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Abstract

Mitotic spindle orientation has been linked to asymmetric cell divisions, tissue morphogenesis and homeostasis. The canonical pathway to orient the mitotic spindle is composed of the cortical recruitment factor NuMA and the molecular motor dynein, which exerts pulling forces on astral microtubules to orient the spindle. Recent work has defined a novel role for NuMA as a direct contributor to force generation. In addition, the exploration of geometrical and physical cues combined with the study of classical polarity pathways has led to deeper insights into the upstream regulation of spindle orientation. Here, we focus on how cell shape, junctions and mechanical tension act to orient spindle pulling forces in epithelia, and discuss different roles for spindle orientation in epithelia.

Introduction

During animal development, complex epithelial structures are formed by regulating the spatiotemporal dynamics of cell rearrangements, cell shape changes and cell division [1–4]. Intensive work highlighted that the orientation of cell division plays essential roles in fate asymmetric cell divisions and tissue architecture, and allowed the establishment of a paradigm for spindle orientation within the cell [3,5,6]. The axis of division is set up by cortical pulling forces acting on astral microtubules (MT) [7,8]. Therefore, the orientation of division can be ensured by locating force generators at specific cell cortex locations. While force generation has been attributed to the MT minus-end directed motor dynein, the recruitment task was assigned to NuMA (Mud in *Drosophila*, Lin-5 in *C. elegans*), which in turn can be recruited to the cortex by LGN (Pins in *Drosophila*, GPR-1/2 in *C. elegans*) and the heterotrimeric G protein Gαi [3]. Recently, the relative roles of NuMA and dynein in force generation for spindle orientation have been further deciphered.

In symmetric cell divisions in epithelia, mitotic spindles are often oriented along tissue symmetry axes [9,10]. Classical observations in single cells led to the proposition of Hertwig's rule, which states that cells divide according to their longest axis [11,12]. While true in many contexts, cell divisions that challenge this rule have been reported [9,13,14]. Recent work has provided novel insights into whether and how epithelial cells adhere to Hertwig's rule, as well as how tension and polarity are integrated by cells to orient their spindles.

In this review, we first discuss how the cortical forces necessary for oriented cell divisions (OCD) are generated within the cell. Second, we describe recent data exploring how cell shape and local or global tissue tension influence spindle orientation in symmetric divisions in epithelia. Finally, we discuss the function of OCD. For more information on spindle orientation in asymmetric divisions and a more complete oversight of OCD molecular regulators, we refer the readers to recent reviews [3,6].

How are spindle orientation forces generated within the cell?

The current paradigm for force generation involves the recruitment of dynein and its cofactor dynactin to the cell cortex [7,8,15–18]. However, recent studies indicate that cortical dynein recruitment is insufficient for OCD [19,20]. Here we discuss recent findings on dynein activity and how its interactors assist dynein in force generation [17,19–22].

Dynein force regulation and spindle orientation

Work in *C. elegans* and mammalian cells has led to the identification of different dynein regulators required for spindle orientation such as LIS-1, Nde1/NdeL1, and a subset of dynactin subunits [23–27]. Recent structural analyses showed that dynein can form a ternary complex with dynactin and a cargo adaptor [28]. *In vitro* reconstitution indicates that this complex is highly processive and capable of transporting cargo over large distances (**Figure 1A**) [29]. Two recent structures of dynein/dynactin complexed with the adapters HOOK3 and BICD2 show that their interaction is facilitated by the coiled-coils of the adapter proteins which run along the length of dynactin [28]. As BICD2 is not essential for spindle orientation in cultured cells, this raises the question whether HOOK3 or another regulator activates dynein during spindle orientation [27,30]. Furthermore, depending on the adapter, one or two dyneins can be part of the ternary complex. Complexes with two dyneins generate higher forces and walk faster on MT [28]. The binding of the second dynein is facilitated by the CAPZβ subunit of dynactin (**Figure 1A**) [28]. Interestingly, recent experiments revealed that CAPZβ regulates spindle orientation *in vivo*, suggesting that the capacity of binding two dyneins contributes to OCD [27].

New roles for NuMA during spindle orientation

The respective roles of dynein and NuMA during spindle orientation have recently been investigated by optogenetically positioning components of the spindle orientation machinery at the cortex [17,22]. In these studies, optogenetic recruitment of NuMA to the cortex was sufficient to orient the mitotic spindle [17,22]. By contrast and in agreement with other studies [19,20], recruitment of the dynein heavy chain/dynactin to the cortex was insufficient to generate pulling forces on the spindle [17,22]. This suggests that NuMA is not only required for dynein recruitment, but also for force production.

NuMA coiled-coil and MT binding domains are essential for spindle orientation

All NuMA homologs have a coiled-coil domain which has been shown to be required for spindle orientation in *Drosophila* and HeLa cells [7,17,31]. Therefore, in the light of the recently established ternary dynein complex structure described above, NuMA is a candidate regulator of dynein activity (**Figure 1B**). Furthermore, NuMA localizes to MT tips in interphase and pro-metaphase cells, and deletion of the domain responsible for this localization (AA:1811-1985 in human) impaired spindle orientation in cultured keratinocytes and *in vivo* [20]. In addition, a second NuMA MT binding domain, (AA: 2002-2115 in human) is also essential for spindle orientation in cultured cells [19,21]. Accordingly, this second MT binding is necessary to orient the spindle upon optogenetic recruitment of NuMA to the cortex [17]. Exactly how NuMA and

its MT binding domains assist in spindle orientation remains to be defined. For instance, MT tip localized NuMA could regulate MT growth dynamics, cortical capture or cortical dwell time which have all been proposed to regulate spindle orientation [3,32,33].

In summary, these recent data illustrate that MT pulling force generation does not solely rely on dynein, but also requires NuMA. Direct analyses of astral MT dynamics and associated forces, combined with the deletion of the aforementioned NuMA domains and optogenetic recruitment will contribute to a better understanding of dynein regulation in spindle orientation.

How do cell shape and tissue tension result in anisotropic spindle orientation forces?

In the first exploration of the influence of cell geometry on division orientation, amphibian eggs were artificially flattened. As a result the first cleavage plane was perpendicular to the long axis of the egg [11,34,35]. Predicting spindle orientation based on the cells' long axis later became known as Hertwig's rule. In depth study of the underlying mechanism was possible after advances in microfabrication enabled standardized regulation of cell shape and the application of defined mechanical stress. Combined with automated measurements of spindle orientation and physical modeling, this led to important advances in linking geometry and division orientation [12,36–38].

By culturing single cells on adhesive micropatterns of defined shape, Théry and colleagues found that it was not cell shape but the distribution of actin rich retraction fibers (RF) connecting the mitotic cell with the substrate which best predicts spindle orientation. RF were proposed to provide a memory of the interphase adhesion pattern of the cell since this pattern controls their distribution [36–38]. The forces for spindle orientation were proposed to be generated in parallel by the NuMA pathway and by RF directed-actin clouds in which Myosin-10 modulates astral MT dynamics [32,36,39]. Following Hertwig's experiments, Minc and colleagues squeezed sea urchin eggs into microfabricated wells which orientated divisions according to the created long axis [12]. They proposed that the applied rotational forces on centrosomes scale with MT length, linking cell geometry with division orientation in single cells [12].

In epithelia, initial studies identified major roles for planar cell polarity (PCP) and apical-basal (AB) polarity in OCD [9,10]. These studies were instrumental in analyzing spindle orientation in multicellular contexts, and in particular with respect to the AB axis. Recent work explored whether and how geometry and tension play roles in regulating spindle orientation in epithelial tissues.

Two axes of spindle orientation in epithelia: apical-basal and planar

In epithelia, mitotic spindles are typically oriented perpendicular to the AB axis, which is critical to maintain daughter cells in the plane of the epithelium. This orientation generally depends on the lateral distribution of pulling forces [40–42]. When the actomyosin machinery necessary for cell rounding is impaired, the geometry can override the lateral pulling forces [40,41,43]. Recently, Box and colleagues found that AB division orientation responds to changes in cell geometry in mouse basal epidermal progenitors. Mutants for the PCP protein Vangl2 fail to close their neural tube, which in turn alters cell geometry, thereby indirectly perturbing division orientation relative to the AB axis [44].

Spindle orientation is also regulated in the plane of the tissue (planar orientation, **Figure 2** top center, **Figure 2A, 3A**), and is usually set according to Hertwig's rule [45–47]. A large variety of cell shapes exist in tissues, ranging from highly anisotropic (elongated) cells to isotropic

(round) cells [7,48–50]. Cells round up during mitosis raising the question of how the interphase long axis is sensed during division [40,51–53]. Furthermore, epithelial cells are subjected to mechanical forces, which can directly affect planar spindle orientation and modulate cell shapes (**Figure 3B**) [4]. Central questions in the field are therefore whether tissue stress is able to orient the spindle, and whether this effect is dependent on cell shape changes.

In single adhesive cells, applying external forces without affecting cell shape was able to induce spindle rotation [36]. Initial studies in the context of Zebrafish epiboly and in cultured epithelial monolayers stretched 30%, also indicated that tension is able to orient cell divisions, while also orienting cell shape [54,55]. In the next section, we discuss recent studies that investigated how epithelial cells sense their interphase shape, as well as how cell divisions are oriented by cell shape and/or tension [7,13,14,48,50,54].

Sensing interphase cell shape in mitosis

Work in the *Drosophila* notum epithelium uncovered a mechanism that translates the interphase long axis into localized forces which can orient the mitotic spindle. This mechanism is based on the localization of Mud/NuMA to tricellular junctions (TCJ, the junctions where three cells come together). This localizes the forces exerted on astral MT to TCJ, leading to spindle orientation according to TCJ distribution, which generally aligns with the cell long axis (**Figure 2B**). In cases where the angle predicted by cell shape and the distribution of TCJ differ by more than 45 degrees, cells divide according to the TCJ distribution showing that the orientation of TCJ is a better predictor for spindle orientation than cell shape [7].

Tension vs shape in spindle orientation

To analyze whether tension can orient the spindle independently of cell shape, a recent study subjected the *Xenopus* embryo animal cap to an uniaxial stretch (20%) with a PDMS based device [50]. The externally applied stretch induced both cell elongation and cell division orientation along the axis of stretch. By comparing cell shape, cell area and the position of the TCJ vertices, the investigators found that TCJ align with the principal axis of local stress and are a better predictor of spindle orientation than global tissue stress [50,56]. Indeed, in cells that do not elongate along the axis of stretch, the spindle is not oriented in the axis of stretch, but according to TCJ distribution (**Figure 2B, 3C**). Mechanistically, TCJ are enriched in Cadherin and LGN, and this distribution is necessary to control planar spindle orientation in this context [50]. In contrast to the findings in *Xenopus* epithelia, dividing cells within MDCK monolayers subjected to low uniaxial stretch (15%), oriented their spindle with respect to the stretch axis, independently of cell shape. The mechanism proposed here involves both Cadherin and the polarization of LGN towards the bicellular junctions (BCJ) that are perpendicular to the axis of stretch (**Figure 3D**) [48]. Therefore, the same molecules mediate the link between the orienting cue (geometry or tension) and force generation by localizing to TCJ or BCJ in *Xenopus* and MDCK cells respectively.

The link between tension and planar spindle orientation has been also studied in developing tissues which undergo elongation or experience localized tensile stress. In the case of the *Drosophila* embryo, cells that are adjacent to high tensile supracellular actomyosin cables divide according to their long axis only if they are highly elongated. By contrast, slightly elongated cells divide according to the tension cue provided by the actomyosin cables, which run perpendicular to cell shape (**Figure 2C**) [13]. Another tension dependent mechanism orienting cell divisions was identified in the elongating *Drosophila* follicular epithelium (FE). In

these cells, the cell long axis is perpendicular to the axis of tissue elongation and thus to the stretch force [14]. The embryo and FE constitute immature epithelia which do not harbor classical BCJ and TCJ septate junctions. No clear crescents or TCJ accumulation of LGN or NuMA could be observed and these proteins were not required for planar spindle orientation [13,14]. In mature epithelia with septate junctions (larval wing disc, pupal wing and notum tissues), Mud is enriched at the level of the tricellular septate junction [7,57]. Therefore, different mechanisms might orient cell divisions according to tension in immature and mature epithelia. In the embryo, the actomyosin cable is proposed to orient the spindle by capturing one centrosome in an actin network, while in the FE, anisotropic cortical stiffness is proposed to bias spindle orientation [13,14].

What emerges from these experiments is that no clear one-size fits all rule can be applied. In highly elongated cells, the predominant cue seems to be cell shape, independently of the mechanism used by cells to interpret this cue [7,13,50]. In cells with moderate anisotropy, different scenarios are possible: i) in immature epithelia or MDCK layers, where OCD regulators do not localize to TCJ, spindle orientation responds to global tension independently of cell shape; ii) in mature epithelia, cells use TCJ bipolarity, which aligns with local tension, as the main cue for spindle orientation (**Figure 2, 3**) [7,13,50]. In this view, one could speculate that TCJ hot spots of LGN or NuMA are more efficient to orient the spindle than other mechanisms that depend on actomyosin forces. Actomyosin based mechanisms remain to be defined in epithelia: could actin clouds and Myosin-10 be involved in spindle orientation like in cultured cells?

Function of mitotic spindle orientation in epithelia

A central question in the field of spindle orientation is the function of OCD in epithelia. Multiple roles have been proposed such as tissue layering, dissipation of tissue stress, changes in tissue morphology, and homeostasis (**Figure 4**) [4,41,47,54,58].

OCD were proposed to be required for axis elongation during Zebrafish gastrulation [9]. The PCP pathway can regulate spindle orientation through Mud and via the Anthrax2 receptor [59,60]. Knock down of the PCP protein Dishevelled resulted in a shortened anterior-posterior axis [9]. Yet, more directly perturbing spindle orientation by impairing dynein or NuMA function did not affect body axis elongation, challenging the idea that OCD are involved in tissue elongation during Zebrafish gastrulation [59,61]. In *Drosophila* wing imaginal disc, cells in the central wing pouch divide preferentially along the proximal-distal axis, while cells in the periphery divide tangentially along the local tissue boundary [10,46,49]. In this tissue, loss of Mud/NuMA leads to the formation of smaller but normally shaped wings (**Figure 4D-G**) [62]. Spindle misorientation can result in basally born cells being lost from the tissue possibly explaining the size reduction [41,62]. As global tissue stress can regulate OCD [10,46,49], the authors proposed that loss of OCD can be buffered by an increase in tissue stress and cell rearrangements contributing to tissue elongation [62]. Therefore, planar division within the wing disc epithelium might serve to aid planar tissue extension and reduce cell loss.

In the developing Zebrafish embryonic surface layer (pre-EVL), Xiong and colleagues proposed that OCD contribute to tissue layering. In this context, cell shape predicts spindle orientation with squamous and cuboidal cells dividing in perpendicular directions (**Figure 4A**) [5]. Cell divisions in cuboidal cells contributed to layering as one of the two daughter cells was displaced from the outer layer into a new layer below. Furthermore, OCD were proposed to buffer changes in the global geometry of the embryo, permitting successful epiboly [4].

In the Zebrafish EVL, cell divisions tend to orient along the axis of tension during epiboly (**Figure 4B**) [54]. In stretched MDCK monolayers, cell divisions reorient according to the axis of stretch (**Figure 3D**, **4C**) [42,55]. These orientations have been proposed to relieve tissue stress by increasing the number of cells along the stress axis [54,55].

Fine tuning AB spindle orientation is essential to ensure faithful epithelial structure. During mouse embryonic epithelial development, a switch from parallel to perpendicular spindle orientation is necessary for progenitor cells differentiation and skin stratification [63]. Impaired AB spindle orientation can also lead to daughter cells being aberrantly displaced out of the epithelial layer [41]. Therefore, OCD have been proposed to prevent tumor initiation and/or tumor spreading. Nakajima and colleagues showed that by perturbing spindle orientation and inhibiting apoptosis, tumor like masses can form in fly wing disc epithelia (**Figure 4G**) [41]. Alternatively, tumor spreading can be prevented by reintegration of cells after misoriented divisions. In the *Drosophila* FE, the embryonic ectoderm, the neuroepithelium and the wing disc, misplaced cells can reintegrate in the tissue (**Figure 4G**) [62,64]. Further exploration of cell reintegration upon division misorientation will help to understand how epithelial tissues can prevent out of plane born cells from becoming tumorigenic. Finally, how epithelial homeostasis and morphogenesis are affected by aberrant cell loss due to AB spindle misorientation remains to be further investigated.

Future directions

Considerable progress has been made in understanding the mechanisms and roles of mitotic spindle orientation during symmetric epithelial cell division. Accordingly, complementary *in vitro* and *in vivo* studies have put forward novel hypotheses. For example, cortical clustering of NuMA is proposed to be critical for spindle orientation in cultured cells, while in epithelial tissues TCJ localization of spindle guidance cues seems to be a predominant mechanism to orient the spindle [17,19]. This suggests that NuMA clustering at TCJ, rather than homogenous cortical localization, could be more efficient to generate pulling forces in epithelia. Better characterization of the upstream regulators of spindle orientation and of the mechanisms of force generation will extend our understanding of the many roles of OCD in multicellular contexts. Another exciting direction will be to explore how the interplay between tissue mechanics, vertex dynamics and apoptosis influences spindle orientation.

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Figure legends

Figure 1: Dynein can bind to dynactin and a cargo adapter such as HOOK3 to form a processive complex (A). Their interaction is facilitated by the coiled-coil in HOOK3. A second dynein can be part of the complex through interactions with the CAPZβ subunit of dynactin. Knock-down of CAPZβ leads to spindle misorientation. NuMA also possesses a long coiled-coil, which is required for spindle orientation. (B).

Figure 2: Planar spindle orientation can be regulated through different mechanisms. Hertwig's rule states that cells divide according to their long axis (A). Cells with Mud or LGN at TCJ divide according to the position of TCJ (B). In the *Drosophila* embryo cells adjacent to the actomyosin cable divide according to cell shape if their cell shape is highly anisotropic, but spindle orientation is directed towards the cable in moderately anisotropic cells (C).

Figure 3: In homeostasis, no apparent global direction of cell division can be observed (A). If a stretch is applied to the tissue, the cells become elongated and divide according to their long cell axis (B). Stretched *Xenopus* animal caps have enriched LGN at TCJ. The anisotropy of the cell as given by TCJ is the best predictor of local stress and cell division orientation (C). Cell divisions in MCDK layers do no follow cell shape, but divide along the direction of stretch by accumulation of LGN on junctions perpendicular to the axis of stretch (D).

Figure 4: Multiple cellular processes have been linked to OCD. In *Zebrafish*, OCD contribute to the formation of multiple tissue layers in the pre-EVL stage (A). The spreading motion of epiboly generates tissue tension which can be mitigated by OCD along the axis of stretch (B). Similarly, OCD can relieve external stress imposed on MCDK layers (C). In *Drosophila,* spindle misorientation causes phenotypes in the plane (D) and along the AB axis of epithelia (E). Spindle misorientation in *mud* mutants leads to smaller wings (F). Furthermore, the loss of AB spindle orientation results in multiple cellular phenotypes. Firstly, a fraction of cells is able to reintegrate into the plane of the tissue. Alternatively, they can be lost through delamination after which the cell can be removed through apoptosis (G). When apoptosis is blocked, these cells can survive and form tumor like masses (G).

Figure 1

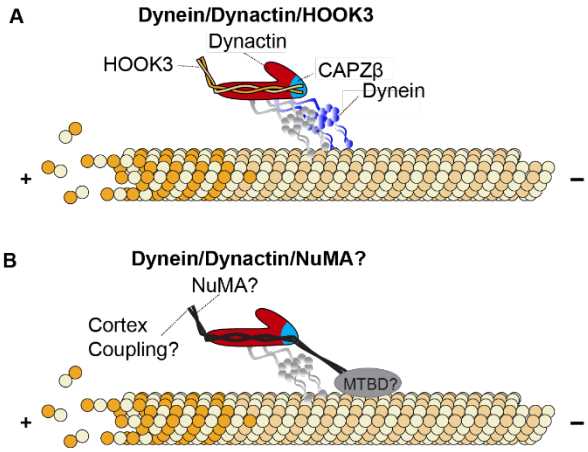


Figure 2

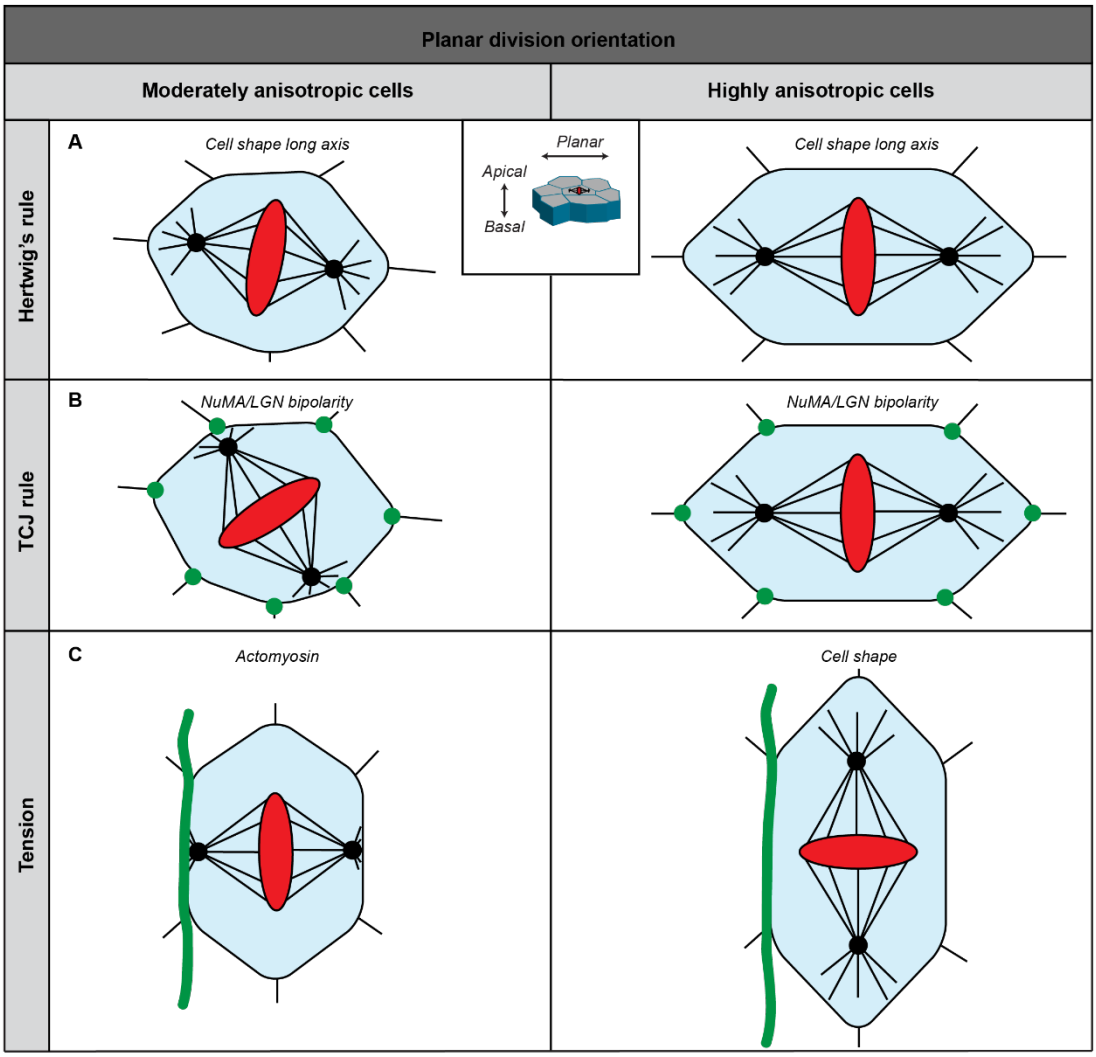


Figure 3

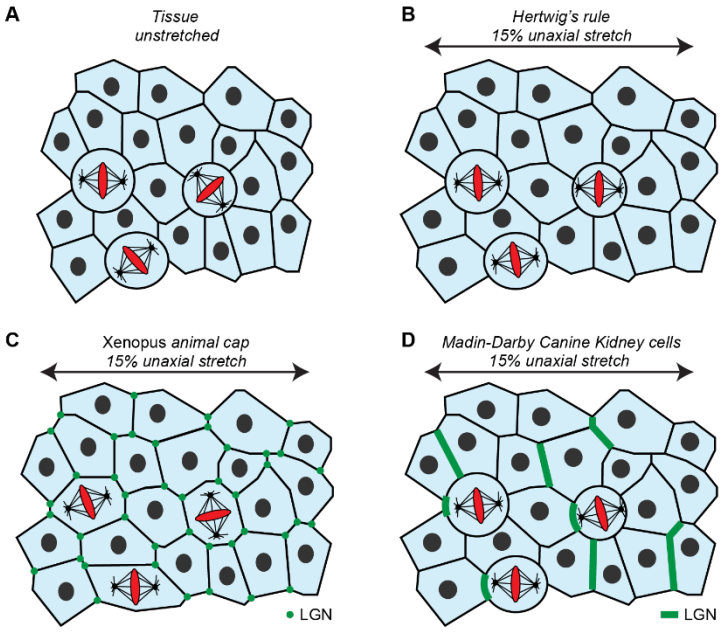
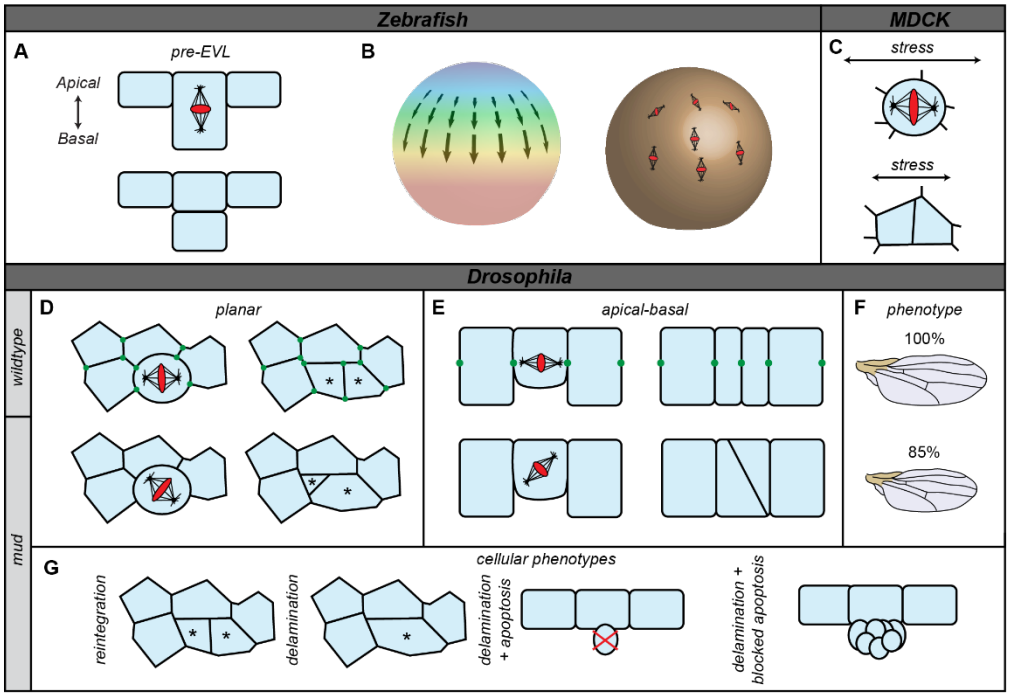


Figure 4



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