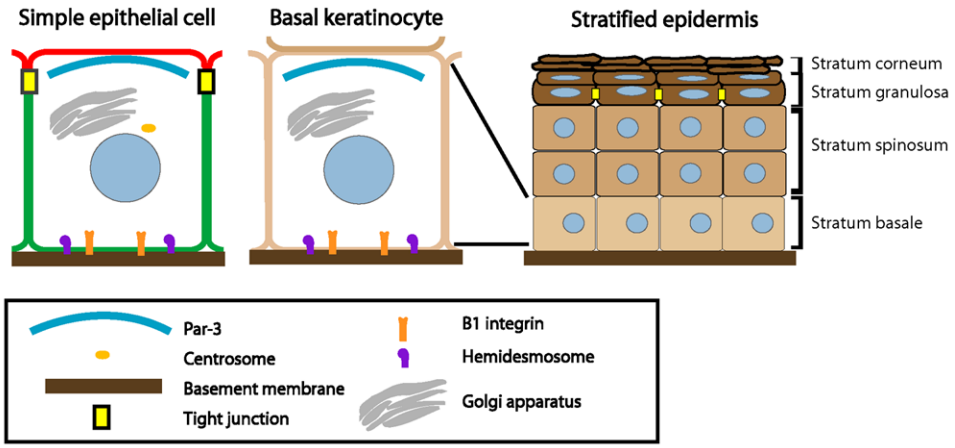

Figure 1.
Comparison of cell polarity in simple epithelial and basal keratinocytes.

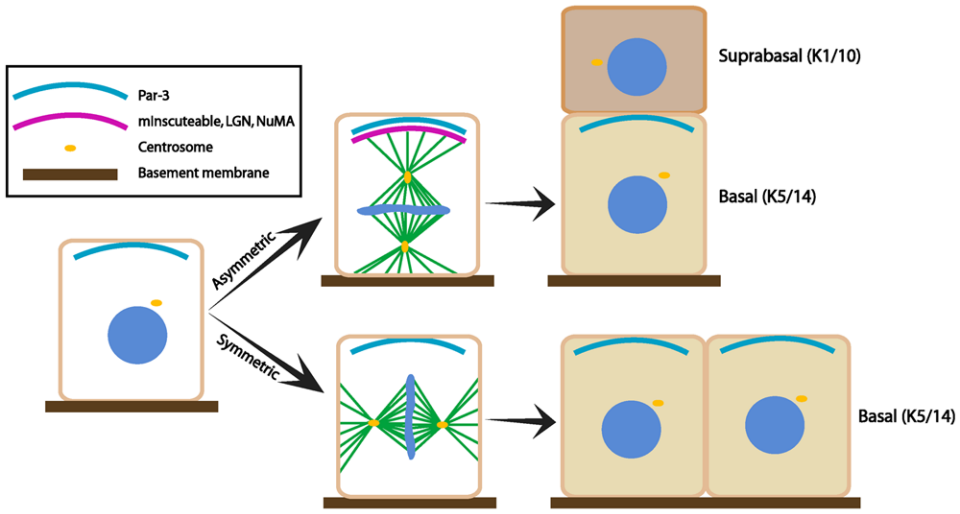

Figure 2.
Model for spindle orientation in symmetric and asymmetric cell divisions.