Review

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Orchestration of tissue-scale mechanics and fate decisions by polarity signalling

Martim Dias Gomes[1,2] & Sandra Iden[1,2,3,*]

Abstract

Eukaryotic development relies on dynamic cell shape changes and segregation of fate determinants to achieve coordinated compartmentalization at larger scale. Studies in invertebrates have identified polarity programmes essential for morphogenesis; however, less is known about their contribution to adult tissue maintenance. While polarity-dependent fate decisions in mammals utilize molecular machineries similar to invertebrates, the hierarchies and effectors can differ widely. Recent studies in epithelial systems disclosed an intriguing interplay of polarity proteins, adhesion molecules and mechanochemical pathways in tissue organization. Based on major advances in biophysics, genome editing, high-resolution imaging and mathematical modelling, the cell polarity field has evolved to a remarkably multidisciplinary ground. Here, we review emerging concepts how polarity and cell fate are coupled, with emphasis on tissue-scale mechanisms, mechanobiology and mammalian models. Recent findings on the role of polarity signalling for tissue mechanics, micro-environmental functions and fate choices in health and disease will be summarized.

Keywords cell fate; cell mechanics; cell polarity; epidermis; polarity proteins
Subject Categories Cell Adhesion, Polarity & Cytoskeleton; Development
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Introduction

Cell polarity refers to the asymmetric shape and uneven distribution of cellular constituents, such as organelles and macromolecules. In epithelial tissues, polarization governs major processes, e.g. the formation of intercellular adhesions responsible for tissue cohesion, the definition of the apical and basal domains and the segregation of fate determinants (Kemphues, 2000; Goldstein & Macara, 2007; St Johnston & Ahringer, 2010; Nance & Zallen, 2011; Campanale *et al,* 2017; Riga *et al,* 2020). Epithelial polarity is also reflected in polarized localization of cytoskeleton components, gradients of soluble molecules or asymmetric distribution of adhesive structures. In invertebrates, conserved polarity proteins of the Crumbs, Par and Scribble polarity complexes (Fig 1A and B) are essential for the establishment of apico-basal polarity and have emerged as important molecules for cell fate decisions (Motegi *et al,* 2020). Links between cell polarity networks and fate specification have been firmly demonstrated in the context of asymmetric cell division, where polarity proteins and the spindle orientation machinery collaborate to drive segregation of fate determinants (Inaba & Yamashita, 2012). Intriguingly, recent findings from epithelial systems have disclosed an unexpected, tight interplay of polarity proteins, adhesion molecules and mechanochemical pathways in tissue organization. Below, we discuss the importance of core apico-basal polarity networks and mechanics in driving fate decisions at tissue scale.

Tissue challenges during development and homeostasis: Intrinsic and extrinsic regulation of cell fate by polarity signalling

Exploring cell polarity across taxa: learning from different systems

Research in the field of cell polarity is characterized by its wide range of model systems utilized, spanning fungi, plants, invertebrate and vertebrate animal models. Prominent studies in lower organisms have revealed intimate links between control of cell polarity and cell fate. Among these, a foundation in the field of cell polarity was the identification of *par*titioning-defective *Par* genes, which, when mutated, cause defects in the first, asymmetric cell division of the *Caenorhabditis elegans* zygote (Kemphues *et al*, 1988) (Fig 2A). Since then, this system has served as a key screening platform to reveal factors required for asymmetric segregation of molecules in dividing cells (Rose & Gonczy, 2014). In *Drosophila melanogaster*, detailed genetic studies in various developing tissues and organs have clarified mechanisms of epithelial polarization, targeted RNA and protein transport, and oriented cell divisions. The neuroepithelium, for instance, hosts remarkably consistent events of cell division, with stem cells engaging polarity proteins to divide asymmetrically to give rise to two daughter cells of different fate (Wodarz, 2005; Homem & Knoblich, 2012) (Fig 2B). Prompted by these findings in invertebrates, the idea of a tight involvement of core polarity components in the control of spindle orientation has

1 CECAD Cluster of Excellence, University of Cologne, Cologne, Germany
2 Cell and Developmental Biology, Faculty of Medicine, Center of Human and Molecular Biology (ZHMB), Saarland University, Homburg, Germany
3 CMMC, University of Cologne, Cologne, Germany
*Corresponding author. Tel: +49 6841 1647912; E-mail: sandra.iden@uks.eu

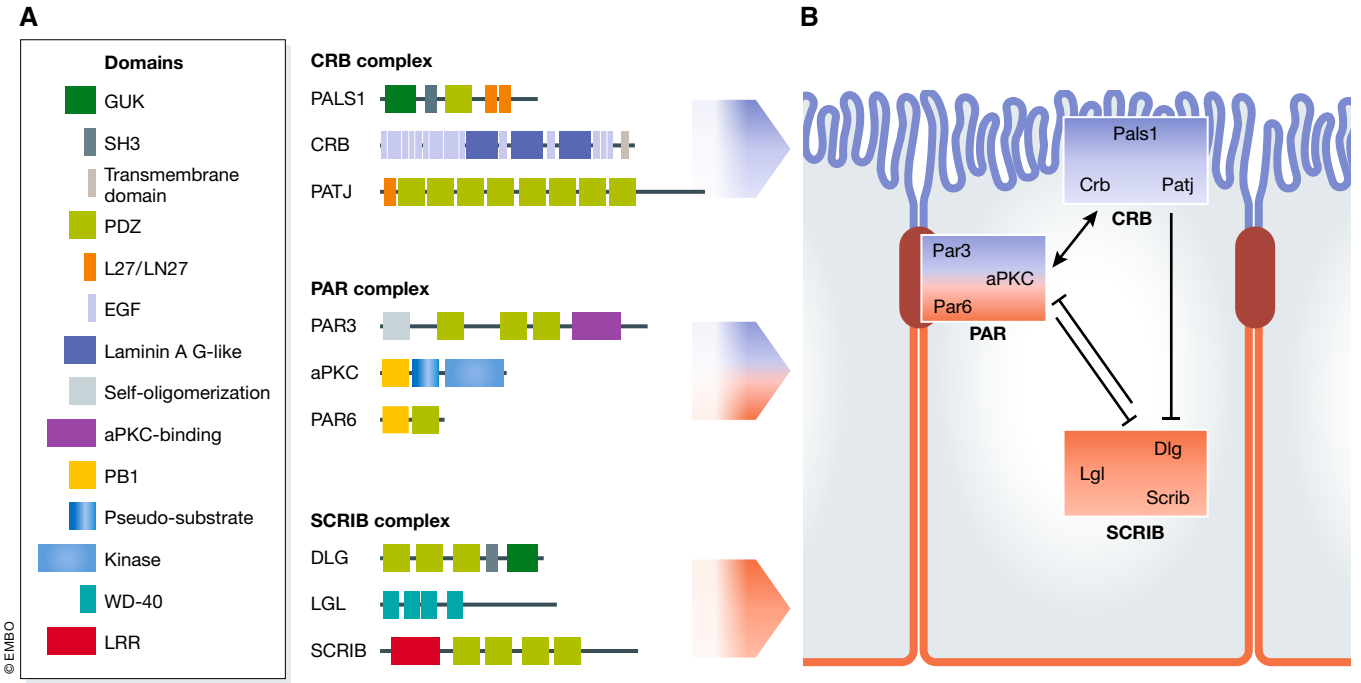


Figure 1. Conserved polarity regulators: molecular overview.

(A) Apical–basal polarity complexes in mammals. There are three main conserved polarity complexes in mammals, the apical Crumbs, the apico-junctional Par and the basolateral Scribble complex. Together, they mediate the establishment of apico-basal polarity. The Crumbs complex is composed of Pals1 and Pals1-associated tight junction (PATJ) homologue proteins. The Par complex consists of partitioning-defective 3 (Par3), partitioning-defective 6 (Par6) and atypical kinase C (aPKC). The Scribble complex includes lethal giant larvae (Lgl), discs large (Dlg) and Scribble (Scrib) proteins. These polarity complexes consist of several scaffold proteins containing protein–protein interaction domains (e.g. PDZ) mediating the binding of different polarity proteins across the apical and basolateral domains. The kinases aPKC and Par1 (not shown) play an important role by promoting the mutual exclusion of proteins from the apical or basolateral domains (see Box 1 for details). (B) Establishment of epithelial apico-basal polarity. The three polarity complexes, Crumbs, Par and Scribble complexes, distribute across the apical and the basolateral domain. During formation of epithelial intercellular junctions, Par3A localizes to the future tight junctions, which is required for the apical targeting of aPKC and Par6. Crumbs3 recruits Pals1, which in turns recruits Par6. When aPKC phosphorylates Par3, Par3 dissociates from aPKC/Par6. Upon Cdc42-mediated Par6 activation, aPKC can phosphorylate Lgl1/2 and Par1 (not shown), excluding them from the apical domain. Conversely, the Ser/Thr kinase Par1b at the basolateral site phosphorylates Par3, preventing its diffusion to the basal domain.

also been embraced for higher organisms. Interestingly, however, while some concepts could be extended to mammalian contexts (e.g. similar molecular interactions between polarity and spindle regulators) (Williams & Fuchs, 2013; Venkei & Yamashita, 2018; Rizzelli *et al,* 2020), the causal relationship between spindle orientation and fate remains a largely open question (Finegan & Bergstralh, 2019; Kotak, 2019; van Leen *et al,* 2020) (Fig 2C). Mechanisms of mammalian cell polarization have been intensively studied in cell cultures, e.g. those of monolayered epithelia and primary cultures of neurons. These systems unravelled roles of polarity proteins in neuronal specification (Nishimura *et al,* 2004; Hapak *et al,* 2018) and epithelial adhesion and barrier function (Iden & Collard, 2008; Roignot *et al,* 2013). Notably, however, polarity genes have widely diversified from invertebrates to mammals (Assémat *et al,* 2008) (Box 1), posing questions of functional redundancy of related genes. Though up-and-coming, there is still a significant gap of knowledge regarding *in vivo* functions of polarity regulators in mammals. Interestingly, as outlined below, recent insights on this came from interdisciplinary studies on cell polarity and mechanobiology, recognizing the joint contribution of these entities in steering cell fate.

Interplay of polarity and mechanics in different tissues

Cell and tissue behaviour were long perceived as the result of genetic and biochemical interactions. Meanwhile, also material properties of tissues are considered to be important for tissue function and behaviour (Xi *et al,* 2019). Cells can sense mechanical forces (mechanosensation), react to and transmit them (mechanotransduction), and the physical and biochemical pathways co-exist and are interdependent (Hannezo & Heisenberg, 2019; Xi *et al,* 2019). Contributing to this recent paradigm change was the realization that cell adhesion and the cytoskeleton are instrumental for sensing and transducing forces, and that morphogenetic processes obey certain mechanical principles known from material science and Newtonian physics (Chen *et al,* 2018). The actomyosin cytoskeleton is fundamental for this, as it is the main source of contractile stresses that are applied via either cell–cell or cell–matrix adhesions (Ladoux *et al,* 2015; Ladoux & Mège, 2017; Kechagia *et al,* 2019). While the presence of polarity proteins at these sites of adhesion precedes the existence of an anisotropic contractile network, the coordination of polarity with adhesion molecules ultimately defines the polarity domains, junction maturation and cellular fate.

Box 1: Mammalian polarity proteins and complexes

The apical Crumbs complex

Mammals express three homologues of the apical transmembrane protein Crumbs (Crb), (Crb1–3), composed of a short cytoplasmic tail and a large extracellular region (not present in Crb3, resulting in a substantially smaller size than Crb1 and Crb2). The cytoplasmic tail contains a FERM-binding motif and a PDZ-binding motif. The extracellular region contains multiple (up to 29) EGF-like and 3 Laminin A G-domain-like repeats.

PATJ is a scaffold protein, composed of a L27C domain, which mediates the interaction with Pals1, and multiple PDZ domain-containing proteins via its PDZ domain PATJ can interact with Par6. There is an additional mammalian homologue, MUPP1, which contains a L27 at the N-terminus and several PDZ domains.

Pals1 (Stardust homologue) is an adaptor protein of 77 kDa, member of the membrane-associated guanylate kinase (MAGUK) family. Pals1 contains two L27C/N-binding domains that mediate binding to Lin7 and Patj, respectively. Additionally, Pals1 has a PDZ domain by which interacts with Crb1 and Crb3, a SH3 domain, and a GUK domain. In mammals, Pals1 is also known as MPP5.

The apico-junctional Par complex

Par3 (Bazooka homologue) is a scaffold protein with several splice variants (180, 150 and 100 kDa). Mammals exhibit two Par3 genes: *Pard3a* and *Pard3b*. Par3 has three conserved regions, CR1, CR2 and CR3, respectively. The CR1 region (or N-terminal domain, NTD) is responsible for self-association via a PB1-like domain. The CR2 region contains three consecutive PDZ domains, which mediate binding to Par6, JAM-A and nectin 1. This region is also involved in phosphoinositide (PIP2/PIP3) association. The Par3A CR3 region comprises the aPKC-binding site, whereas Par3B has been reported not to bind to aPKC isoforms. The C-terminus of Par3 can bind to Kif3a. Par3A is target of different kinases (ERK2, LIMK; Par1, PP1) and phosphatases, with aPKC-mediated phosphorylation at S827 regulating the stability of the aPKC-Par3 interaction.

aPKC is a serine/threonine kinase and member of the larger PKC kinase family. Mammals express two related isoforms: aPKCiota (in humans; lambda in mice) and aPKCzeta, as well as an N-terminally truncated form, PKMzeta. aPKCs contain a PB1 domain (mediating interaction with the PB1 domain of Par6), a C1 domain (comprising a pseudosubstrate sequence involved in self-inhibition) and the C-terminal kinase domain. aPKC differs from classical and novel PKC kinases by being calcium insensitive since it is lacking the C2 domain.

Par6 is an adaptor protein. Three Par6 genes have been identified in mammals: *Pard6a*, *Pard6b* and *Pard6g*. Pard6 is composed of a PB1 domain, interacting with aPKC, a semi-Crib-motif by which interacts with Cdc42/ Rac1 and a single PDZ domain that mediates binding to Par3A and Crumbs3.

The basolateral Scribble complex

Scrib (Scribble homologue) is a cytoplasmic LAP family protein of 175 kDa. It contains sixteen LRR domains, which are necessary for its basolateral targeting, two LAP domains by which Scrib interacts with Lgl, a linker region, and four PDZ domains The PDZ3 and 4 are involved in ZO2 binding.

Dlg (discs large homologue) is a scaffold protein of the membrane-associated guanylate kinase (MAGUK) family. There are two splice variants (alpha and beta) that differ in their N-terminus: the first contains a palmitoylation site, whereas the latter includes an L27 domain responsible for self-association and binding to MPP proteins. In mammals, there are five disc large genes (*Dlg1–5*) but *Dlg1* is most closely related to the *D. melanogaster Dlg*. Dlg1 is a protein of approximately 120 kDa and contains three PDZ domains by which it binds to GluR1, APC and PTEN. This is followed by an SH3 domain, which enables Dlg1 to interact with the Lin2 serine/threonine kinase (CASK). The Dlg1 C-terminus harbours a GUK domain.

Lgl (lethal giant larvae homologue) is an adaptor protein. Two Lgl genes have been identified in mammals: *Llgl1* and *Llgl2*. Lgl is composed of several WD40 domains. There are other two very related proteins to Lgl in mammals: the syntaxin-binding protein 5 (Lgl3) and syntaxin-binding protein-like (Lgl4).

Polarity, mitotic spindle orientation and mechanical cues in non-mammalian systems

The cell division plane is typically established by spindle positioning right before chromosome segregation. Several cues can contribute to spindle orientation that are either of cell-intrinsic origin, such as centrosome and spindle anchoring position via the action of molecular motors, or of extrinsic origin, like exposure to a differential micro-environment, mechanical stimuli inducing elongation (e.g. stretch), or adhesion asymmetry (Finegan & Bergstralh, 2019). So far it has been difficult to dissect the various contributions that guide spindle orientation, especially at a tissue-scale level. Moreover, the exact consequences of spindle orientation for cell fate, morphogenesis and homeostasis appear to be highly context-dependent (Wodarz, 2005; da Silva & Vincent, 2007; Cabernard & Doe, 2009; Lu & Johnston, 2013; Finegan *et al,* 2019; Loyer & Januschke, 2020). The fly embryonic neuroepithelium represents a long-standing model in which division orientation is seamlessly coupled to fate determination (Fig 2B). Apical polarity cues such as Bazooka (Baz, in mammals termed Par3) or aPKC are essential for the establishment of apico-basal asymmetry in neuroblasts (NBs) and for spindle positioning. Baz/aPKC, via their interactions with the spindle regulators Pins and Mud, sequester astral microtubules, thereby retaining the centrosome apically and orienting the spindle (St Johnston & Ahringer, 2010; Inaba & Yamashita, 2012; Venkei & Yamashita, 2018). Additionally, division orientation can be modulated by changes in external factors and tissue mechanics (Fig 3A–C). External cues provided by the last-born NB, for example, help orient the spindle of subsequent divisions and stabilize NB-cortex glia interactions, thereby promoting stress resistance of the stem cell pool (Loyer & Januschke, 2018). Micro-environmental guidance of spindle orientation is also evident when developing tissues undergo large deformations: *D. melanogaster* embryo segmentation is characterized by extension of the germ band, a process that requires coordination of cell intercalation, junction or cortical tension remodelling (mediated by Baz/Par3 and Myosin II) and oriented cell divisions. During germ band segmentation, supracellular mechanical boundaries are created, with a defined actomyosin-based boundary cable that "traps" the spindle pole and hence orients cell divisions perpendicular to tension-bearing mechanical boundaries (Scarpa *et al,* 2018) (Fig 4A).

Cells engage actomyosin and adherens junctions (AJs) when dividing planar to the tissue elongation axis (Fig 4B), whereby cortical tension couples the spindle orientation machinery to actual spindle positioning (Lam *et al,* 2020). When the actomyosin

network is disturbed or when AJs are disrupted, cells fail to orient their spindle along the planar axis (Finegan *et al,* 2019). Stretching *Xenopus laevis* embryonic animal caps further showed that, similar to reports in *D. melanogaster* pupal notum (Bosveld *et al,* 2016), spindle positioning largely follows the positions of tricellular junctions (which are polarized according to the axis of the principal local stress) and depends on cadherins and polarization of the spindle machinery (i.e. LGN) to tricellular junctions. In this system, mechanical stress induced mitotic entry in a myosin-dependent manner rather than directly affecting spindle

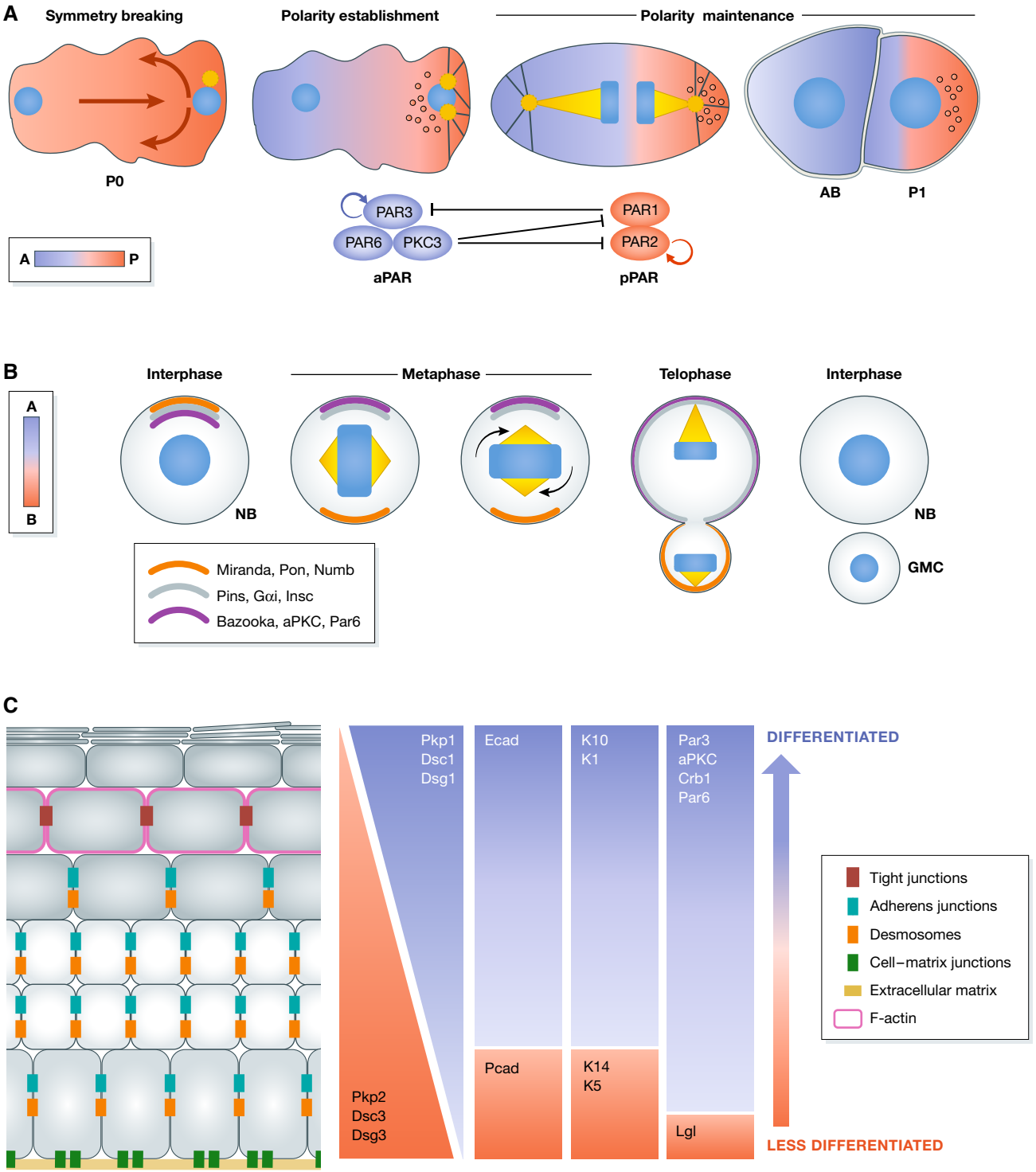


Figure 2.

Figure 2. Coordination of cell polarity and cell fate determination: examples.

(A) The sperm entry site determines the anterior–posterior axis and gives rise to the first polarized cell of the *Caenorhabditis elegans* embryo (P_0) (Nance & Zallen, 2011). Initially, PAR3, PAR6 and PKC3 (the aPKC homologue in *C. elegans*) are localized across the plasma membrane; however, a symmetry breaking event induces their anterior localization, hence referred to as the anterior polarity proteins (aPAR). PAR1 and PAR2 are evenly distributed within the cytoplasm and upon symmetry breaking are concentrated at the posterior pole, thus collectively referred to as the posterior polarity proteins (pPAR). The phosphorylation of pPAR proteins by PKC3 results in the establishment of the zygote a-p polarity. aPARs are under constant inhibition by pPARs, i.e. PAR1 phosphorylates PAR3 and removes it from the cortex. The basis for this sorting process is the existence of actomyosin flow directed to the anterior part of the embryo, passively transporting the aPar proteins. After sorting, the actomyosin flow finally diminishes (in a Par-dependent manner), which culminates in two domains with distinct Par polarity (polarity establishment) (Goehring *et al,* 2011; Gross *et al,* 2019). P_0 cells eventually undergo an asymmetric cell division that produces a blastomere (AB) and a germline cell (P_1). The two daughter cells will not only inherit different polarity proteins but also fate determinant molecules and structures like P-granules, RNA multi-phase condensates that become restricted to the posterior side and are segregated to the P_1 germline cell (Goldstein & Macara, 2007; Hoege & Hyman, 2013; Rose & Gonczy, 2014; Illukkumbura *et al,* 2020). (B) The neuroblast (NB), the *Drosophila melanogaster* neural stem cell, sustains its self-renewal capacity as a result of an asymmetric cell division (ACD). The two daughter cells display different fates and morphologies: a large pluripotent cell and a smaller one, committed to differentiation, called the ganglion mother cell (GMC). NBs are apico-basally polarized and exhibit asymmetry of polarity proteins. Baz (Par3), aPKC and Par6 are enriched apically and restrict the localization of basal fate determinants. This is achieved by aPKC-mediated phosphorylation of Numb and its scaffold protein Miranda, which displaces both proteins from the apical site to the basal cortex. Subsequently, the polarity and the spindle orientation machineries jointly mediate the segregation of apical and basal fate determinants. Inscuteable (Insc) binds Baz and Pins (LGN in mammals), which in turn connect $G_{\alpha i}$ to microtubules together with Mud (NuMA in mammals), a dynein-binding protein. Together, they promote spindle reorientation along the apical–basal axis and segregation of fate determinants (Prehoda, 2009). (C) The murine epidermis displays apico-basal polarization with differential expression of polarity proteins across the epidermal layers (Simpson *et al,* 2011; Niessen *et al,* 2012; Niessen *et al,* 2013; Ali *et al,* 2016). Likewise, cell adhesion and cytoskeletal components distribute asymmetrically within the epidermis. The basal layer expresses both P- and E-cadherin, while in the suprabasal layers, E-cadherin dominates. Similarly, desmosomal components distribute across the apico-basal axis: the basal layer expresses desmocollin 2/3, desmoglein 3 and plakophilin 2, whereas desmocollin 1, desmoglein 1 and plakophilin 1 are largely confined to the suprabasal layers. Tight junctions are restricted to the last viable layer, the stratum granulosum (SG2) and participate in the epidermal barrier. Likewise, the epidermal cytoskeleton exhibits apico-basal polarization. Intermediate filaments vary in their set-up across the epidermis, with specific expression of keratin K5/K14 heterodimers in the basal layer and K1/K10 dimers in suprabasal layers. Actin is enriched in the SG2 layer, associated with the formation of a junctional actomyosin ring (Simpson *et al,* 2011; Baroni *et al,* 2012). Intracellular organelles such as the centrosome, Golgi apparatus and cilia are also apico-basally polarized in the skin epidermis (Muroyama & Lechler, 2012). The skin also manifests planar cell polarization of hair follicles across the anterior–posterior and proximal–distal axis (Devenport, 2014).

orientation (Nestor-Bergmann *et al,* 2019). Together, these reports emphasize that spindle orientation results from the interplay between cell shape, mechanics and spindle machinery but that the individual contributions of any of these processes vary from tissue to tissue.

Polarity, mitotic spindle orientation and mechanical cues in mammalian systems

Above studies focused mostly on lower organisms, but how about the control of spindle orientation in mammals? There, the level of complexity is raised by gene diversification (and hence often redundancy of mammalian isoforms) and differential requirements during morphogenesis and homeostasis. An intriguing example for this is the role of LGN in spindle orientation. LGN localization is cell-type specific (Morin *et al,* 2007; Konno *et al,* 2008; Shitamukai *et al,* 2011; Matsuzaki & Shitamukai, 2015), and in the murine oral cavity, LGN shows distinct distributions depending on the type of oral epithelium. It localizes apically in the palate, buccogingival and ventral tongue epithelia, promoting perpendicular divisions and stratification, but in the dorsal tongue, LGN resides basally or

Figure 3. Analogies in symmetry breaking and self-organization between early embryogenesis and epithelial tissues.

(A) In the *Caenorhabditis elegans* one cell embryo, establishment of anterior and posterior polarity domains is achieved by antagonistic interactions between the polarity components of both domains and contributions by anterior-directed cortical actomyosin flow. The shear stress generated by the actomyosin flow acts on the cytoplasm and leads to the passive transport of cytoplasmic components (Illukkumbura *et al,* 2020). PAR network interactions maintain a polarized state, following the segregation of the aPAR to the anterior site, while the pPAR remains posterior. Polarization of the embryo is therefore the result of continuous diffusion of PAR molecules between areas where they can associate with each other and areas promoting their dissociation. This phenomenon can be described by a reaction–diffusion model, in which the PAR components transit between the cytoplasm and a membrane-associated state where they laterally diffuse until being detached by an antagonist (Goehring *et al,* 2011; Rodriguez *et al,* 2017; Gross *et al,* 2019). This type of intracellular self-organization forms the basis of various polarization events in small systems (Ganguly *et al,* 2012; Niwayama *et al,* 2016; Stückemann *et al,* 2017). In epithelial tissues, self-organization manifests at a supracellular level and can drive cell sorting, tissue organization and pattern formation (Kondo & Miura, 2010; Shyer *et al,* 2015, 2017). (B) Tissue fluidity as an organizational principle in morphogenesis. Tissue morphogenesis results from the combination of forces driving tissue movements (e.g. pressure, collective cell movements) and the material properties of the tissue (viscoelasticity). Symmetry breaking events will initiate body axis formation (e.g. anterior–posterior, dorsal–ventral) and progressive polarization. Tissues with different viscoelastic properties will respond differentially to external forces. Despite apparent high viscosity of tissues, if a rheological threshold is reached ("yield stress"), they will undergo a "solid" to "fluid" transition and will behave like fluids. Tissue fluidity depends on cell rearrangements such as cell intercalations and turn-over of junctional complexes. Anterior–posterior gradients of the yield stress have been implicated in body axis elongation (Mongera *et al,* 2018) and in gastrulation (Petridou *et al,* 2019) of the zebrafish. Fluidization in tissues due to mitosis or apoptotic events is thought to affect tissue viscoelasticity (Ranft *et al,* 2010) and tissue compression (Krajnc *et al,* 2018). In mammalian epithelia, state transitions have been implicated in the pathophysiology of asthma (Park *et al,* 2015) and fate decisions (Miroshnikova *et al,* 2018). (C) Intercalation is the process in which cells actively exchange their neighbours. There are two main types of planar intercalations that occur during morphogenesis: T1 transitions and rosettes. Both types require that actomyosin exerts force (i.e. cortical tension) on adherens junctions, thereby defining the junctional length (i.e. elongation or shrinking of the cell contact). Cells constantly fluctuate their cell–cell adhesions as a result of actomyosin dynamics, in order to delay or speed up cell rearrangements and consequently the neighbour exchange rate (David *et al,* 2014). Neighbour exchange is an essential behaviour of tissues and contributes to many processes such as tissue bending (Mason *et al,* 2013; Shook *et al,* 2018), elongation (Lienkamp *et al,* 2012) and wound healing, but can also modulate tissue fluidity (Rauzi *et al,* 2015; Curran *et al,* 2017; Tetley *et al,* 2019). There are several constraints that dictate an optimal cell arrangement. Cells must maintain their function (e.g. barrier performance), sustain their growth-to-differentiation balance (i.e. self-renewal) and be able to dynamically interact with their neighbours (Tetley & Mao, 2018).

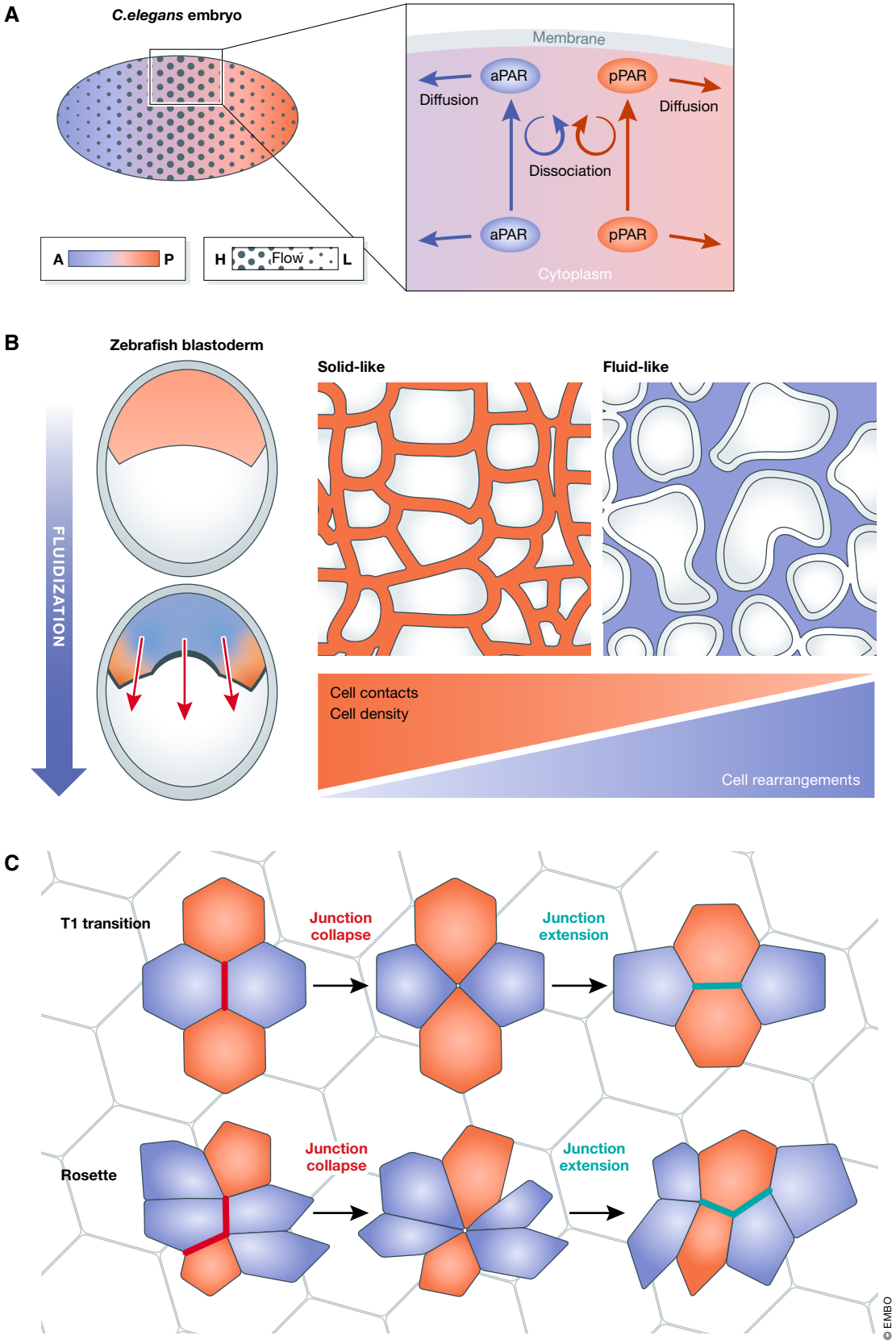


Figure 3.

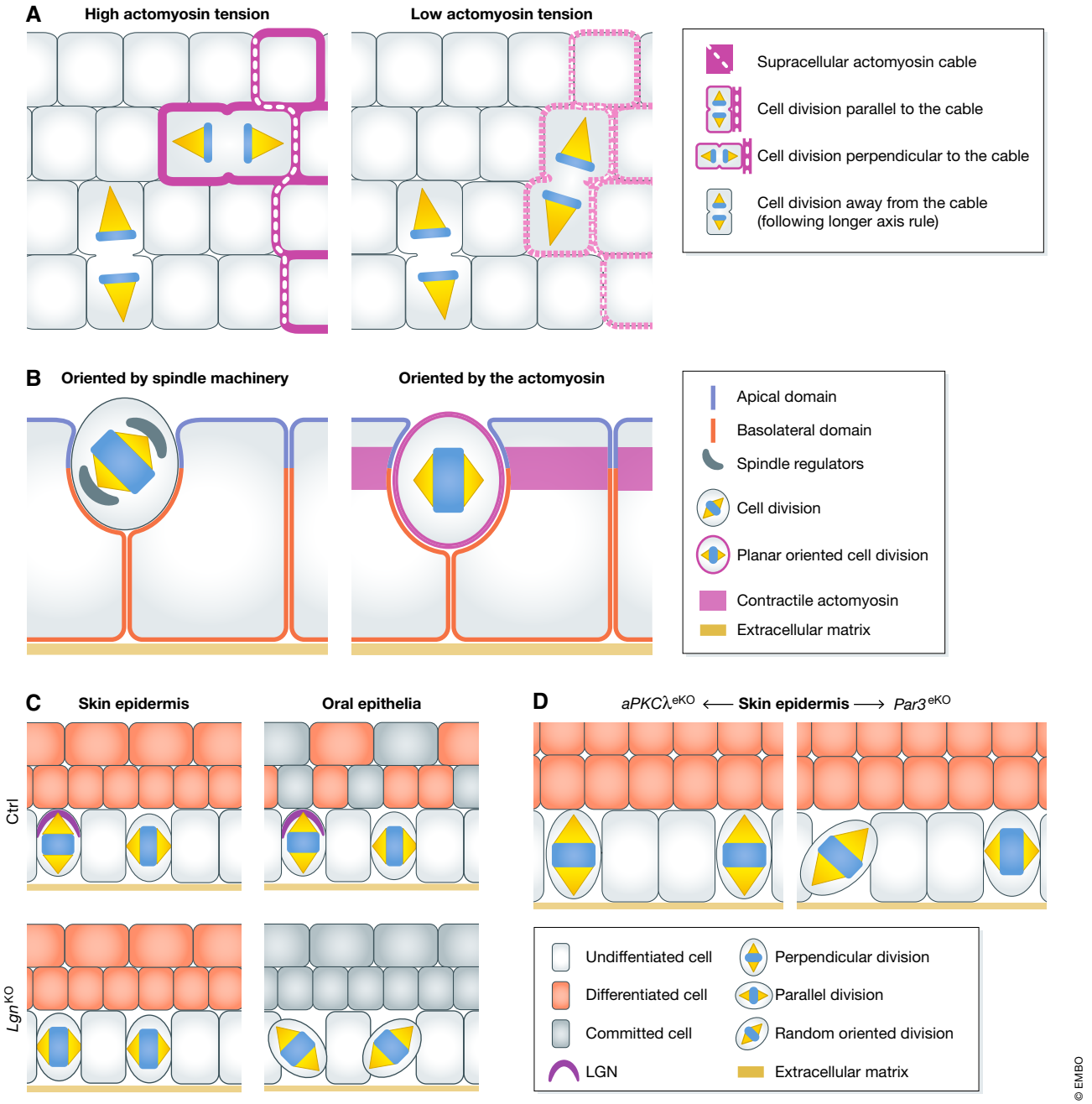


Figure 4. Intrinsic and extrinsic cues in spindle orientation and fate decisions.

(A) In the *Drosophila melanogaster* germ band, spindle orientation of boundary cells is guided by the topology and anisotropy of the tensile actomyosin network, whereas inner cells not facing the boundary divide along their longer axis (Scarpa *et al*, 2018). (B) In the *D. melanogaster* follicular epithelium, spindle orientation is not coupled to tissue elongation, challenging the tissue context dependency of these mechanisms. Inhibition of mitosis in follicular cells does not affect tissue extension but impairs the optimal packing of the cells. Interestingly, in this context Pins/Mud are necessary for the apico-basal positioning of the spindle (left) but not for its planar positioning (right) (Finegan *et al*, 2019). (C) In mammals, deletion of the spindle regulator LGN differentially affects mitotic spindle orientation, perhaps based on its apical vs. lateral localization in different epithelia (Byrd *et al*, 2016; Lough *et al*, 2019). In the mouse skin epidermis, LGN loss elicits mostly planar cell divisions, and in the oral tongue epithelium, it randomizes the spindle orientation. In these epithelia, LGN deficiency reduces differentiation or stratification, whereas LGN is not required for embryonic hair follicle development (Byrd *et al*, 2016). See text for details. (D) In the adult murine epidermis, aPKCλ deletion results in increased perpendicular divisions and epidermal differentiation (Niessen *et al*, 2013). Contrarily, epidermal Par3 deletion results in more planar and random divisions and increased differentiation (Ali *et al*, 2016).

laterally, promoting tissue expansion via planar cell divisions (Byrd *et al,* 2016) (Fig 4C). This suggests an exciting possibility that fate can be steered towards self-renewal or differentiation depending on the polarization of LGN. Moreover, LGN inactivation impaired the formation of filiform papillae, a ventral tongue appendage. In contrast, disrupting LGN in the embryonic epidermis did not affect the formation of hair follicles (Byrd *et al,* 2016), main epidermal appendages analogous to the papillae. Thus, spindle regulators have distinct functions in different tissues, supporting the idea that some of these genetically conserved players perform different tasks depending on the respective epithelial framework. Further, in the skin epidermis LGN participates in a tension-dependent correction mechanism of spindle orientation. During telophase, LGN collaborates with AJ tension sensitive proteins, such as α-catenin and afadin, to perpendicularly orient spindles (Lough *et al,* 2019). This mechanism suggests an extra layer of control over spindle orientation, where the spindle machinery itself cooperates with mechanoresponsive cortical elements to position the spindle.

Gene inactivation studies in mammalian epidermis further revealed intriguing and in part non-overlapping functions of apical polarity proteins in this barrier-forming epithelium: whereas epidermal aPKCλ knock-out mice showed more perpendicular spindle orientation (Niessen *et al,* 2013), epidermal Par3 deletion resulted in random and planar spindle orientation in different epidermal compartments (Ali *et al,* 2016), albeit both mutations led to increased differentiation (Niessen *et al,* 2013; Ali *et al,* 2016) (Fig 4D). This presents an example in mammalian epithelia where spindle orientation is seemingly not coupled to fate determination. Interestingly, NuMA-mutant mice showed increased planar cell divisions and an inability to differentiate (Seldin *et al,* 2016), thus implicating NuMA-mediated spindle orientation in fate determination during epidermal homeostasis. These findings therefore emphasize that cell shape, cell polarity signals and tissue mechanics can steer cell fate, whereby the relation of spindle orientation with fate is highly context-dependent.

Coupling of polarity, cytoskeleton and tension anisotropy during early lineage commitment in mammalian embryos

The previous section highlighted that tension anisotropy can serve as an important polarizing cue and is able to control spindle orientation. Does this concept also apply to directional movement of cells? Can differences in tension define the asymmetric positioning of cells? Indeed, for example in the early mouse blastocyst, the inner cell mass (ICM) is established by translocation of non-polarized cells to the centre of the embryo, clearly separating from the trophectoderm, which is composed of polarized cells. Several polarity proteins are apically enriched in trophectoderm cells, and loss-of-function experiments suggested a role for Par3 (Plusa *et al,* 2005), Par6B (Alarcon, 2010; Hirate *et al,* 2013; Lim *et al,* 2020) and aPKC (Plusa *et al,* 2005; Hirate *et al,* 2013; Maître *et al,* 2016) isoforms in lineage allocation. For long, spindle orientation was considered the major mechanism of asymmetric cell division during ICM allocation. However, recent findings challenged this view. Samarage *et al* (2015) reported that apical constriction driven by heterogeneity in cortical tension – rather than spindle orientation – was responsible for ICM rearrangement. The first embryo cell divisions are randomly oriented, whereas between the 8- and 16-cell stage, following their own division or that of their neighbours, some embryonic cells constrict their apical site and translocate to the embryo's centre. The apical constriction process relies on polarized myosin II enrichment around constricting cells. Remarkably, cell–cell adhesion seems dispensable for the constriction process, but cortical tension in the neighbouring cells does affect the inner cell movement (Samarage *et al,* 2015). Recently, the asymmetric inheritance of keratins (K) was reported as a very early mechanism directing the lineage segregation in mouse embryos. The stochastic expression of K8 and K18 filaments in a subset of cells at the 8-cell embryo stage establishes an intercellular polarity, with one daughter cell inheriting an apical pool of poorly diffusing keratin polymers, while the other cell will remain largely keratin-free. The apical, outside K8/K18-positive cells will give rise to the trophectoderm, while the K8/K18-negative cells will contribute to the ICM. Disrupting keratin networks by K8/K18 knock-down reduced apical PARD6B and PKCζ and prevented trophectoderm specification. *Vice versa*, PARD6B depletion impaired keratin polarization at the apical site and instead promoted the symmetrical inheritance of keratins. This indicates that the apical localization of keratins and classical polarity proteins creates a polymer-based polarity memory as an early event during lineage specification (Lim *et al,* 2020). Interestingly, also alterations in embryo size by means of blastocoel volume oscillations and concomitant changes in trophectoderm cortical tension contribute to asymmetric fate determination in mouse blastocysts (Chan *et al,* 2019). Together, these data emphasize the role of polarity, cytoskeleton and tension anisotropy for early lineage commitment in mammalian embryos.

Integrating cell and tissue polarity, mechanics and fate decisions: Insights from mammalian skin

Mammalian epidermal polarity and mechanics during morphogenesis

The mammalian skin epidermis is a prime example of an apicobasally polarized, multi-layered tissue (see Fig 2C). Polarity proteins are expressed throughout the epidermis, albeit with layer-specific localization patterns (Niessen *et al,* 2012). For example, aPKC and Par3 in the basal layer can be detected both in the cytoplasm and diffusely at peripheral membranes, whereas within the stratum granulosum II (SG2) they show distinct localization to tight junctions (TJs). The differential localization suggests distinct functions and signalling events in basal vs. suprabasal epidermal layers (Niessen *et al,* 2012). A central question in the field of skin biology is how cell division of stem/progenitor cells in the basal layer is coupled to differentiated fate of suprabasal epidermal cells to ensure self-renewal and skin barrier function. Spindle orientation has been implicated in this process, coordinating epidermal expansion and stratification during morphogenesis (Poulson & Lechler, 2010; Williams *et al,* 2011). Moreover, as the case in invertebrate systems, epidermal cells appear to engage different polarity proteins to orient mitotic spindles (Williams *et al,* 2014). Yet, despite causing alterations in spindle orientation during epidermal morphogenesis, ablation of Par3 or aPKC isoforms did not prevent early epidermal stratification (Niessen *et al,* 2013; Williams *et al,* 2014; Ali *et al,* 2016), opening the possibility that alternative mechanisms to spindle orientation play a role in epidermal morphogenesis and stratification. Interestingly, evidence emerges that polarization of the actomyosin tension and of cell–cell adhesion contributes to coupling of self-renewal and differentiation of progenitors. Miroshnikova *et al* (2018) proposed a model based on the concept that proliferation in the basal layer results in epithelial jamming (for

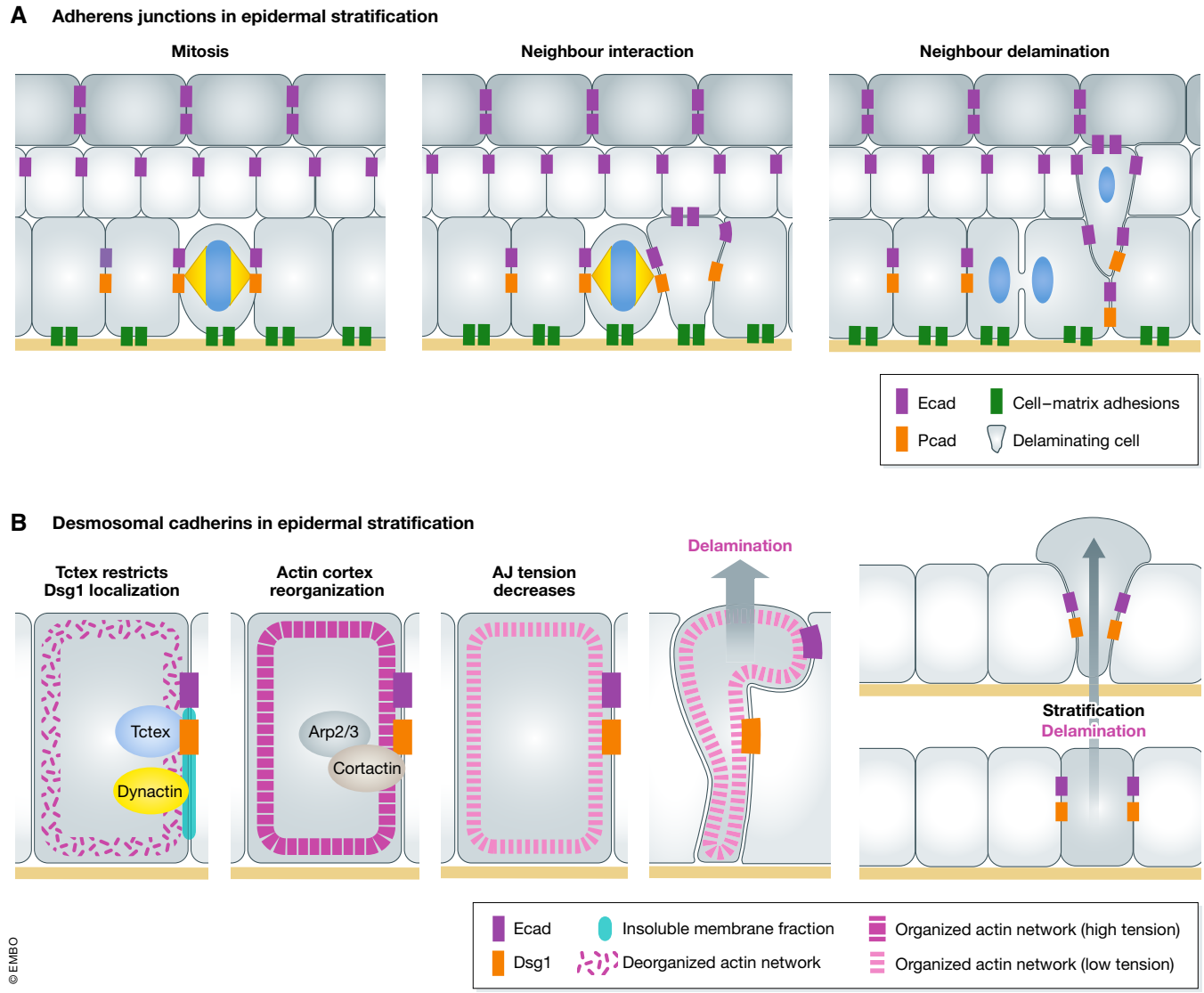


Figure 5. Compartmentalized epidermal cell–cell adhesion molecules and tissue mechanics in skin epidermal morphogenesis and fate.

(A) Cellular interactions in developing epidermis: a mitotic division compresses its direct neighbours. The compressed cell changes its cadherin-based adhesion profile, weakens its substrate adhesions and delaminates (Miroshnikova *et al*, 2018). (B) Desmoglein 1 promotes actin cortex reorganization via Arp2/3 and cortactin, which decreases cortical tension. A softer actin cortex promotes keratinocyte delamination (Nekrasova *et al*, 2018). See text for details.

state transitions, see Fig 3B). The compressive forces generated polarization of cell shapes and stresses, reducing locally the cortical tension and increasing cell–cell adhesion via E-cadherin. Interestingly, the delamination rate was higher in close proximity to cell divisions, thus sustaining the average cell density (Miroshnikova *et al*, 2018) (Fig 5A). Another recent study utilized 3D organotypic keratinocyte cultures to show that the actin nucleator Dia1 promotes epithelial jamming in the basal layer. Dia1 is predominantly expressed in basal keratinocytes where it is thought to enhance lateral cell–cell adhesions and hence columnar cell shape (preprint: Harmon *et al*, 2021). Distinct expression of actin modulators thereby may help instruct keratinocyte shape anisotropy across stratified epidermal tissues.

The coordination of cell–cell junctions with cell mechanics is particularly evident during apico-basal polarization of intercellular junctions. E-cadherin, albeit ubiquitously expressed, has been shown to couple localized actomyosin contractility at AJs with spatiotemporal control of EGF receptor activity to restrict TJs to the upper SG2 layer (Rübsam *et al*, 2017). These data thus implicate this classical cadherin in apico-basal polarization of junctional tension and barrier function. Interestingly, also other cell–cell adhesion molecules seem to contribute to tension anisotropy across epidermal layers. For example, Dsg1 is a desmosomal cadherin that shows a polarized distribution across the tissue axis, with increasing levels from basal to suprabasal layers. When Dsg1 expression is initiated in basal epidermal layers, this triggers actin reorganization, leading to

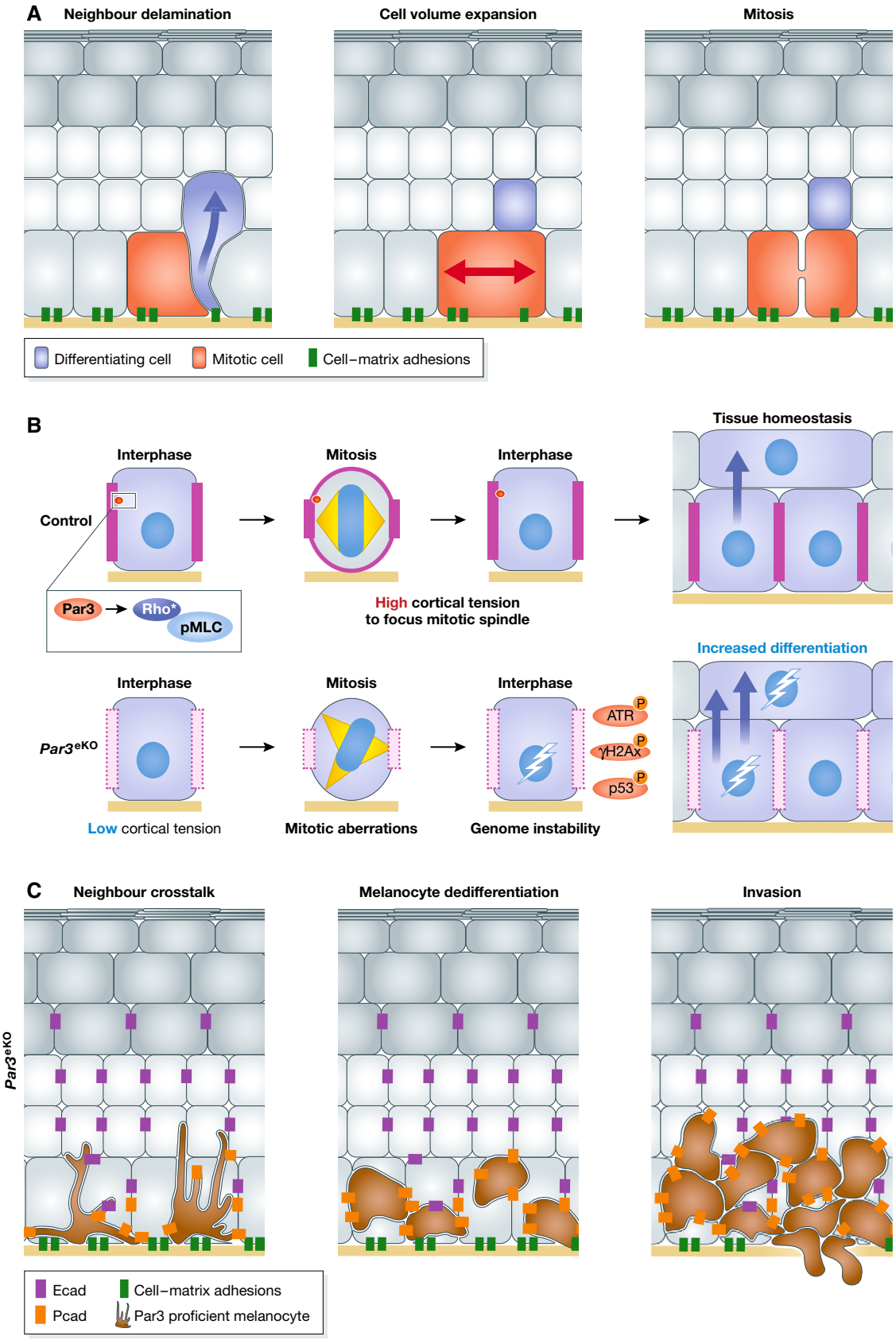


Figure 6.

Figure 6. Epidermal fate decisions and tissue mechanics in adult epidermis.

(A) In adult ear epidermis, differentiation of a basal layer keratinocyte changes the aspect ratio of the neighbour cell, now compelled to fill the gap. Cell elongation triggers mitotic division, which equilibrates cell numbers (Mesa *et al,* 2018). (B) Epidermal Par3 acts upstream of Rho/actomyosin contractility to promote intrinsic force generation and mitotic rounding, thereby maintaining mitotic accuracy and cellular fitness at the genomic level. Loss of Par3 alters keratinocyte mechanics resulting in mitotic infidelity and accumulation of DNA damage and p53, which in turn fuels differentiation (Dias Gomes *et al,* 2019). (C) The loss of Par3 in keratinocytes leads to upregulation of surface P-cadherin in keratinocytes. Altered cadherin engagement in keratinocyte–melanocyte interactions promotes melanocyte proliferation and phenotypic switch, providing a niche for melanocyte transformation (Mescher *et al,* 2017). See text for details.

reduced cortical tension at AJs. Following the drop of tension at AJs, basal cells delaminate to the suprabasal layer. This switch might be mediated by a molecular crosstalk between desmosomal and molecular motors (Dsg1, Tctex1/dynein cargo adaptor and actin/actin-modulating proteins) (Nekrasova *et al,* 2018) (Fig 5B). Remarkably, ectopic expression of Dsg1 in simple epithelial cells is sufficient to form a multi-layered epithelium (Nekrasova *et al,* 2018), implicating Dsg1 in key events mediating the stratification of epithelia.

Patterned cell rearrangements are also important during the morphogenesis of epidermal appendages such as hair follicles. The planar polarization of hair follicles relies on supracellular movements and coordination of the epidermal epithelium with the dermal condensate (DC). Placode polarization is associated with radial movements and planar cell polarity (PCP) control of Rho and myosin II activity. Sonic hedgehog (Shh) signalling instructs progenitor fates together with the PCP machinery, inducing cell rearrangements and neighbour exchanges (mainly by junctional remodelling and cell intercalation) in an actomyosin-dependent manner. These supracellular movements also reposition progenitors along the A–P axis and displace the DC, leading to asymmetric DC signalling and cell fate asymmetry (Cetera *et al,* 2018). Moreover, hair follicle fate specification was recently shown to depend on mechanical tension in suprabasal epidermal layers, with high contractility of differentiated keratinocytes counteracting the commitment of basal layer cells towards hair follicle fate (Ning *et al,* 2021), further highlighting links between tissue polarity, mechanics and fate.

Mammalian epidermal polarity and mechanics in tissue homeostasis

Similar to this emerging picture in the developing epidermis, it becomes more and more evident that polarity, adhesion and mechanical forces also shape epidermal homeostasis in the adult organism, though not necessarily through control of spindle orientation. Mesa *et al* (2018) imaged and tracked basal stem/progenitor cells in the adult ear epidermis *in vivo* and found that neighbouring cells were more likely to acquire opposite fates. When one cell differentiated, a cell in its vicinity was subsequently more likely to divide than a non-direct neighbour, thereby coordinating basal cell delamination and differentiation with the expansion of neighbouring cells (Mesa *et al,* 2018). The differentiation event is thought to provide space in the basal epidermal layer, allowing neighbouring stem/progenitor cells to progress through the cell cycle and thus coupling differential cell fate independent of spindle orientation (Fig 6A) (Mesa *et al,* 2018). Of note, this contrasts the findings of Miroshnikova *et al* (2018) in embryonic epidermis, where mitosis precedes differentiation (Miroshnikova *et al,* 2018), suggesting context dependency (e.g. cell density and proliferation rate). Next to such neighbour interactions, apico-basal polarity proteins were recently implicated in control of epidermal differentiation in adult epidermis, albeit through mechanical control of mitotic accuracy rather than spindle orientation. Epidermal deletion of Par3 elicits DNA damage accumulation and p53 expression in the hair follicle stem cell compartment and throughout the epidermis (Dias Gomes *et al,* 2019). Intriguingly, the DNA damage in Par3-deficient epidermal keratinocytes was a consequence of altered mechanical properties including reduced actomyosin activity, causing insufficient mitotic rounding, low mitotic fidelity and increased genome instability. Reconstituting RhoA activity or directly enhancing myosin activity was sufficient to rescue mitotic fidelity and to prevent DNA damage-dependent p53 induction (Fig 6B). Additionally, in an *in vitro* stratification model the altered viscoelastic properties biased $Par3^{KO}$ cells towards suprabasal layers, which could be reverted by enhancing actomyosin contractility (Dias Gomes *et al,* 2019). Together, these different studies showed how coordinated cellular behaviours, polarity proteins and cell mechanics contribute to the homeostasis of stratified epithelia.

Next to intraepithelial mechanisms, the skin epithelium can also impinge on the fate of neighbouring tissue-resident cell types such as epidermal melanocytes. Par3 inactivation in surrounding keratinocytes, in addition to the mechanical changes mentioned above, induced melanocyte dedifferentiation, altered motility and increased melanoma formation and invasion. The loss of Par3 in keratinocytes led to upregulation of the basal layer cadherin P-cadherin, which was required and sufficient to mediate melanocyte proliferation and phenotype switch (Mescher *et al,* 2017) (Fig 6C). This study thus demonstrates that polarity networks in the epithelial microenvironment can modulate the fate of adjacent cell types through direct cell–cell adhesion. Furthermore, these findings raise the question if polarity protein-mediated cellular mechanics contribute to tissue-scale instruction of fate determination in the epidermis and perhaps other epithelia.

Tissue-level decisions in regeneration, degeneration and cancer

Polarization and mechanics in wound-induced regeneration

Epithelial tissues occasionally face acute damage, for instance during wounding. In such situation, the epithelial barrier is compromised, putting the organism at risk due to an increased exposure to environmental factors. Therefore, the ability to repair the tissue and regain homeostasis is of fundamental importance for survival; however, the regenerative capacity varies immensely among species. Some organisms can regenerate seamlessly even large parts of their body (e.g. hydra and planarians), while others have limited regenerative potential that further declines with age (e.g. humans). Cell mechanics and the actomyosin cytoskeleton are fundamental to wound closure and tissue repair in most epithelia (Guzmán-Herrera & Mao, 2020), yet the exact mechanisms governing directed wound

closure *in vivo* are only beginning to unfold. Here, we focus on the tissue-scale aspects of wound healing from a polarity perspective.

In the *D. melanogaster* imaginal disc, reduction of junctional tension (e.g. by lowering myosin activity) increases cell intercalation events and tissue fluidization, which in turn accelerates wound closure (Tetley *et al,* 2019). Interestingly, wounds display a supracellular actomyosin network characterized by anisotropic myosin distribution, heterogeneous actin density and actin cables of varying thickness among different cell–cell contacts. This supracellular actin and myosin heterogeneity partially stems from the wound topology itself and leads to stochastic actomyosin contractions. Myosin activity further relies on the polarization of tension-mediated strain-activated ion channels (SAICs). Thus, wounding induces strain, which activates SAICs that in turn cause stochastic actomyosin contraction, a process that may foster stress dissipation (Zulueta-Coarasa & Fernandez-Gonzalez, 2018). Moreover, injured cells produce mitochondrial reactive oxidative species (ROS), which elicit polarization signals leading to actin and myosin anisotropy. Src kinase acts as a ROS sensor and mediates the trafficking and removal of E-cadherin by endocytosis, thereby "weakening" cellular junctions, which is required for localized actomyosin remodelling (Hunter *et al,* 2018). Together, these results depict a mechanism by which damaged cells induce a tissue-scale polarity response in order to heal epithelial wounds.

Importantly, there is also increasing evidence for a role of polarity signalling in mammalian wound healing. Following injury, the shape and polarity of cells at or close to the wound changes significantly (Paladini *et al,* 1996; Aragona *et al,* 2017). A range of *in vitro* studies indeed suggested a role of mammalian polarity proteins for efficient epithelial migration and wound closure (Iden *et al,* 2012; Trepat & Sahai, 2018). Such functions could be confirmed in part in murine models of tissue repair: the embryonic epidermis of Scribble mutant mice, for instance, shows defective wound healing, likely due to an inability to recruit Cdc42 and Rac1 to the cell edge (Dow *et al,* 2007). Moreover, aPKCλ, but not the aPKCζ isoform, has recently been implicated in polarizing the wound edge of keratinocytes *in vitro* and in efficient closure of large skin wounds *in vivo*, although by so far unknown mechanisms (Noguchi *et al,* 2019). Meanwhile, single-cell transcriptomics revealed complex heterogeneity, high plasticity and fate changes during epidermal wound healing (Joost *et al,* 2018; Haensel *et al,* 2020; Phan *et al,* 2020). Longitudinal imaging of skin reepithelization after wounding further revealed a dynamic tissue-scale programme that steers efficient wound healing: keratinocytes at the wound edge migrate directionally towards the wound centre, a process facilitated by Rac1. In the adjacent mixed zone, cells both migrate and proliferate, orienting their division axis towards the wound during collective movement. Such coordination of proliferation, division orientation and polarized migration promotes the local expansion of the epithelium, culminating in its repair (Park *et al,* 2017).

Altered cell polarity in tissue dysfunction and cancer

Next to tissue regeneration, changes in cell and tissue polarity also occur during other processes of disrupted homeostasis, such as tissue degeneration and tumorigenesis (Mescher & Iden, 2015). With increasing organismal age, alterations of biochemical and mechanical properties are associated with stem cell depletion (Matsumura *et al,* 2016; Liu *et al,* 2019), niche dysfunction (Segel *et al,* 2019) and dysplasia (Wolfenson *et al,* 2019).

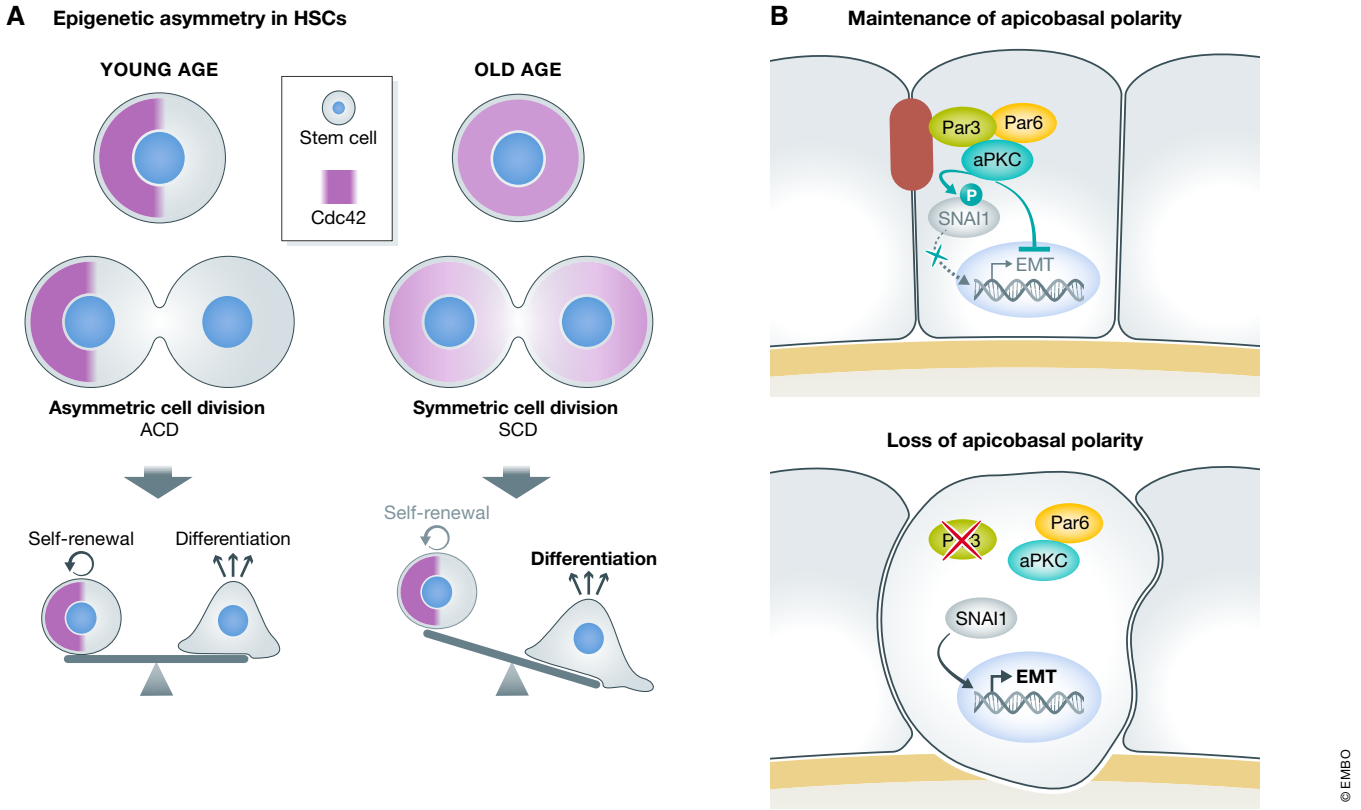
Polarity in loss of tissue fitness and ageing

How do tissues maintain homeostasis following stochastic mutations or damage? Quality control mechanisms exist that evaluate and feedback cell fitness, resulting in maintenance or removal of damaged cells from a tissue. Next to immune surveillance mechanisms, a well-studied process governing quality control is cell competition. The basic principle behind it is that cells in a high fitness state cause the elimination of unfit cells. The cell polarity field contributed to the formation of cell competition concepts, with many studies reporting how aberrant polarity signalling triggered such events in lower organisms and cell culture (Merino *et al,* 2016; Vishwakarma & Piddini, 2020). Mutations for Scribble and Dlg in fly epithelial cells resulted in their elimination via JNK and TNF signalling (Igaki *et al,* 2009; Yamamoto *et al,* 2017; Caria *et al,* 2018). Related mechanisms may also play a role in mammals. In MDCK cells, depletion of Scribble sensitized cells to crowding. Scribble knock-down cells, when crowded by their wild-type neighbours, were eliminated by cell death. This hypersensitivity to cell–cell contact was mediated by a ROCK-p38-p53 axis upstream of cell death, directly connecting alterations in mechanical properties, sensibility to crowding and upregulation of low-fitness markers (Wagstaff *et al,* 2016). This, together with the finding that loss of Par3 in epidermal cells leads to DNA damage-driven p53 upregulation and increased differentiation (Dias Gomes *et al,* 2019), emphasizes intriguing links between different polarity networks and p53-mediated cell fitness, with the outcome of p53 action (i.e. apoptosis *vs* differentiation) likely depending on the specific epithelial traits.

Moreover, cell fitness has been linked to cell division orientation. Hematopoietic stem cells (HSCs), for instance, divide asymmetrically to generate progenitors that undergo either myeloid or lymphoid fate to give rise to all cells of the hematopoietic system, ranging from megakaryocytes to B lymphocytes. Although HSCs do not display a clear morphological polarity, they show asymmetry at the level of the microtubule cytoskeleton and activity of small Rho GTPases, such as Cdc42. Interestingly, cell division orientation of HSCs depends on Cdc42 activity, and aged HSCs exhibit reduced polarization, associated with lower Cdc42 activity and increased symmetric division. Comparing the epigenetic landscape of young *vs* old HSCs and their transcriptome revealed distinct profiles. Daughter cells of young HSCs, which maintained Cdc42 activity and frequently divided asymmetrically, showed differential allocation of epigenetic histone marks for open chromatin, correlating with stem cell potential. Progeny of old, symmetrically dividing HSCs instead presented with lower self-renewal capability and reduced potential to generate differentiated cells of the lymphoid lineage, implicating links between cell polarity and control of epigenetic features (Fig 7A) (Florian *et al,* 2018). Similar connections between loss of regenerative potential and altered cell division polarity have been reported in the retina (Quinn *et al,* 2018; Kraut & Knust, 2019; Kujawski *et al,* 2019) and muscle tissue (Dumont *et al,* 2015).

EMT, early oncogenic lesions and hijacking of cell architecture

Epithelial–mesenchymal transition (EMT) is an important process in development but is also associated with cancer cell invasion. Through complex cellular reprogramming, epithelial cells acquire key mesenchymal, migratory characteristics as a result of activation of distinct transcription factors (such as Snail, Twist and Zeb), ECM remodelling and loss of apico-basal polarity, which culminates in



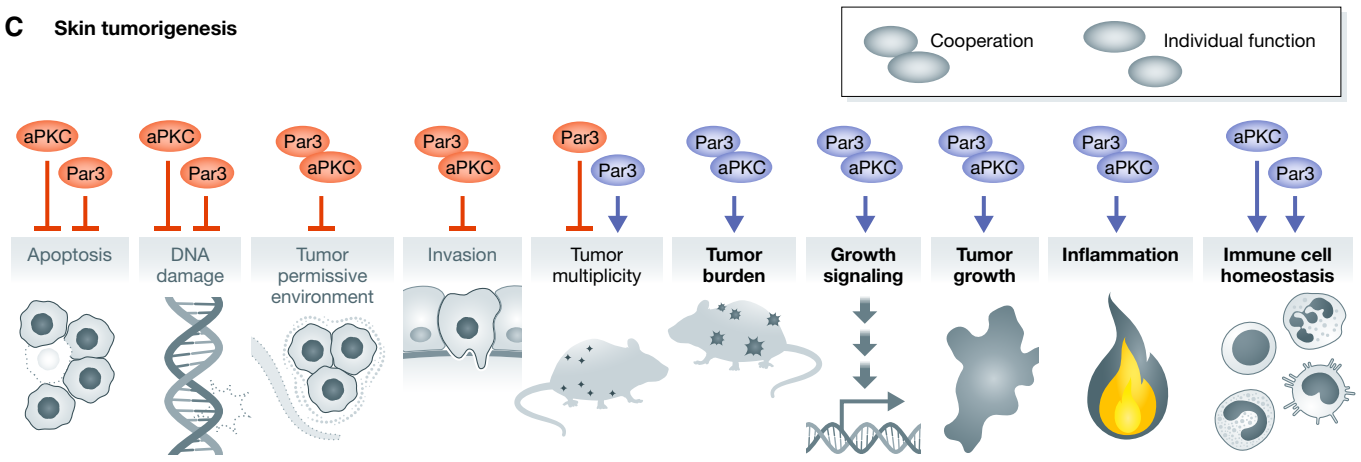


Figure 7. Polarity, tissue degeneration, EMT and cancer.

(A) Asymmetric cell division of hematopoietic stem cells (HSCs) includes the polarized segregation of Cdc42, which is important for the maintenance of stemness and is associated with polarized signatures of epigenetic marks. An age-related shift towards symmetric cell division (SCD) in HSCs diminishes self-renewal capacity (Florian *et al,* 2018). (B) Core Par complex polarity proteins ensure cyto-architecture and prevent epithelial-to-mesenchymal transition (EMT). The kinase aPKC phosphorylates the transcription factor SNAI1, promoting its degradation and preventing EMT (Jung *et al,* 2019). (C) Cooperative and independent *in vivo* functions of Par3 and aPKCλ in skin tumorigenesis based on oncogenic Ras-driven mouse tumour models (Iden *et al,* 2012; Vorhagen *et al,* 2018).

alterations of cell–cell adhesion and of the cytoskeleton (Moreno-Bueno *et al,* 2008; Godde *et al,* 2010; Lamouille *et al,* 2014). aPKCλ and Par6 have been reported to participate in EMT in small cell lung cancer (Regala *et al,* 2005; Gunaratne *et al,* 2013), whereas Par3 loss promotes tumour cell invasion in the mammary gland (McCaffrey *et al,* 2012; Xue *et al,* 2012) and skin cancers (Iden *et al,* 2012) *in vivo*. The role of Par3 complex proteins in EMT was recently further supported in a 3D model of primary mammary epithelial

cells. When cultivated at 3D conditions that allowed full apico-basal polarization, Par3 and aPKC localized to TJs. When Snai1 was induced at these conditions, aPKC phosphorylated Snai1, targeting it for degradation. Instead, when apico-basal polarity was not pronounced, as the case in 2D cultures of mammary cells, or upon loss of Par3, aPKC could not phosphorylate Snai1, leading to stabilization and nuclear translocation of Snai1, followed by expression of EMT-related genes (Jung *et al,* 2019) (Fig 7B). These data implicate polarity signalling in the maintenance of cyto-architecture and in preventing EMT.

Intriguingly, cancer cells can also control cyto-architecture and cell mechanics by hijacking actomyosin signalling for optimal cell division. Activating *RasV12* in mammary cells is sufficient to enhance mitotic rounding, which increases the ability to divide under confinement, thereby perhaps providing an advantage to mutant cells when dividing in stiff environments (Matthews *et al,* 2020). Likewise, induction of EMT was recently shown to alter cell mechanics in a cell cycle-dependent manner, with a mitosis-specific enhancement of cortical mechanics and rounding (Hosseini *et al,* 2020). Oncogene-mediated shaping of the cytoskeleton may also instruct the overall tumour tissue architecture beyond the cellular scale. Messal *et al,* (2019) found that the growth direction of neoplastic lesions in tubular single-layered epithelia is in part determined by the lumen's physical properties (Messal *et al,* 2019). Lesions in small ducts mostly grew outwards, while those in large ducts predominantly grew inwards, with the growth direction correlating with epithelial curvature and mechanical changes in transformed cells: ductal epithelial cells usually showed apically polarized F-actin and pMLC2 (phospho-myosin light chain II), whereas upon *KRas*-induced transformation, this asymmetry was lost and pMLC2 was redistributed, resulting in altered mechanical properties (Messal *et al,* 2019). Another recent study in murine multi-layered epidermis explored the significance of tumour architectures typically observed in non-invasive basal cell carcinoma (BCC) and more invasive squamous cell carcinoma (SCC). Fiore *et al,* (2020) found that oncogenic mutations in Shh signalling (common in BCCs) differentially affected the mechanical properties of early tumours and their micro-environments when compared to mutations in the Ras signalling pathway (common in SCCs). Ectopic activation of Shh signalling resulted in softer basement membranes and promoted BCC-like bud-type overgrowths. *HRas* mutations instead were associated with higher basement membrane stiffness and predominantly led to SCC-like tissue folding and invasiveness. Notably, these two types of lesions differed in their levels of actomyosin tension in suprabasal layers, at interfaces to healthy cells (being high at bud but lower at fold borders), and in their ability to remodel the basement membrane (Fiore *et al,* 2020). Together, these studies reinforce the importance of controlling cytoskeletal polarization and distinct architectures at both cellular and tissue levels and underline that mechanical properties of tissues can fundamentally affect dysplastic outcome.

Polarity signalling, cancer models and tailored therapeutics

The majority of cancers arise from epithelial cells, and the various stages of tumorigenesis are often accompanied by dramatic changes in cell and tissue architecture, posing the question whether polarity networks also play functional roles in malignancies. Loss-of-function studies in *D. melanogaster* provided clear evidence for a tumour-suppressive role of polarity proteins (Bilder, 2004; Elsum *et al,* 2012; Khursheed & Bashyam, 2014; Mescher & Iden, 2015). Meanwhile, various studies in mouse models and organoids also unravelled a tight connection between (altered) polarity signalling and oncogenic signalling in mammals (Mescher & Iden, 2015). Against initial predictions from fly models, however, mammalian polarity proteins, such as aPKC, Par3 and Lgl2, were shown to act as both pro-oncogenes and tumour suppressor genes (Iden *et al,* 2012; Garg *et al,* 2014; Marques *et al,* 2016; Vorhagen *et al,* 2018; Saito *et al,* 2019). In a *Ras*-driven two-stage skin cancer model, Par3 and aPKC collaborate in promoting skin tumorigenesis through ERK, Akt and Stat signalling, while also possessing independent functions: Par3 is a tumour suppressor in certain skin tumours (e.g. keratoacanthoma) (Iden *et al,* 2012; Vorhagen *et al,* 2018), whereas loss of aPKC causes a stronger induction of apoptosis in keratinocytes than Par3 inactivation (Vorhagen *et al,* 2018) (Fig 7C). Similarly as in flies, Scribble acts as a tumour suppressor in this skin cancer model (Pearson *et al,* 2015), although its subcellular localization seems important in counteracting some but not all properties of cancer cells (Stephens *et al,* 2018), highlighting the complexity of polarity protein functions in mammalian cancer. Comprehensive reviews on polarity proteins and cancer can be found here (Saito *et al,* 2018; Stephens *et al,* 2018; Reina-Campos *et al,* 2019; Fomicheva *et al,* 2020). Notably, also genetic disruption of spindle orientation (via expression of a NuMA mutant) was recently shown to cooperate with oncogenic KRas in causing strong skin tissue overgrowth (Morrow *et al,* 2019), further implicating regulated cell division orientation in cancer.

Clearly, polarity networks can be altered or hijacked in different types of human cancers. Studies on cell polarity signalling in the context of cancer were fuelled by the development of several animal models for human cancers. The $Lkb1^{KO}$ mouse represents a model for Peutz–Jeghers syndrome (Miyoshi *et al,* 2002), Par3 epidermal knock-out combined with carcinogen-induced *HRas* mutations is a model for keratoacanthoma (Iden *et al,* 2012; Vorhagen *et al,* 2018), and *D. melanogaster* Hippo or Scribble mutants can be used to mimic tissue overgrowth for drug screening (Humbert *et al,* 2008; Elsum *et al,* 2012; Snigdha *et al,* 2019). There is growing evidence supporting the potential use of inhibitors against polarity kinases such as aPKC (Butler *et al,* 2015), LKB1 (Par4) (Momcilovic & Shackelford, 2015; Ciccarese *et al,* 2019) or MARK1 (Par1c) (Levy *et al,* 2013; Voura *et al,* 2019). For example, in BCC, the most common skin cancer, inhibition of aPKCλ is arising as coadjuvant for conventional therapy (Mirza *et al,* 2017). BCCs in advanced stage often utilize the Hedgehog pathway (Hh) to maintain their growth and survival, and inhibition of Smoothened (a transmembrane Hh signalling component) presents a clinically validated strategy (Oro, 1997; Mirza *et al,* 2017). Downstream of Smoothened, the Gli transcription factors of the Hh pathway are deacetylated by HDAC1/2, which regulates their chromatin association and consequently Gli-dependent transcription (Canettieri *et al,* 2010; Coni *et al,* 2013). Targeting HDAC1/2 has been useful in some cancers but its dose-related cytotoxic effects impede its use for BCC. aPKCι phosphorylates Gli1 to promote the Gli1-HDAC1/2 association (Mirza *et al,* 2019), and a small molecule aPKC inhibitor PSI, when combined with lowered doses of HDAC inhibitors, prevents BCC proliferation *in vitro* and in patient-derived explants (Mirza *et al,* 2017). Interestingly, Gli1 either associates with the nuclear lamina

or chromatin in an aPKC-dependent manner, thereby changing "nuclear polarization" and modulating signal amplification in BCCs (Mirza *et al,* 2019). Thus, inhibiting aPKC might represent a strategy to treat Smoothened inhibitor-resistant BCCs. In summary, targeting polarity signalling – and in particular polarity kinases – has promising anti-cancer potential.

Conclusions & perspectives

Polarity-regulated cell fate decisions underlie tissue formation and maintenance. Although the core polarity machineries are well conserved from invertebrates to mammals, there are clear differences how these molecules exert their functions with respect to cell fate. Findings from diverse model systems remarkably illustrate that, next to the apico-basal axis, polarity also manifests at many other levels, such as adhesion asymmetries or the anisotropy of forces. Moreover, it becomes increasingly clear that spindle orientation is not the sole mechanism how mother cells segregate fate determinants. Regarding self-renewal, it is exciting that distinct intrinsic and extrinsic parameters are essential to maintain tissue stem cell pools and their niches. Additionally, polarity proteins regulate stem/progenitor cell behaviour and modulate how they interact with their neighbours, keeping tissues in check. Ultimately, mammalian tissues show a considerable tolerance to loss of cyto-architecture, steering self-renewal and differentiation according to their needs to maintain overall homeostasis. During ageing or disease, these properties decline, rendering tissues more susceptible to dysplasia.

In the future, to better understand mechanisms that safeguard tissue integrity it will be crucial to further map the details and dynamics of tissue polarity networks. Spurred by techniques such as single-cell RNA sequencing, it will be possible to trace cell lineages and to deconstruct and reconstruct polarity networks during differentiation. Additionally, despite great advances, our current molecular view of the core polarity complexes is still incomplete and rather static. Some polarity complexes – either because they are transient or underrepresented – might be very difficult to detect experimentally. Novel approaches including single-cell biochemistry, endogenous gene tagging and super-resolution imaging might unveil new dynamics of the different polarity complexes and amend our current views on how polarity proteins cooperate with cytoskeletal, mechanochemical and signalling entities in development, health and disease.

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Author contributions

MDG drafted the text and figures with inputs from SI. Both authors revised and finalized the manuscript for publication.

Conflict of interest

The authors declare that they have no conflict of interest.

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