Review

Cell PRESS

Special Issue – 3D Cell Biology

Epithelial organization, cell polarity and tumorigenesis

Luke Martin McCaffrey[1] and Ian G. Macara[2]

[1] Department of Oncology, Rosalind and Morris Goodman Cancer Research Centre, McGill University, Montreal, Canada
[2] Center for Cell Signaling, Department of Microbiology, University of Virginia School of Medicine, Charlottesville VA 22908, USA

Epithelial cells comprise the foundation for the majority of organs in the mammalian body, and are the source of approximately 90% of all human cancers. Characteristically, epithelial cells form intercellular adhesions, exhibit apical/basal polarity, and orient their mitotic spindles in the plane of the epithelial sheet. Defects in these attributes result in the tissue disorganization associated with cancer. Epithelia undergo self-renewal from stem cells, which might in some cases be the cell of origin for cancers. The PAR polarity proteins are master regulators of epithelial organization, and are closely linked to signaling pathways such as Hippo, which orchestrate proliferation and apoptosis to control organ size. 3D *ex vivo* culture systems can now faithfully recapitulate epithelial organ morphogenesis, providing a powerful approach to study both normal development and the initiating events in carcinogenesis.

Epithelial organization and development

The epithelium was probably the first tissue type to have arisen in the evolution of multicellular animals, and is the first differentiated structure to emerge during embryogenesis. Its organization depends on three attributes: intercellular adhesion to form a super-cellular structure; mitotic spindle orientation to ensure the formation of a sheet rather than a blob of cells; and apical/basal polarity to distinguish the outside surface from the inside surface of the sheet. These characteristics might even predate the rise of the metazoa, since the slime mould *Dictyostelium discoideum* can form a fruiting body that contains a polarized layer of cells [1], which express certain catenins, although not the cadherin adhesion proteins, nor Par polarity proteins. Elaboration of the basic epithelial characteristics in animals has led to the organization of epithelia into complex structures, such as stratified epithelia and branched tubules and alveolae that can contain multiple cell types, arising from stem cells or lineage-specific progenitors. In addition to cadherin-based adherens junctions, other cell-cell connections have evolved, either to provide a tight, regulated seal between the external and internal environments (tight junctions in vertebrates, septate junctions in flies), or to provide mechanical strength (desmosomes). The lipid and protein makeup of the apical plasma membrane of epithelial cells is quite different from that of the basolateral membrane, and provides for distinct functions, including polarized influx and efflux of ions and molecules, and signal detection.

Epithelial cells differ from mesenchymal cells in that daughter cells do not separate from one another during cytokinesis, and proliferation can continue even when the cells are in contact with neighbors [2]. Moreover, apical/basal polarity is retained during epithelial cell division. These factors ensure that epithelial sheets can expand during development, and retain their intercellular contacts, thereby preventing leakage of luminal contents, or fragmentation of the sheet. However, the absence of contact inhibition means that epithelial tissues need other mechanisms to limit proliferation, and ensure that the correct organ size is achieved. Organisms also need quality control mechanisms to protect themselves against defective epithelial cells that might disrupt the integrity of the tissue. Loss of adhesion can induce apoptosis through inhibition of the Akt survival pathway; but, in addition, at least in *Drosophila*, disruption of apical/basal polarity can trigger a cell non-autonomous response in which either neighboring cells or circulating hemocytes release factors that induce apoptosis in the target cells [3,4]. Recently, other mechanisms that orchestrate epithelial development have come to light, including the Hippo signaling pathway [5]. Failure of these controls on epithelial growth and integrity might be predicted to contribute to carcinogenesis; importantly, the majority of human cancers arise from epithelial cells and their progenitors.

In this article, we will review recent work on epithelial stem cells, mitotic spindle orientation and asymmetric cell divisions, and the genetic control of the epithelial lineage. We will also highlight mechanisms of epithelial growth control, the signaling networks that impinge on polarity proteins, and their connections to cancer. Finally, we discuss the problems with traditional 2D cell cultures for studying both epithelial cell biology and cancer, and the value of 3D and organotypic *ex vivo* cultures that more closely recapitulate *in vivo* behavior.

Epithelial stem cells

Several epithelial organs are known to develop from multipotent stem cells that can generate all of the diverse cell types that make up the tissue. The epithelium of the small intestine arises from actively cycling Lgr5+ stem cells in the base of the crypts, and 'mini-guts' can be generated in 3D *in vitro* cultures from single Lgr5+ stem cells [6].

Corresponding author: Macara, I.G. (igm9c@virginia.edu).

0962-8924/$ – see front matter doi:10.1016/j.tcb.2011.06.005 Trends in Cell Biology, December 2011, Vol. 21, No. 12

Similarly, hair follicles arise from multipotent stem cells [7], and entire mammary glands can be regenerated from single stem cells implanted into the cleared fat pads of host mice [8,9]. In the latter case, however, the identity of the stem cell remains uncertain, although it is associated with basal cell markers. Much of our understanding of how stem cells generate diverse progeny has come from studies in *Drosophila*, in which neuroblasts and SOP cells undergo asymmetric cell divisions, such that one daughter cell retains 'stemness' while the other becomes a lineage-specified progenitor [10]. Apical/basal polarity proteins — Par3, Par6, and atypical protein kinase C (aPKC) — play key roles in both the distribution of cell fate determinants, and in the spindle pole alignment necessary for segregation of these determinants between the daughter cells; defects in this distribution can result in stem cell tumors. The *Drosophila* embryonic neuroblast derives from an epithelial cell that delaminates in a basal direction from the ectodermal sheet.

Par3/Par6/aPKC forms a complex at the apical surface of the neuroblast that functions both to exclude cell fate determinants necessary for neuronal differentiation, and to recruit factors that attach astral microtubules to the cell cortex during mitosis. One key protein involved in attachment is Partner of Inscuteable (Pins). Attachment at the apical cortex ensures correct spindle orientation, such that the fate determinants are segregated into the basal daughter cell, while the Par proteins remain in the apical stem cell [10].

There has been a general assumption that this mechanism of asymmetric division is conserved and applies to mammalian stem cells and progenitors. Yet there is scant evidence in support of such an assumption. Although basal epidermal cells use this mechanism to divide vertically so as to generate a stratified epithelium [11], stem cells can also produce diverse cell types through a stochastic decision to either divide into two stem cells, or to divide into two progenitors (Figure 1). Detailed analyses of Lgr5+ cells in intestinal crypts support strongly the stochastic, symmetric division model [12,13]. An independent study of the inheritance of labeled DNA [14] has proposed the opposite conclusion — that the stem cells consistently undergo asymmetric divisions, in which one daughter preferentially inherits the parental DNA strands — but these results have been questioned recently [15]. Asymmetric inheritance of DNA (the 'immortal strand' hypothesis) was proposed long ago as a potential mechanism through which stem cells could escape the accumulation of potentially deleterious mutations. However, in most cases no such asymmetry has been detected unequivocally.

Do carcinomas originate from epithelial stem cells? The cell of origin for most epithelial cancers has not yet been established definitively [16], but several studies point to a stem or progenitor cell origin. For example, in a mouse model of intestinal cancer Lgr5+ stem cells rapidly generated adenomas upon deletion of the APC tumor suppressor, whereas tumorigenesis by deletion of APC in later progenitor cells of the intestinal epithelium was very inefficient [17]. Lineage tracing has also defined a luminal stem cell as the cell of origin for prostate cancers, in a mouse $PTEN^{-/-}$ model [18]; an epidermal progenitor is the

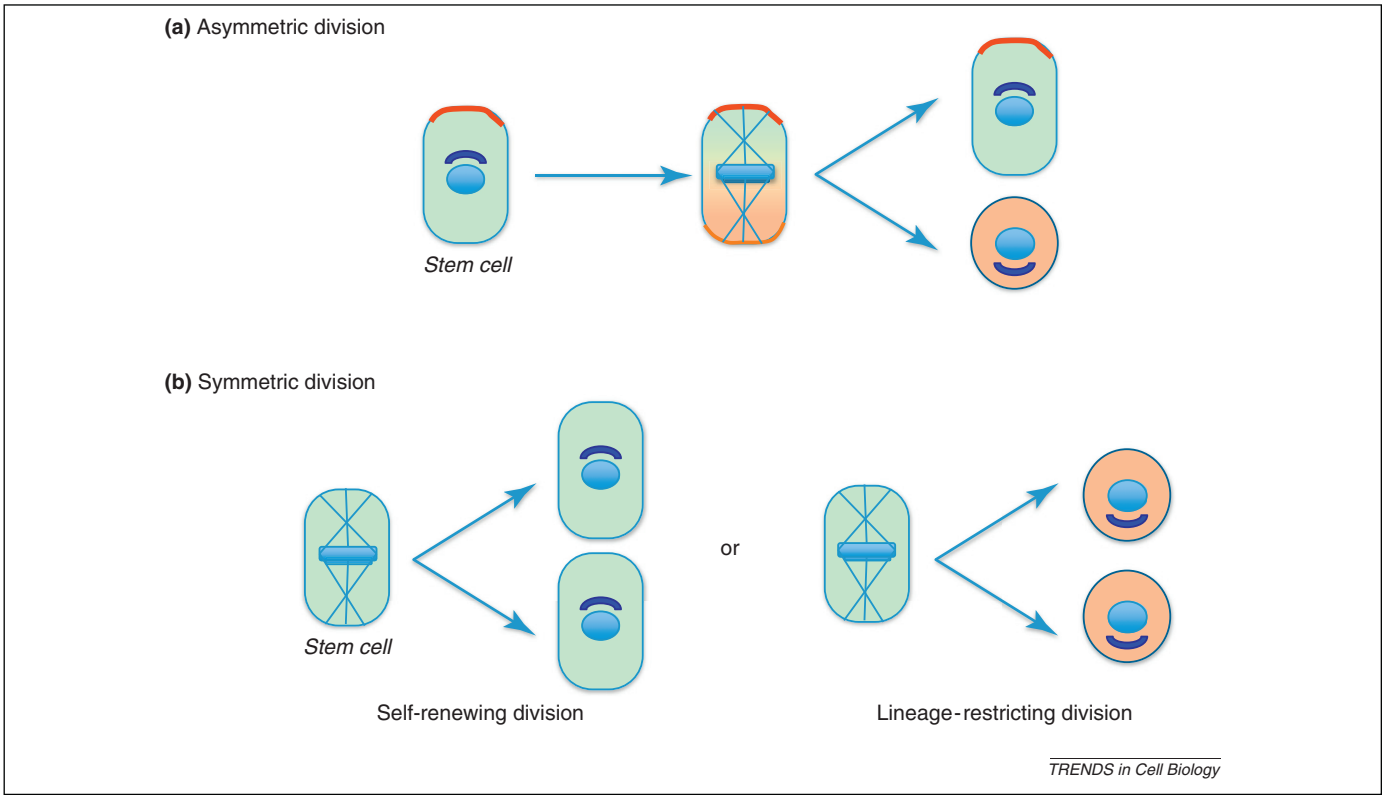

Figure 1. Modes of stem cell divisions to generate cellular diversity. **(a)** Generating cellular diversity through asymmetric divisions. In *Drosophila* neuroblasts and sensory organ precursor (SOP) cells, cell diversity is achieved through asymmetric divisions controlled by the asymmetric distribution of stem cell maintenance factors (red line) and cell fate determinants (orange line). **(b)** Generating cellular diversity through symmetric divisions. Mouse intestinal stem cells divide symmetrically to give rise to two stem cells, each of which can differentiate stochastically within the niche. Symmetric divisions could also give rise to two differentiated daughter cells, without self-renewal of the stem cell.

cell of origin for basal cell carcinomas in skin [19]. Some breast tumor subtypes, such as those with Brca1 mutations, are thought to arise from luminal progenitors [20,21]. Is lineage specification hierarchical and unidirectional, however, or can fully differentiated, polarized epithelial cells revert to a progenitor status, which could then give rise to tumors? If plasticity in lineage determination can occur, can the same progenitor give rise to multiple types of tumor, or can different progenitors produce tumors with similar phenotypes? Perhaps the differences in conditions that identify a high frequency of tumor initiating cells (TICs) versus those that find only rare TICs in a tumor relate not to the actual number of pre-existing TICs but to the ability of cells to revert to a stem-cell-like status when exposed to a particular environment. Lineage tracing experiments will provide the only definitive approach to these questions, but there is experimental support for plasticity, and oncogenic transformation can drive the conversion of human mammary epithelial cells into a stem-like state [22].

Genetic control of the epithelial lineage

Evidence that cell lineage reprogramming is possible was provided recently by the demonstration that mouse fibroblasts can be converted directly into functional neurons, through the ectopic expression of three transcription factors [23]. The conversion of epithelial cells to cells with a mesenchymal phenotype (epithelial-mesenchymal transition or EMT), and the reverse process (MET) both occur during embryogenesis, and have been the subject of intense study because of possible connections to metastasis. Yet transdifferentiation of fibroblasts to epithelial cells has not received much attention. Remarkably, the adenovirus E1A protein can induce an epithelial phenotype in human fibroblasts and a variety of transformed cells, although the underlying mechanism has not been identified [24]. A number of endogenous factors are known to be required for the formation and/or maintenance of epithelial identity. For example, the p63 transcription factor is essential for epithelial stem cell survival and for the formation of stratified epithelia [25], and GATA-3 is required for maintenance of luminal epithelial cells in the mammary gland [26,27]. In addition, a network of microRNAs and transcription factors regulates a slew of proteins necessary for intercellular adhesion and cell polarity. In particular, the miR-200 family and the ZEB1 transcription factor appear to be key players in epithelialization [28]. MiR-200 is expressed at high levels in epithelial cells and suppresses the expression of the stemness factor BMI1, the growth factor TGFβ, and the transcription factor ZEB1 [29]. TGFβ has been known for many years to induce EMT in certain epithelial cell types, and can induce ZEB1 expression. Activation of the TGFβ receptor can also result in phosphorylation of the polarity protein Par6, which mediates the dissolution of epithelial tight junctions. High ZEB1 blocks the epithelial phenotype by repressing genes involved in intercellular adhesion (E-cadherin), apical/basal polarity (Crumbs, Patj, Lgl1 and Lgl2) and basement membrane proteins (LAMA3, LAMB3) [30]. It also inhibits expression of the miR-200 family of micro-RNAs in a double-negative feedback loop (Figure 2) [29]. Interestingly, the p53 tumor suppressor induces expression of miR-200c [31]. Very recently, $Brca1^{mut/+}$ breast tissue was found to express abnormally high levels of the Slug transcriptional repressor, which has been implicated in EMT [32]. EMT markers are, however, characteristic of basal (myoepithelial) cells in the mammary gland, and Slug promotes basal cell differentiation, whereas silencing of Slug results in up-regulation of luminal markers. Epithelial-mesenchymal transitions might, therefore, be sometimes better described as being epithelial-myoepithelial transitions. What remains uncertain is whether other cell lineages can be switched to an epithelial identity simply by forcing expression of miR-200, or by blocking expression of ZEB1 or Slug. As is true for fibroblast/neuron reprogramming, multiple factors will most probably be required to drive cells fully into the epithelial lineage.

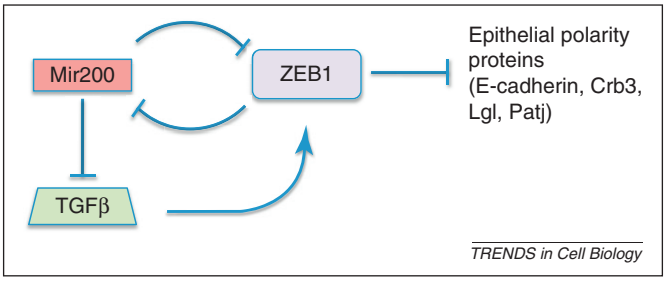

Figure 2. Genetic control of epithelial polarity. The transcription factor ZEB1 negatively controls the expression of several apico-basal polarity and epithelial determinants, including E-cadherin, Crb3, Lgl2, and Pals1. Epithelial character is maintained by miR-200, which negatively regulates ZEB1 directly and through TGF-β. Through a feedback loop, ZEB1 also inhibits miR-200 expression, allowing genetic control of epithelial plasticity during development and tumor progression.

Spindle orientation and cancer

Even after growth is complete, many epithelial tissues continually turn over the cells of which they are composed. The most striking example is the intestinal epithelium, for which the lifetime of differentiated cells is only 4–5 days. Perhaps it is because of this continuous proliferation that epithelial tissues are the predominant source of cancers in humans. One of the earliest events following the acquisition of oncogenic mutations by an epithelial cell is the escape of its progeny from the sheet to form multilayered, disorganized polyps or similar structures. There are various mechanisms by which escape might occur (Figure 3). First, loss of intercellular adhesion caused, for instance, by an epithelial-mesenchymal transition (EMT) that represses E-cadherin expression, could enable cells to crawl out of the epithelial sheet either apically or basally, or be expelled by normal neighbors. Second, in the absence of EMT, loss of adhesion to the basement membrane could enable neighboring wild type cells to extend under the detached cell, pushing it up and out of the monolayer. Third, misorientation of the mitotic spindle could result in daughter cells being generated perpendicularly to the epithelial sheet instead of parallel to it, thereby resulting in multilayering even though intercellular adhesions are retained. Interestingly, a subset of the same proteins that orient spindles vertically in *Drosophila* neuroblasts and in basal cells of the mammalian epidermis is also required to orient spindles horizontally in epithelial sheets. This subset includes Par3, Par6, aPKC, Cdc42, and

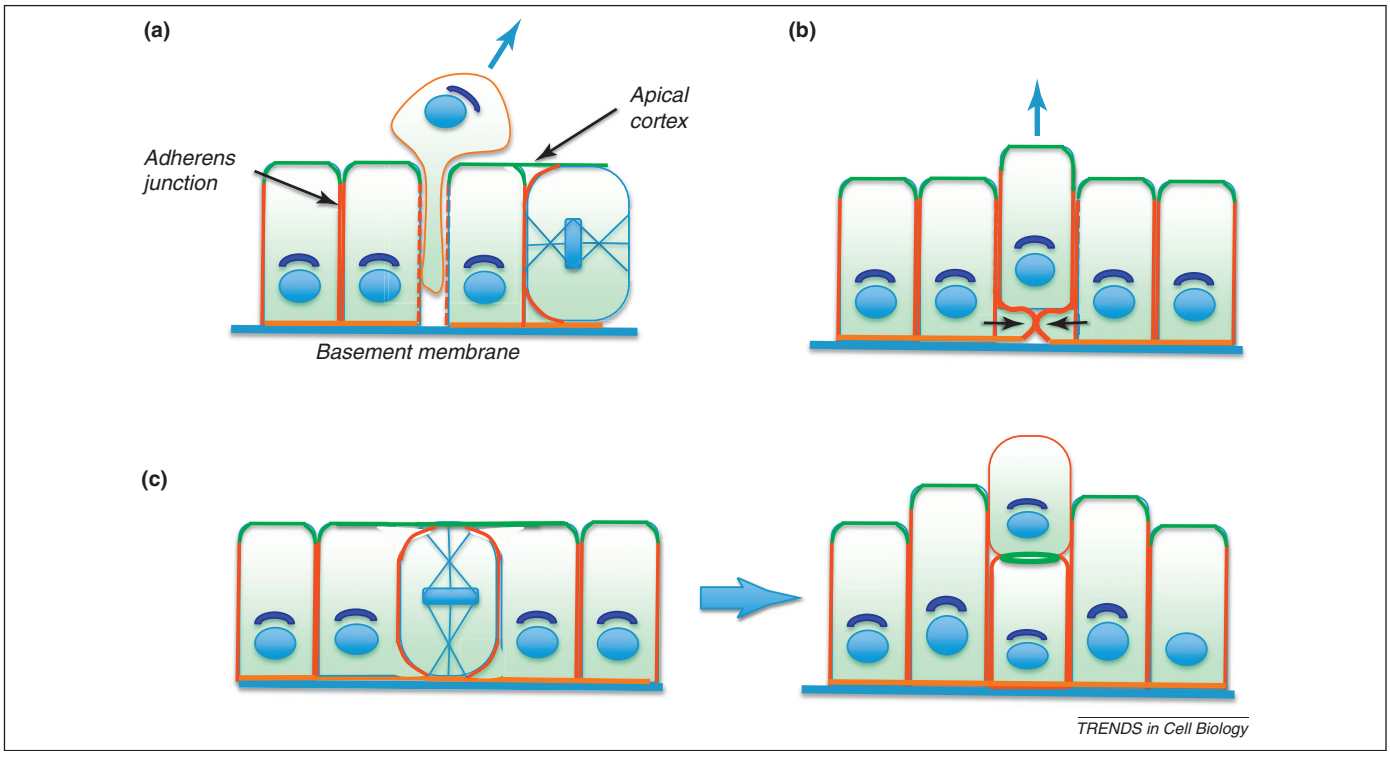

Figure 3. Models for loss of epithelial organization in cancer. **(a)** Disruption of cell-cell contacts. Down-regulation of adhesion molecules or EMT induction would allow cells to migrate out of the epithelial sheet. **(b)** Disruption of cell-matrix adhesion. Loss of contact with the basement would allow neighboring cells to spread into the gap, pushing the detached cell out of the plane epithelial sheet. **(c)** Disruption of spindle orientation. Epithelial cells normally divide within the plane of the epithelial sheet to maintain the epithelial layer. Divisions perpendicular to the plane of the epithelium would result in stacked daughter cells.

Pins. However, whereas all of these proteins associate in a crescent at the apical surface of the neuroblast, they are segregated in different regions of the epithelial cell: Par3 is at the apical/lateral boundary (at tight junctions in mammalian epithelia), while aPKC, Par6, and Cdc42 are apical, and Pins is lateral [33]. Silencing any of these proteins causes a substantial defect in spindle orientation, as does the loss of exchange factors for the small GTPase Cdc42, Tuba and Intersectin [34–38].

Mitotic spindles are oriented by the attachment of astral microtubules to the cell cortex, and in epithelial cells aPKC blocks such attachments to the apical surface, thereby preventing vertical orientation of the spindles. Par3 is required for the localization of aPKC to the apical surface, and Cdc42-GTP is necessary for the activation of aPKC at the apical surface. Therefore, with the loss of these proteins, aPKC can no longer function to exclude astral microtubule attachment, and spindles can become misoriented (Figure 4). The mechanism of attachment by astral microtubules to the cell cortex is not understood completely, but in addition to the Pins protein, NuMA, dynein, and the G-protein alpha subunit, Gαi, are also required [10]. Pins is diffusely cytoplasmic in interphase cells, but at the onset of mitosis NuMA is released from the nucleus and can bind to and trigger a conformational change in Pins, enabling this protein to associate with membrane-bound Gαi [39]. NuMA contains a microtubule-binding domain, although it cannot simultaneously interact with both Pins and tubulin, indicating that other factors are also necessary for attachment. Atypical PKC functions by phosphorylating Pins, resulting in the recruitment of 14-3-3, and disassociation from Gαi [36]. One could imagine that defects in any part of this process could result in spindle orientation defects, and consequent disruption of epithelial sheets.

Epithelial growth control, apoptosis and cancer

Restraints on epithelial cell proliferation are necessary to limit tissue growth, and depend on cell density. One proposed mechanism involves the sequestration at tight junctions of a transcription factor, ZONAB, which is required for cell cycling. As the cell density increases in an epithelial sheet and junctions mature, more ZONAB is sequestered from the nucleus to the junctions together with a binding partner, the cell division kinase CDK4 [40], thereby reducing proliferation.

Another important sensor of cell density is the adherens junction protein α-catenin, which functions in this capacity through coupling to the Hippo signaling pathway [41,42]. The Hippo pathway was first discovered in *Drosophila* as a pivotal regulator of organ size, and orchestrates the balance between proliferation and apoptosis [5]. It has also been shown more recently to perform similar roles in mammals. A protein kinase cascade, which includes multiple tumor suppressor genes, ultimately phosphorylates and inactivates the transcription co-activators Yorkie/Yap1 and TAZ (Figure 5). Unexpectedly, phospho-Yap1 is sequestered at adherens junctions through association with α-catenin, via 14-3-3. In the epidermis, loss of α-catenin results in the nuclear accumulation of Yap1 and increased cell proliferation [41,42]. How cell density controls the α-catenin/14-3-3/Yap1 interaction remains unclear, but another Hippo pathway component, Merlin, also binds directly to α-catenin and is required for stable

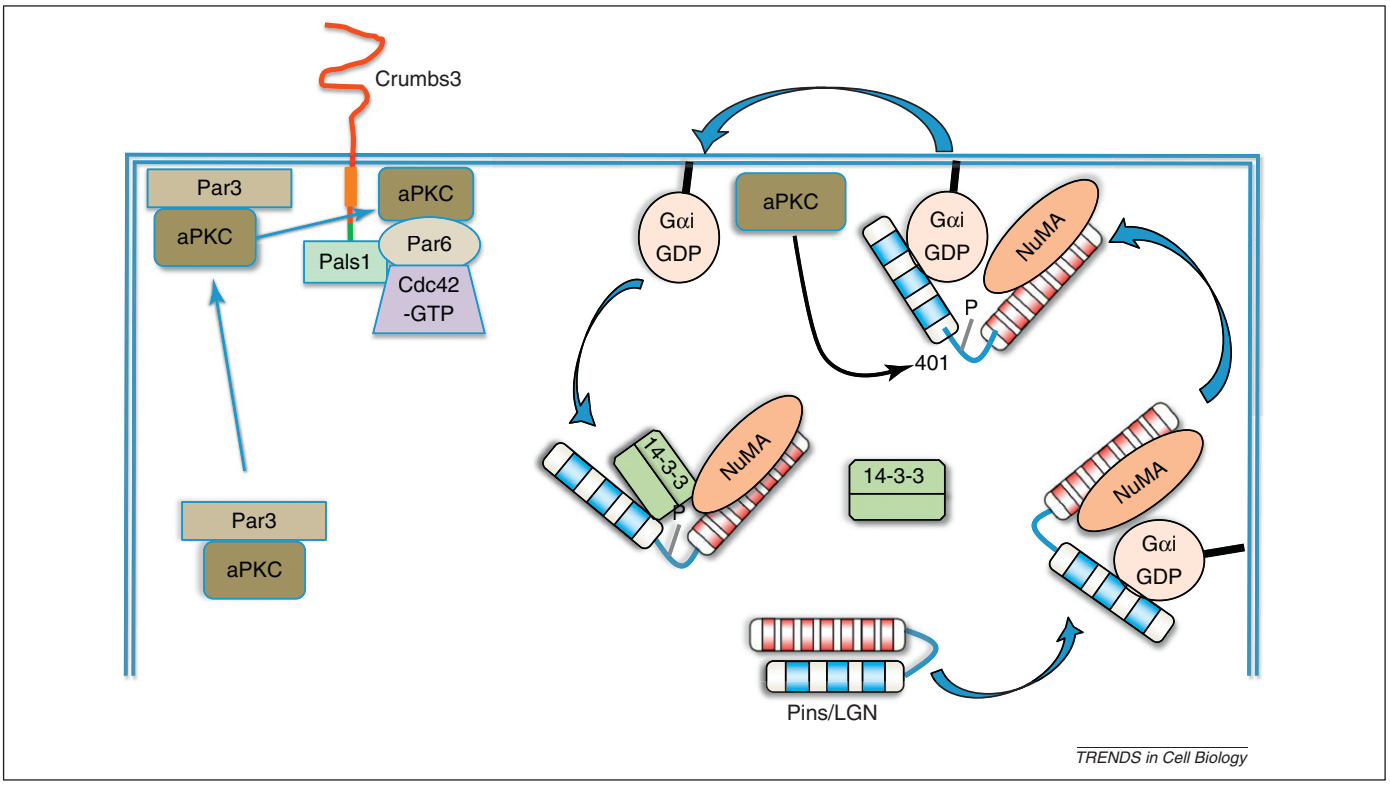

Figure 4. Regulation of spindle orientation. The Par polarity complex regulates spindle orientation during cell division. Epithelial cells divide within the plane of the epithelium by orienting the spindle poles towards the lateral membranes. Spindle poles are anchored to the cell cortex through the interaction of LGN/ NUMA and $G\alpha_i$. Anchoring of spindle poles to the apical membrane is inhibited by apically localized aPKC. aPKC phosphorylates LGN on Ser401, which recruits 14-3-3 to disrupt the association between LGN and $G\alpha_i$, thereby releasing LGN from the apical domain. Par3 and Par6 control spindle orientation by recruiting aPKC to the apical membrane.

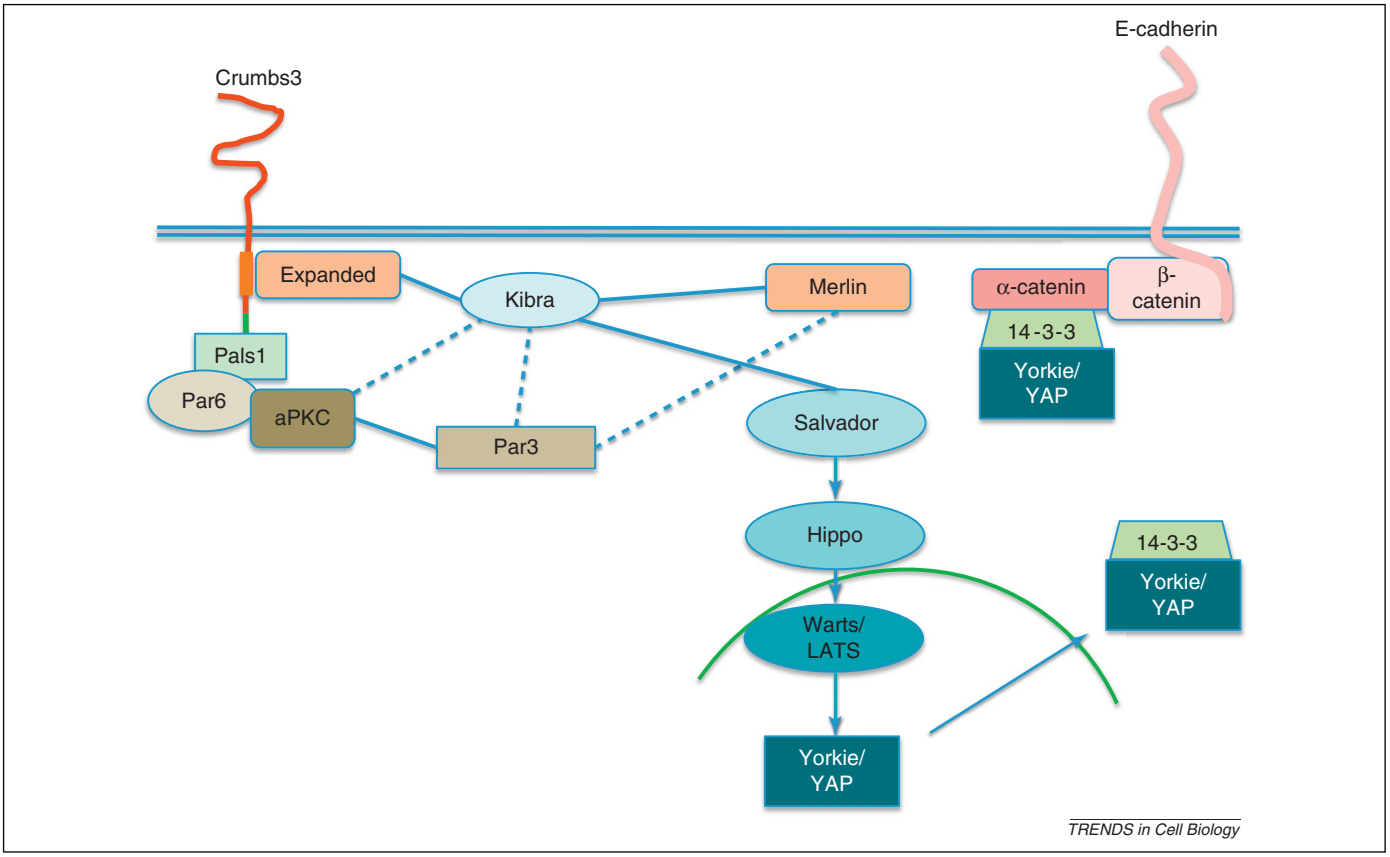

Figure 5. Control of epithelial growth by the Hippo pathway. A signaling cascade involving Salvador, Hippo, and Warts controls phosphorylation and nuclear localization of the transcription factors Yorkie/YAP and TAZ to regulate epithelial growth. Epithelial integrity is monitored by cell-adhesion complexes (E-cadherin, α-catenin) and by the Par and Crumbs polarity complexes through the adaptor Kibra.

adherens junction formation [43]. Merlin couples α-catenin to Par3 during junction maturation in keratinocytes, a process that might be important for the positioning of tight junctions apical to the adherens junctions. We speculate that Merlin might in some way regulate Yap1/14-3-3 association with α-catenin.

Importantly, certain components of the Hippo pathway can bind to apical/basal polarity proteins. For example, the *Drosophila* protein Expanded binds to the intracellular domain of Crumbs, a transmembrane protein that defines the identity and size of the apical domain in epithelial cells [44–47]. The over-expression of Crumbs causes a mislocalization of Expanded and is sufficient to induce hyperproliferation of epithelial cells in *Drosophila* [48]. Interestingly, loss of Crumbs in *Drosophila* embryos also results in the incorrect localization of Expanded, with a consequent dysregulation of Hippo signaling and tumorigenesis. In mouse epithelial cell lines, Crumbs3-mediated apico-basal polarity sequesters SMADs in the cytoplasm through the Hippo pathway, which inhibits TGFβ signaling and TGFβ-induced EMT [49].

An important component of the Hippo pathway is Kibra, which binds to many pathway components including Expanded, Merlin, Warts, Hippo, and Salvador, but how the interactions are regulated remains unclear. Kibra also binds to the Par complex and localizes to tight junctions and the apical membrane [50]. Kibra is a substrate for aPKC phosphorylation [51], but it can also negatively regulate aPKC activity to control the exocytosis of apical membrane components [50]. A similar mechanism can function in cell migration, with an aPKC/Kibra/exocyst complex regulating focal adhesion dynamics at the leading edge by recruiting ERK [52]. In mammalian epithelial cells, Kibra forms a complex with both aPKC and the discoidin domain receptor 1 (DDR1). DDR1 is a receptor tyrosine kinase that is activated by collagen. Collagen dissociates Kibra from DDR1, but Kibra remains bound to aPKC and regulates growth through MAPK signaling [53]. Interestingly, Par3 and Par6 can also associate with DDR1, at least in a squamous cell carcinoma (SCC) cell line [54]. This complex is independent of collagen; instead, DDR1 and the Par complex are co-dependent for localization to cell-cell junctions and mediate actomyosin remodeling and intercellular adhesion. In the absence of DDR1, the cells lose their capacity for collective migration [54]. We speculate that these connections could play important roles in the misorganization, overgrowth, and invasion of epithelial cancers.

Other potentially important connections between polarity proteins and epithelial growth control have also been discovered recently. Mammalian Par3 binds to a protein called ASPP2, which was originally discovered as a regulator of the apoptotic functions of the nuclear tumor suppressor protein, p53. Surprisingly, however, ASPP2 is predominantly at tight junctions in epithelial cells, and Par3 and ASPP2 localization to tight junction is interdependent [55,56]. Are there connections between Par3, ASPP2 and p53? We speculate that upon disruption of epithelial polarity, ASPP2 is released and promotes p53-dependent apoptosis in depolarized cells. However, ASPP2 also has anti-apoptotic functions, since it promotes dephosphorylation of the TAZ co-activator by recruiting the PP1 phosphatase, thereby triggering TAZ-dependent gene expression, which enhances proliferation [57]. Moreover, a closely related protein, ASPP1, inhibits the interaction of the Hippo pathway kinase Lats1 with Yap1, thereby promoting the transcription of Yap1-dependent targets and inhibits apoptosis [58]. These data point to an extensive network of regulatory interactions between the apical/basal polarity machinery and Hippo-mediated control of organ size. An important area for future research will be to further understand how this network functions both in normal development and in cancer.

Cell polarity defects, cell competition and compensatory proliferation

Defects in the cell polarity machinery can trigger another mechanism distinct from Hippo that couples epithelial cell proliferation and apoptosis. The loss of the Scribble (Scrb), Discs-Large (Dlg) or Lethal giant larvae (Lgl) genes in the imaginal discs of *Drosophila* larvae causes both a disruption of normal epithelial architecture and over-proliferation [59]. Interestingly, the mutant cells initially grow more slowly, in part because of increased apoptosis induced by neighboring normal cells; but they fail to exit from the proliferative cycle as is required for pupation. Growth arrest is probably triggered by ecdysone, but the link to the polarity proteins remains to be identified.

The apoptotic program induced by neighboring wild type cells is termed cell competition, and can eliminate small clones of defective or slowly growing cells, thereby preventing tumor growth or other developmental problems [60]. To fill the gap left by apoptotic cells, and maintain epithelial homeostasis, a compensatory proliferation program is stimulated in neighboring cells. This program is controlled by JNK-dependent signaling pathways. Before dying, damaged cells secrete factors that stimulate adjacent cells to proliferate. If, however, the damaged cells are prevented from dying, for example by inhibiting apoptosis, these 'undead' cells continue to secrete proliferation signals, leading to hyperplastic overgrowth.

Loss of cell polarity can trigger apoptosis so as to eliminate damaged cells. Disruption of either the Par or Scribble complexes leads to JNK-mediated apoptosis and engulfment [3,61]. Interestingly, however, the Par-complex specifically induces JNK-dependent compensatory proliferation and causes tissue overgrowth in response to radiation damage and tissue overgrowth, whereas Scribble-activated JNK does not [62]. This differential response suggests that JNK activation *per se* does not drive compensatory proliferation, but the pathways mediating JNK-activation are important determinants. The Par and Scribble complexes signal to JNK by distinct mechanisms: disrupting the Par complex activates JNK through Rho1/Rok/myosin, whereas JNK is activated by increased endocytosis of Eiger when Scribble is disrupted [62].

The Par3 and Scribble polarity gene mutants show a striking cooperativity with dominant oncogenes to promote invasive tumorigenesis in *Drosophila*. Instead of undergoing apoptosis, polarity-defective cells that express oncogenic Ras hyper-proliferate and escape from the epithelial sheet to form metastatic tumors [3,63]. Remarkably, this

proliferative response can even occur when the oncogene is in a neighboring cell rather than in the defective cell, through a mechanism involving secretion of a cytokine and the activation of a JAK/STAT signaling pathway [61,64].

Whether cell competition occurs in mammals has not yet been tested unambiguously, although the signaling pathways involved in competition (c-myc, TGFβ, JNK) are all conserved between flies and mammals, and *in vitro* co-cultures of normal and transformed cells recapitulate several features of cell competition [65,66].

The relationships between cell competition, polarity, proliferation, and apoptosis can be context-dependent. Loss of Par3 in the mouse mammary gland causes increased apoptosis and decreased ductal outgrowth [67]. In this system, there is also an increase in proliferation, but whether this occurs through a process of compensatory proliferation, as in *Drosophila*, remains to be determined. Par6 can also affect signaling to apoptosis. Expression of a dominant-negative Par6 – one that cannot bind aPKC – leads to increased apoptosis in ErbB2-activated cells [68]. In this case, blocking normal Par-complex function does not directly activate a pro-apoptotic signal, but rather uncouples ErbB2-mediated anti-apoptosis signaling.

Cell polarity and cancer

Loss of cell polarity has been widely assumed to be a key step in cancer progression, but the majority of primary human carcinomas retain epithelial characteristics such as intercellular adhesions and tight junctions [69], although loss of E-cadherin and/or α-catenin correlates closely with invasiveness. The expression of a number of polarity proteins is altered in human cancers, but in unpredictable ways. For example, Par6 and aPKC are often upregulated, whereas Lgl2 is down-regulated [30,70,71]. Loss of the Scribble polarity protein has been reported to increase tumor growth in a *myc* model of mammary cancer [72], and Scribble is down-regulated in a number of epithelial cancers, but is over-expressed in others [73]. Most of the polarity complexes interact with and reciprocally regulate the activity of other polarity proteins: aPKC phosphorylates Lgl2, for instance, and negatively regulates its activity [33]. It is likely, therefore, that disrupting the balanced expression of any number of polarity proteins could have a similar effect on promoting tumorigenesis. The localization of polarity proteins can also be affected in human cancer; aPKCι is mislocalized from the apical membrane in both breast and ovarian cancers [70,74]. Since restriction to specific membrane domains is an essential component of polarity, mislocalization rather than changes in expression level could be important in tumorigenesis. For example, the loss of aPKC from the apical membrane would disrupt normal epithelial architecture, a characteristic of pre-invasive adenomas. We speculate that loss of normal spindle orientation and escape from apoptosis are the primary drivers of initial tumor growth. Strikingly, however, the invasive edges of carcinomas often lose epithelial character, and express mesenchymal markers that have also been associated with cancer stem cells [75]. Similar behavior has been observed in *Drosophila* models of cancer [76], and suggests that cell non-autonomous effects of the neighboring stroma are key to understanding cancer cell invasion and dissemination.

2D versus 3D models of epithelial polarity and carcinogenesis

Classically, *in vitro* studies of cancer cells have involved cultures of monolayers on plastic dishes, despite the landmark work of Mina Bissell showing that the extracellular matrix and the 3D organization of breast cancer cells has profound effects on their behavior and gene activity. 3D culture systems have now become much more common, both for established cell lines and for primary tissue explants or stem cell-based organotypic cultures. One goal of these systems is to provide an environment in which normal tissue morphogenesis can be recapitulated, and a remarkable example is the formation of 'mini-guts' from single Lgr5+ intestinal stem cells [6]. When cultured in Matrigel with the correct mix of factors, the stem cells eventually give rise to structures containing the normal complement of intestinal epithelial cell types, organized in the correct pattern. Another recently developed 3D Matrigel system demonstrated the organization of undifferentiated mouse embryonic stem cell aggregates into fully patterned optic cups [77]. Fragments of other tissues, including mammary glands, salivary glands, skin, and kidney, can also be grown in organotypic cultures, and undergo branching morphogenesis and other changes that reflect normal developmental programs. The advent of lentiviral transduction provides enormous potential to manipulate cells in these systems, as a rapid screen for gene function.

3D systems can also provide a powerful method to study tumor invasion. Squamous cell carcinomas have been modeled from human primary epithelial cells transformed with oncogenic Ras and plated onto an intact basement membrane over a matrix containing stromal fibroblasts [78]. The transformed cells invade through the basement membrane into the matrix. What is particularly striking about this study, however, are the results of a transcriptome analysis, which showed a strong correlation with the gene expression pattern for actual squamous carcinomas, but no correlation with the expression pattern of identical transformed epithelial cells grown in 2D culture.

Even established cell lines behave differently when cultured in 3D versus 2D systems. Two widely used lines are the MDCK canine kidney epithelial cells and MCF10A human breast cells. Both can form cysts when grown in 3D matrices of collagen or Matrigel [79,80]. The mature cysts comprise a single layer of cells, the apical surface facing inwards and enclosing a central lumen. MDCK cells exhibit classical apical/basal polarity, with tight junctions at the apical/lateral boundary, and microvilli decorating the apical surface. In collagen, they initially form a disorganized ball of cells, until the outermost layer has secreted sufficient laminin to drive polarization [81]. These cells form apical surfaces that express the anti-adhesive protein podocalyxin. They disengage from internal cells, which then undergo apoptosis, resulting in a cleared lumen. Interestingly, however, if suspended in Matrigel, which is very rich in laminin, an MDCK cell will generate a lumen immediately after the first cell division, at the site of the

midbody. MCF10A cells have been described as being of basal origin, but they express E-cadherin, which is a characteristic of luminal epithelial cells, so their phenotype is ambiguous. They do not create tight junctions, and there is no evidence of cortical polarity, but they polarize in the limited sense that the Golgi apparatus is situated on the luminal side of the nucleus [80]. When MDCK cells are grown in 2D cultures, aPKC is predominantly localized to the tight junctions, and the Par3 polarity protein is required for normal tight junction assembly [82]. In this process, Par3 sequesters the Rac GTPase exchange factor, Tiam1, and prevents its inappropriate activity, thereby suppressing tight junction formation. Yet in the same cells, when grown in 3D culture, aPKC is spread over the apical surface, and instead of controlling tight junction assembly, Par3 determines the orientation of mitotic spindles [36]. Thus, simple changes in the geometry of cell growth can change the signaling pathways that control cell phenotype. Importantly, the function of Par3 in 3D culture more closely recapitulates its function *in vivo*, during murine mammary gland development, than does Par3 in monolayer cultures. Differences in behavior have also been noted in MCF10A cells, where silencing of the Scribble polarity protein has no phenotype in 2D cultures, but inhibits normal acinar morphogenesis through reduced apoptosis [72].

We conclude that 3D cultures can, under the right circumstances, provide highly accurate, biologically relevant systems for the analysis of organ or tissue morphogenesis that are readily amenable to genetic and environmental manipulation. Organotypic *ex vivo* systems are likely to be of particular importance in the analysis of the tumor micro-environment and for therapeutic development, where mouse models do not always recapitulate tumor behavior seen in humans, and cancer cell lines grown as monolayers on plastic bear little to no resemblance to actual cancer tissue *in situ*.

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