Highlights

- Tissue-level tension, cytoskeletal tension, and stiff substrates for 2D culture generally promote cell cycle progression and division, whereas confining environments or compression inhibit cell growth, leading to delayed or halted cell cycle progression and division.
- Cells dividing in confining environments generate extracellular forces to drive major morphological changes which are necessary for proper division completion, during mitotic rounding, division elongation, and postdivision spreading.
- Extracellular forces generated during cell division contribute to cell migration and tissue-scale processes important in development, including tissue growth, invagination, luminogenesis, and epithelial stratification.
- Cancer cells are able to undergo cell cycle progression and cell division within the confining tumor microenvironment.

Main text:

Dividing cells mechanically interact with their microenvironment

Cell cycle progression and cell division are fundamental biological processes that drive development, wound healing, and are critical for replenishing cells in mature tissues. Disruption of these processes can often lead to the development of different diseased states, such as cancer. In their native context, cells interact with other cells and the **extracellular matrix** (ECM, see Glossary), which provides both biochemical and mechanical cues to direct cell behavior, and often a three-dimensionally confining context that restricts changes in cell morphology. In this review, we will describe our emerging understanding of the mechanical interactions between cells and their surrounding microenvironment during cell cycle progression and division. The first section will focus on how mechanical forces and mechanical properties of matrices affect cell cycle control mechanisms, and, complementarily, how cells regulate their own mechanics throughout the cell cycle leading up to mitotic entry. At mitotic entry, cells orient their spindle axis in response to geometric and mechanical cues. For an in depth discussion of division orientation regulation, we defer the reader to a recent review on the topic [1]. In the second part, we describe how dividing cells generate **extracellular forces**, or forces applied to the surrounding microenvironment, to complete division successfully. Finally, it is emerging that cell division is not only important for generating additional cells, but that the division process itself contributes to morphological processes involved in development and maintenance of homeostasis. In the final section, we discuss ongoing work on the role that cell division forces play in contributing to larger tissue-scale processes.

Mechanotransduction and cell cycle progression

While the focus on cell cycle regulation has historically been on biochemical cues, it is becoming increasingly appreciated that mechanical cues play a key role. Cell cycle progression begins at division completion. Cells will typically either enter the G0 or quiescent phase, in which they do not divide, or the G1 phase of the cell cycle. During G1, cells grow in size, as they must typically double in size to maintain a stable cell size over many divisions. Once cells pass the restriction or G1/S checkpoint, they transition to S phase, during which DNA replication occurs. At this point cells are generally committed to completing the cycle, and transition to a second growth phase (G2) before undergoing division. Progression through the cell cycle is primarily controlled by cyclin-dependent kinases (Cdks) and their regulators [2]. Though expression of Cdks remains fairly constant, they are only functional when bound to a corresponding cyclin

protein. Cyclin transcription levels vary depending on cell cycle stage. Different sets of cyclin-Cdk complexes drive cells through cell cycle progression. Cyclin-Cdk complexes can be inhibited by Cdk-inhibitory proteins, such as p21 and p27. Additionally, different cyclin-Cdk complexes are regulated by a variety of other control mechanisms. For example, cyclin B-Cdk1 is regulated through inhibitory phosphorylation by Wee1, and dephosphorylation of an inhibitory site by Cdc25. Transcription of various cyclins, and the activity of Cdk inhibitors, are regulated by a variety of intra- and inter-cellular biochemical cues. In this section we review how mechanical forces, matrix mechanical properties, and cell mechanical properties affect these well-studied cell cycle control mechanisms.

Tissue-level tension promotes cell cycle progression

Within many different cell-types and contexts, stretch and tensile forces lead to the activation of a wide array of **mechanosensors** and subsequent activation of **mechanotransduction** pathways, which act to promote cell cycle progression (Fig. 1) [3–9]. Cell stretching is relevant in many *in vivo* contexts, such as lung wall stretching, dilation of arterial walls, gut tract activity, skin deformation, and growth. Within quiescent epithelial monolayers, uniaxial strain application leads to activation of yes-associated protein (YAP), a transcriptional regulator associated with cell growth, mechanotransduction, and cell cycle reentry into G1 [5]. This is followed by ß-catenin-mediated transcription and G1 to S phase transition. Cell cycle progression due to mechanical strain is dependent on E-cadherin [5,8]. E-cadherin is a transmembrane protein which transmits mechanical forces across cells, and is also mechanically connected to the actin cytoskeleton via adaptor proteins, primarily α-catenin. The dependence on E-cadherin suggests that E-cadherin, or an associated protein, is detecting changes in tension that activate downstream signaling and eventually YAP. Indeed, work suggests that α-catenin undergoes a conformational change under tension [10]. This indirectly leads to the recruitment and deactivation of LATS1/2 kinases, and thus YAP activation [9,11,12]. In other works, the stretch-activated ion channel Piezo1 has been associated with increased proliferation for cells paused at early G2 [3]. Applying uniaxial strain to epithelial monolayers leads to Piezo1 activation and an influx of intracellular calcium, leading to ERK1/2 phosphorylation, and eventually cyclin B transcription. Cycle progression upon strain application is observed with other cell types as well. For example, within sub-confluent adipose-derived stem cells, uniaxial strain promotes proliferation through activation of ß1 integrin [6].

Analogous to sensing externally applied stretch, cells are subject to endogenous tensile forces from neighboring cells and the ECM. Within low-density monolayers, cells are subject to stretch through E-cadherin connections with neighboring cells. Both stretch and low-density monolayers have been associated with E-cadherin-dependent YAP activation [9,11,12]. For cells in G2, low-density monolayers and externally applied stretch also leads to increased tension across E-cadherin, followed by degradation of Wee1, CDK1 activation, and mitotic entry (Donker et al., unpublished, bioRxiv). Within 3D contexts, cells are subject to tensile forces in additional ways. In developing *Drosophila*, faster-growing cells eventually exhibit reduced cytoskeletal tension when pushing out against slower-growing cells, leading to activation of the Hippo pathway, and consequently deactivation of Yorkie, the *Drosophila* analog of YAP [13]. Fluid pressure within lumens can also increase tension transmitted across cells, analogous to the stretching of a balloon filled with water. Epithelial cells forming an acini in 3D experience greater tensional stresses across the cell layer than 2D monolayers due to fluid pressure [14]. This increase in stress leads to increased tension transmitted across E-cadherin adhesions, and is sufficient to promote proliferation. Together, these studies show that cells are subject to tensile forces in many ways, leading the activation

of a variety of mechanosensors, including E-cadherin, piezo1, and integrin ß1, which in turn activate cell cycle control mechanisms to promote cycle progression at both G1 and G2.

Cell contractility correlates with cell cycle progression

Cell contractility in 2D culture studies are also linked to cell cycle progression through some of the same cell cycle control mechanisms as with tissue-level tension, consistent with the expectation that tissue-level tension results in increased intracellular tension and impacts cytoskeletal geometry [6,9]. Both cell and nuclear area growth rate correlate strongly with the duration of G1, with cell tension also increasing throughout the cell cycle, and serving as the strongest predictor of S-phase entry [15]. These studies suggest that key cell cycle events are dependent on observed cell geometry and contractility trends. Cells exert **traction forces** on their underlying substrate through actomyosin contractility and formation of **focal adhesions** (FA). Both actomyosin contractility and traction forces are necessary for centriole separation and centrosome duplication [16]. Other work has shown that nuclear flattening, which is dependent on myosin II-mediated tension, leads to the activation of the transcription factors TEAD and API, inducing G1 to S transition [17].

Cell contractility is also regulated downstream of cell-cycle control mechanisms, highlighting the complex interactions between the two processes. Measurements of various cell types show focal adhesion area increases during the G1 and S phases, and decrease during the G2 phase [18]. Regulation of adhesions was found to be dependent on activation then deactivation of CDK1-cyclin complexes. CDK1-cyclin complexes phosphorylate the formin FMNL2, a protein that initiates actin polymerization, leading to stress fiber formation. In agreement with this, measurements show that both as single cells and monolayers, epithelial cells increase their traction forces during G1, and either maintain or decrease these forces during G2 [15,19]. Overall, cell cycle progression is associated with distinct trends in cell geometry and contractility, which is often necessary for cells to progress through the cell cycle.

Stiffer substrates promote cell cycle progression

Adherent cells spread to a greater extent and generate greater traction forces with increased substrate stiffness in 2D culture, due to enhanced actomyosin contractility and formation of focal adhesions. Consequently, stiffer matrices are typically found to promote cell proliferation, often in an actomyosin contractility-dependent manner [8,20–22]. Matrix stiffness varies widely between soft tissues, from 100s of Pascals (Pa) to 100s of kPa, and changes during development, wound healing, and disease progression, making the impact of matrix stiffness on cell division a significant topic [23]. Increased stiffness leads to increased focal adhesion kinase (FAK) association with activated integrins, followed by Rac activation, and then cyclin D1 transcription, to drive S-phase entry [22]. This pathway is conserved across mammary epithelial cells, osteoblasts, mouse embryonic fibroblasts, and muscle cells. Increased stiffness has also been shown to drive cell cycle progression through ERK1/2 phosphorylation in endothelial cells [20], increased epidermal growth factor signaling in keratinocytes [21], and YAP activation in mammary epithelial cells [24]. Overall, stiffer substrates are associated with increased cell spreading, mature focal adhesions, and cytoskeletal tension, driving cell cycle progression.

Tissue-level compression inhibits cell cycle progression

Compressive forces generally inhibit cell cycle progression. Biaxial confinement introduced to sarcoma cells through microfluidic channels of varying dimensions resulted in reduced cell and nuclear areas, and inhibition of cell cycle progression [25]. Similar results were found with other cell types and compression modalities, such as hydrostatic compression, which refers to uniform 3-dimensional

compression. Cancer cell spheroids exposed to hydrostatic compression exhibited reduced volumes and increased expression of the p27 cell cycle inhibitor, and a subsequent reduction in proliferation [26]. The impact of compression on cell cycle progression could be in part due to deactivation of tension-dependent mechanosensors, discussed previously, or other means. For example, biaxial planar compression of both fibroblasts and epithelial cells leads to cell cycle arrest in S phase specifically due to disassembly of the actin cytoskeleton, which induces activation of checkpoint kinase 2, and the tumor suppressor p53 [27].

Confinement from the ECM due to cell growth has also been linked to cell cycle control mechanisms. In a recent 3D culture study, cell spheroids were confined within **viscoelastic** nanoporous hydrogels, which exhibit some characteristics of **elastic** solids, and some of **viscous** liquids [28,29]. In more elastic hydrogels, cell growth is blocked by the confining microenvironment and cell cycle progression does not proceed. When the hydrogel is more viscoelastic and exhibits sufficient **stress relaxation**, cells are able to deform the matrix and undergo G1 phase growth. Growth leads to activation of the mechanosensitive calcium ion channel TRPV4, activating the PI3K pathway, and consequently p27 inhibition, allowing S-phase entry [29]. Taken together, various modalities of compression or confinement have been found to inhibit cell cycle progression, due to actin cytoskeleton disassembly or lack of mechanosensor activation, leading to the activation of various cell cycle inhibitors, such as p27 and p53.

Generation of extracellular forces during cell division

While tension and compression within tissues regulates cycle progression, the process of cell division itself generates extracellular forces on tissues and the ECM. Starting at mitotic entry, cells undergo dramatic morphological changes that are required for proper segregation of genetic and cytoplasmic materials. Cells prepare for division by transitioning from their native morphology (i.e. flat, cuboidal, columnar, or 3D spread) to a rounded shape in a process known as mitotic rounding. Then, after passing the spindle assembly checkpoint, the sister chromosomes begin separating, which is accompanied by elongation of the dividing cell along the division axis. Elongation continues as the dividing cell ingresses at the center and undergoes cytokinesis. The newly divided daughter cells then transition back to their native morphology. In many contexts that are fully or partially confining, such as a dense 3D matrix or crowded epithelial monolayer, cells must generate extracellular mechanical force to enable these striking morphological changes to occur. Here we review what is known about extracellular force generation during cell division.

Mitotic rounding

Mitotic rounding begins at mitotic entry when cells de-adhere from their underlying matrix and transition to a spherical geometry, due to a combination of actomyosin contractility, manipulation of osmotic gradients, and increased cell stiffness [30–33]. **Atomic force microscopy (AFM)** measurements show these morphological changes generate extracellular forces on the order of 100 nanoNewtons (nN) [30,31]. In epithelia, mitotic rounding is linked to both outward and inward extracellular forces (Fig. 2AB) [34–36]. In squamous or relatively flat monolayers, adjacent cells are pulled in as the dividing cell rounds up and reduces its cross-sectional area. (Fig. 2A) [34,35]. Additionally, this is accompanied with a decrease in monolayer tension adjacent to the dividing cell, which aids mitotic rounding [15]. In order to maintain epithelial barrier integrity, tension associated with rounding has been shown to reinforce cell-cell adhesions through vinculin recruitment at E-cadherin adhesions [34]. Force generation patterns and strengthening of adhesions seems to be context dependent, however. In cuboidal epithelia, mitotic rounding is associated with outward force generation [36] (Fig. 2B). In *Drosophila* pseudostratified

epithelia, weakening of cell-cell junctions through downregulation of E-cadherin was found to be necessary for dividing cells to undergo rounding [37]. Measurements of force generation due to cell shortening along the apical-basal axis have not been made, though these forces have been implicated in lumen and villi formation (see Tissue-scale processes section). The specific underlying mechanisms of force generation during rounding have been studied extensively in the past, especially in the context of single cells, so we defer the reader to a recent review for a more detailed discussion of this topic [38].

Division elongation

The dividing cell continues generating extracellular forces to undergo morphological changes during division elongation at anaphase onset, during which genetic and cytoplasmic materials are separated into the two daughter cells. Division elongation is strictly required for division to occur successfully [39]. In environments that are highly mechanically confining so that dividing cells are unable to deform their environment and elongate, division fails. Cancer cells dividing within 3D confining, inert, nanoporous alginate hydrogels initially generate **anisotropic** forces to drive elongation via interpolar spindle elongation (Fig. 2C) [39]. Forces originate from motor proteins such as kinesin-5 pushing apart cross-linked interpolar microtubules, and are concentrated along the division axis. In addition, due to conservation of cell volume during elongation, inward forces generated by the contractile actomyosin cytokinetic ring during cytokinesis, also generate outward forces along the division axis. This is analogous to a water balloon elongating as it is squeezed at the center. For cells cultured in collagen matrices, dividing cells continue to generate forces to drive elongation using both of these mechanisms, rather than generating space for elongation via matrix degradation or remodeling [40].

Evidence for division elongation forces has been observed in epithelia as well. *In vitro* studies suggest that cell division forces are generated solely by the dividing cell, with the movement of neighboring cells not being involved (Fig. 2D) [35]. Division elongation during epithelia was found to be more dependent on cytokinetic ring contraction relative to interpolar spindle elongation. However, in epithelia subject to stretch, dividing cells tend to divide along the direction of stretch [41]. Stress measurements show that in this case cell division leads to stress dissipation along the division axis, which could suggest that cells are passively elongating, due to internal tension within the monolayer, rather than actively pushing outward. It is not entirely clear how forces generated during division elongation are transmitted to neighboring cells. Measurements of E-cadherin tension in *Xenopus* embryos using FRET-based sensors show that global E-cadherin tension is unchanged during cytokinesis, which could indicate other mechanisms of force transmission [42]. However, it should be noted that measurements were taken from only the ingressing portion of the membrane, and division in *Xenopus* is morphologically different from other epithelia since dividing cells do not undergo rounding. Overall, dividing cells generate anisotropic outward extracellular forces to drive elongation, yet how these forces are transmitted to neighboring cells remains unclear.

Postdivision spreading

After division completion, newly formed daughter cells transition back from a rounded shape to their native morphology, which could involve substantial force generation. Forces driving daughter cell reintegration are important for maintaining epithelial integrity and for cells to carry out their normal functions. In epithelial monolayers, daughter cell spreading further exerts outward forces on the environment to create room for re-integration into the monolayer (Fig. 2E) [35]. Measurements reveal force generation during spreading is on the same order of magnitude to that of mitotic rounding.

Postdivision spreading is thought to be similar in nature to normal cell spreading, which is powered by the formation of cell-matrix adhesions and actomyosin contractility [43,44]. Within epithelial monolayers, neighboring cells extend protrusions underneath the dividing cell during mitotic rounding, and retract them during daughter cell re-spreading [15]. This suggests a potential role in neighboring cells assisting rounding or re-spreading. In some contexts, forces generated during cell re-spreading are sufficient to aid in cell abscission when the cytokinetic ring is disrupted [44]. Taken together, these studies have identified the generation of substantial extracellular forces during mitotic rounding, division elongation, and postdivision spreading.

Cell division guides tissue-scale processes

Cell cycle progression and cell division directly generate forces which change the stress landscape within tissues, and thus potentially contribute to or drive tissue-scale processes. Recent work has shown that cell division is associated with cell movement, tissue elongation, invagination, lumen expansion, and stratification of epithelia. However, in some cases it is still unclear whether force-generating mechanisms from cell division, coupled with regulation of cell division orientation and position, play an active role. Here we discuss ongoing work on how mechanical forces associated with cell division contribute to tissue-scale processes, and which topics require further research.

Cell movement

Cell division and migration are intertwined within a variety of model organisms, connected largely through mechanical forces. Within chick embryos, a combination of pulling forces exerted by the cytokinetic ring of dividing cells on neighboring cells, and low levels of cortical actomyosin, leads to neighboring cells being pulled in between the resulting two daughter cells and forming a new cell-cell contact (Fig. 3A) [45]. This cell division-induced intercalation event is necessary for cells to be rearranged correctly prior to gastrulation, and is observed in other contexts, including intestinal organoids and early mouse embryos [46]. Though cell division drives cell intercalation, it is not completely clear how cell division orientation is regulated to achieve correct cell rearrangements prior to gastrulation. Cell division has also been associated with multi-cellular scale movements in other contexts, consisting of vortices within monolayers, or cell migration within intestinal villi (Fig. 3A) [47–50]. In these contexts, on average, neighboring cells along the division axis tend to migrate away from the division site, while neighboring cells along the perpendicular axis tend to migrate toward the division site. Though these migration patterns are dependent on cell division, it is not entirely clear which underlying mechanisms link these two processes. Collective cell migration is replicated in models which treat cell division as an active source of pressure or energy generated in the monolayer along a specific axis [47,48]. These works suggest that migration patterns arise due to division elongation, which generates anisotropic outward forces along the division axis [35,39,40]. Other modeling work shows that the location alone, rather than the orientation of the dividing cell, is important in inducing collective cell migration [49], suggesting an alternative mechanism underlying cell division-mediated migration. Work done in developing embryo models suggest that cell division increases tissue fluidization due to cell-cell contact disassembly during mitotic rounding, which could promote collective cell migration [51,52]. In zebrafish embryos specifically, cell division-induced migration was dependent on N- and E-cadherin-based adhesions, due to their role in modulating epithelial tension [53]. These works suggest that cell migration is dependent on cell division due to regulation of cell-cell adhesions, which in turn modify properties of the epithelium, rather than forces originating from division directly. Finally, work done in single-cell contexts shows that newly divided daughter cells tend to migrate away from each other, along their axis of division [44,54]. This suggests

that it is possible that migration patterns are induced because the newly divided cells initiate migration along their division axis, independent of cell division forces. Overall, though it is clear migration patterns are often associated with division events, the connection depends upon the context, and additional study on the molecular mechanisms connecting cell division to tissue-level migration patterns is needed.

Oriented division can drive tissue elongation

With cell division resulting in a re-distribution of cell mass due to a parent cell transitioning into two daughter cells along the division axis, the controlled orientation of multiple division events can potentially drive or aid tissue elongation during embryogenesis. Within the germband of developing *Drosophila*, division events are oriented along the anterior-posterior axis due to tissue-level tension [55]. This leads to an increase in surface area along the anterior-posterior axis, aiding tissue elongation. In this case, it is possible that tissue-level tension both orients division events and drives division elongation. In other contexts, such as the developing *Drosophila* follicular epithelium, dividing cells are oriented by tissue-level tension specifically at the apical plane [56]. In this case, outward forces generated during mitotic rounding or division elongation along the central or basal plane could be necessary to complete cell division, and consequently tissue elongation [35]. Finally, other evidence suggests that though oriented divisions are observed during tissue elongation, they are not always necessary [57]. In the *Drosophila* wing, oriented divisions occur in the direction of tissue elongation and tension. After inhibiting Mud, a protein that mediates the link between the actin cortex and the mitotic spindle, cells continued to divide but were not oriented in the direction of tissue elongation. Increased cell movement compensated for the lack of oriented divisions, resulting in normal wing development. Similarly, within the developing *Drosophila* follicular epithelium, oriented divisions were found to not be necessary for tissue elongation, but were important for the development of hexagonal cell shapes and cell packing [56]. Oriented divisions in the direction of tissue expansion has been observed in other model organisms, including airway tube morphogenesis in mice [58]. More work is needed to determine in which contexts coordinated division events are required for outward force generation or are simply relaxing tissue-level tension, addressing whether oriented divisions drive tissue elongation, or are simply a consequence of tissue-level stress.

Invagination / villi formation

Also linked to cell division are invagination and villi formation, during which a 2D epithelial layer folds inward or outward to form a more complicated 3D structure. A combination of apical constriction as well as outward forces generated by dividing cells during rounding are implicated [59]. In developing *Drosophila* trachea, dividing cells aid invagination when they are not on the side of apical constricting cells [60]. Outward forces generated during mitotic rounding cause the epithelial layer to buckle and push outward on the basal side (Fig. 3B). Similarly, during ectodermal divisions of developing *Drosophila*, divisions are localized adjacent to a group of apically constricting cells [61]. Thus the outward pushing forces generated during division help the constricting cells pull the monolayer inward and form an invagination. Complementary to these studies, research has shown that epithelial invaginations precede and aid in the formation of villi. During embryogenesis within mice, dividing cells are associated with apical invaginations within intestines [62]. During mitotic rounding, cells shorten in height along the apical-basal axis, leading to forces that pull the epithelium inward (Fig. 3C). Key pending questions remain on whether division orientation is important for these processes, and whether division forces from elongation or postdivision spreading contribute to invagination as well.

Lumen expansion

Cell division has been linked to lumen expansion, due to similar force-generating mechanisms implicated in villi formation. Lumens are hollow openings within multicellular structures. Within columnar epithelia, dividing cells often exerting pulling forces on their environment as they shorten during rounding [63]. Within developing zebrafish ear epithelia, these pulling forces are exerted on the apical side of the cell which faces the lumen, thus helping to expand the space in between cells (Fig. 3D). Thus, forces associated with cell division are relevant to lumen expansion in zebrafish ear epithelia, but it is not clear if this is broadly applicable.

Stratification of epithelia

Development and maintenance of a stratified epithelium, such as in skin, involves the movement or division of cells from a lower to upper epithelial layer. Recent work shows that regulation of cell division orientation plays an important role in the development of new epithelial layers. In-plane division events leads to cells being replenished within a lower basal epithelial layer, whereas perpendicular division results in asymmetric cell division, with one daughter cell differentiating and moving into an upper layer [64]. Within mutant mouse embryos, cells dividing in low-density epithelia divide within the plane, due to being stretched in the planar direction (Fig. 3E) [64]. As proliferation continues, crowding within the epithelium leads to an increased cell aspect ratio. Taller and thinner cells orient to divide perpendicular, with one daughter cell moving into the epithelium layer above. However, it is unclear whether forces generated during perpendicular cell division play an active role in driving the second daughter cell to move into an upper cell layer. Within a crowded epithelium, cell division has also been associated with delamination of adjacent cells into upper epithelial layers, due to weakening of cell-cell junctions during mitotic rounding (Fig. 3F) [65]. Given that delamination and differentiation are also associated with tissue stress anisotropy and compressed monolayers [65], it would be interesting to further explore whether active anisotropic compressive forces generated during division elongation contribute to adjacent cell delamination [35]. Overall, these studies highlight the role of cell division in epithelial stratification.

Concluding remarks

Cells biochemically and mechanically interact with their microenvironment. Mechanical cues from the microenvironment, including tension, compression, ECM stiffness and viscoelasticity regulate various aspects of cell behavior, including cell cycle progression and cell division. In turn, as individual cells progress through the cell cycle and divide, they generate and apply forces on neighboring cells and the ECM. Cell division has also been linked to larger tissue-scale processes, such as migration, tissue elongation, invagination, luminogenesis, and the formation of stratified epithelia.

While this emerging body of work has compellingly linked cell division mechanics to tissue scale processes, more work needs to be done on determining whether and how mechanical forces link cell division to some of these processes, and which aspects of cell division force generation are responsible for contributing to these tissue-scale processes (see Outstanding Questions). Further, the connection between mechanical regulation of cell division and cancer remains unclear (Box 1). Multiple studies show that cancer cells are able to generate greater levels of force during division, and are more resistant to a confining environment, which is prevalent in tumors [27,66–68]. Future work may address the critical questions of whether and how mechanical cues from the microenvironment might lead to tumor progression. In summary, the mechanical aspects of cell cycle progression and cell division are critical for cell and tissue biology, and this link represents an area that is ripe for future study.

Outstanding questions

- How does cell division lead to longer-length scale collective cell migration, and what role does this play in development and disease?
- What role do forces generated during division elongation and post-division spreading play in tissue scale processes during development or maintenance of homeostasis?
- Do cancer cells generate greater forces to grow during the cell cycle, undergo division elongation, or spread after division, in more confining tumor microenvironments?
- Do changes in the mechanical properties of the ECM lead to DNA damage or cell division errors?

Box 1: Mechanical regulation of cell division in cancer

Cancer is characterized by abnormal and uncontrolled cell growth and division. Recent studies suggest some interesting aspects of mechanical regulation of cancer cell cycle progression and force generation during cancer cell division. Cancer cells are often resistant to some cell cycle control mechanisms due to inactivity of tumor suppressor genes or overactivity of oncogenes, but appear to retain some mechanosensitive cell cycle control mechanisms. The tumor microenvironment is stiffer than normal tissue due to increased deposition of ECM proteins and ECM remodeling (Fig. I) [69]. In addition to increased stiffness creating a more confining environment, most solid tumors are under increased solid stress (i.e. hydrostatic compression) [70]. As with normal cell types, cancer cells do exhibit cell cycle inhibition in confining microenvironments (see Main Text) [25,26,29].

However, there are some exceptions. For example, biaxial planar compression results in S-phase arrest of both fibroblasts and epithelial cells due to the tumor suppressor p53, but the same arrest is not observed for multiple cancer cell lines [27]. More work is needed on how compression regulates cell cycle progression in cancer cells in different contexts.

Furthermore, the increasing confinement encountered by growing tumors indicates that cancer cells must generate greater forces to drive morphological changes during cell division. Indeed, though cancer cells are softer in interphase, transformed cells, as well as cells that have undergone epithelial to mesenchymal transition, are stiffer during mitosis [66,67]. This increased stiffness reduces cell deformation upon compression, making dividing cells more resistant to mechanical confinement during mitotic rounding. It is unknown how division elongation or postdivision spreading forces might vary in normal versus cancer cells. One study showed that metastatic cell lines exhibit greater levels of kinesin-5 relative to normal cell lines [68]. These cells exhibit a longer spindle and faster spindle elongation rate, which in principle could generate greater forces during division elongation. Further biophysical measurements are needed to understand the forces of tumor cell division.

Cancer cells are also marked by high levels of genomic damage and genomic instability. Confined cell migration can induce errors in DNA replication [71]. Additionally, the tissue microenvironment affects the frequency of chromosome segregation errors [72], and increasing levels of confinement lead to abnormal or failed cell division [31,73]. Contrastingly, cells expressing oncogenic Ras are more resistant to cell division errors under confinement [66]. Future work may address how mechanical cues lead to cancer progression, through errors in DNA replication, DNA damage, or cell division errors.

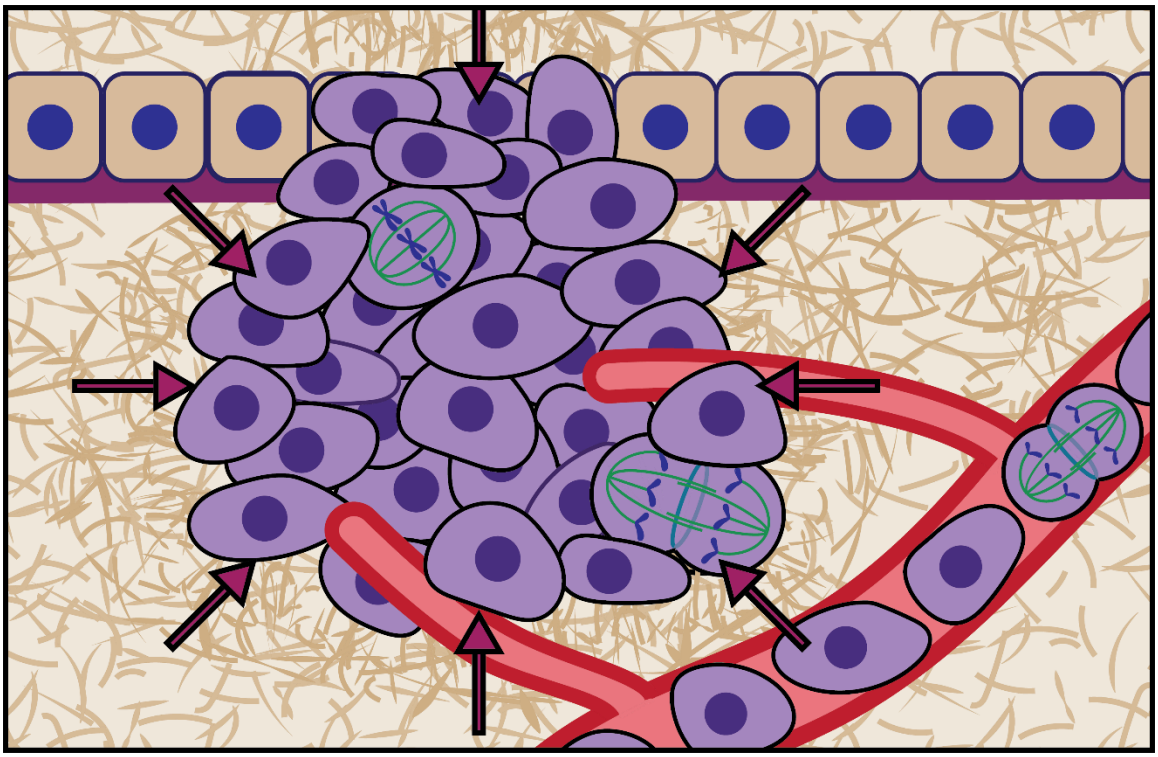

Figure I. Cancer cells grow and divide in an increasingly confining environment due to increased solid stress, a stiffer ECM, and confinement due to the vasculature. Adapted from [39].

Glossary

Anisotropic: a physical property that varies depending on orientation or direction.

Atomic force microscopy: an instrument which can be used to apply and measure forces on a micro/nano-scale sample using a flexible cantilever.

Elastic: a property of a material allowing it to reversibly resist deformations upon application of a force, independent of its applied rate.

Extracellular forces: forces generated by a cell that are propagated to neighboring cells or the extracellular matrix.

Extracellular matrix (ECM): a 3D matrix, often consisting of collagens, proteoglycans, and glycoproteins, spanning gaps between cells and tissues, which provides structural support and biochemical cues to cells.

Focal adhesions: protein complexes which mechanically link a cell's internal actomyosin cytoskeleton to the extracellular matrix.

Mechanosensor: a biomolecule that can sense a change in force and relay this change to a downstream signaling pathway.

Mechanotransduction: the process in which cells to convert a mechanical signal into a biomechanical response.

Stress relaxation: a material property in which mechanical stresses decrease over time after an imposed deformation. Viscoelastic materials exhibit stress relaxation.

Traction force: forces applied by the cell onto the ECM. On 2D substrates, traction forces are often exerted within the substrate plane, whereas in 3D forces can be more varied.

Viscoelasticity: property of a material exhibiting both elastic and viscous behavior, depending on the rate and magnitude of applied force.

Viscous: a property of a material in which the resistance to deformation is solely dependent on the rate of force application.

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Declaration of Interests

The authors declare no competing interests.

Figures

Figure 1

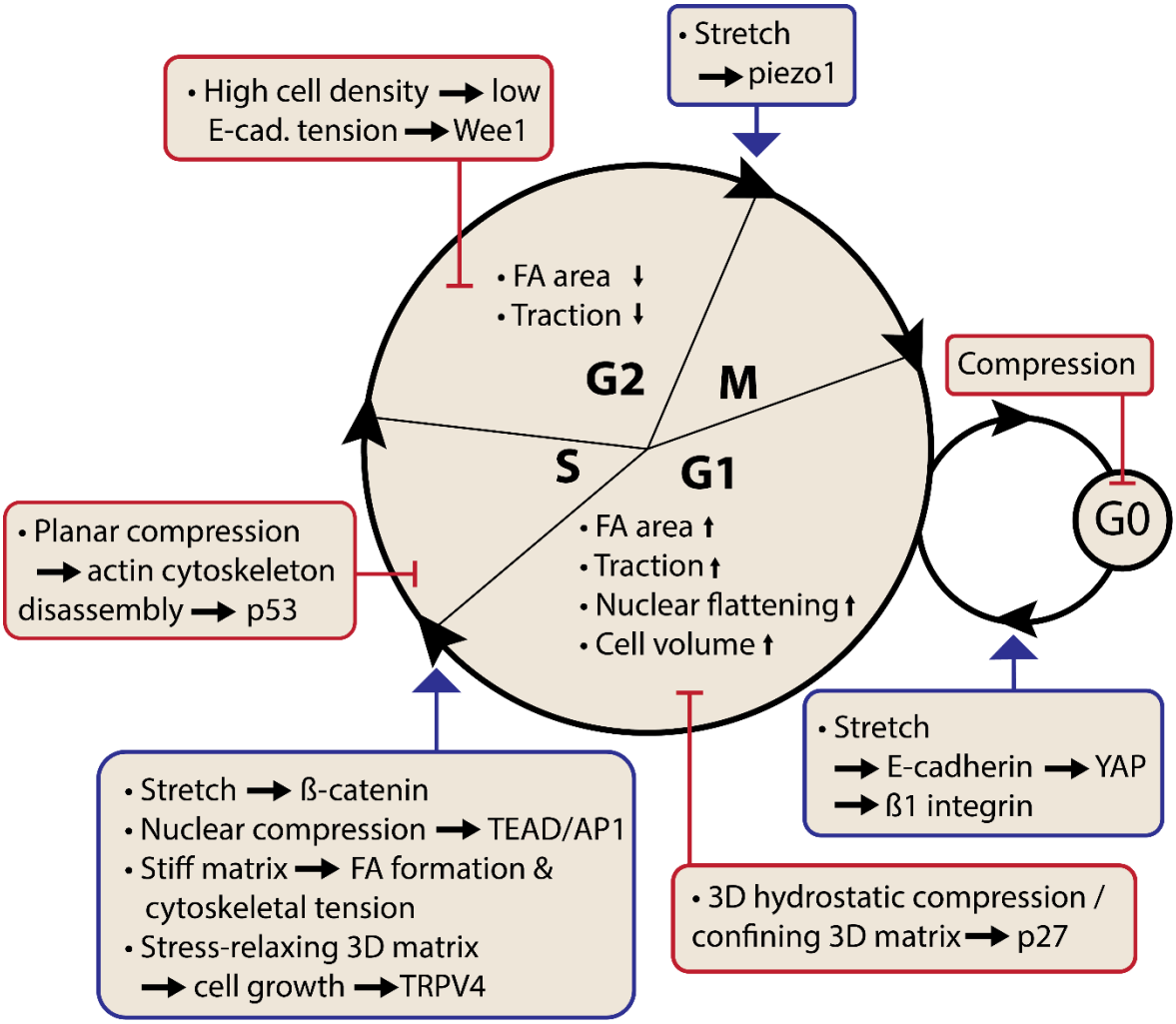


Figure 1. Mechanical Regulation of Cell cycle Progression. Summary of recent work on how tension, compression, and extracellular matrix mechanics regulate cell cycle progression at various stages. Changes in cell properties are denoted within the circle, and applied mechanical perturbations, with their corresponding effect on cell cycle progression (blue arrows) or inhibition (red lines), are shown outside. Tissue tension can promote G0-G1 transition through β1 integrin signaling and YAP activation, G1-S transition through β-catenin, and G2-M transition through activation of piezo1. Similarly, intracellular tension (contractility) and spreading promotes G1-S transition. Conversely, compression inhibits cell cycle progression, in part due to the lack of activation of tension-dependent proliferation pathways. Changes in matrix mechanical properties including increased stiffness and increased stress relaxation, in 3D culture, can promote cell cycle progression through promotion of spreading and contractility and activation of a TRPV4-PI3K-p27 signaling axis, respectively. Note: relations depicted are dependent on biological context.

Figure 2

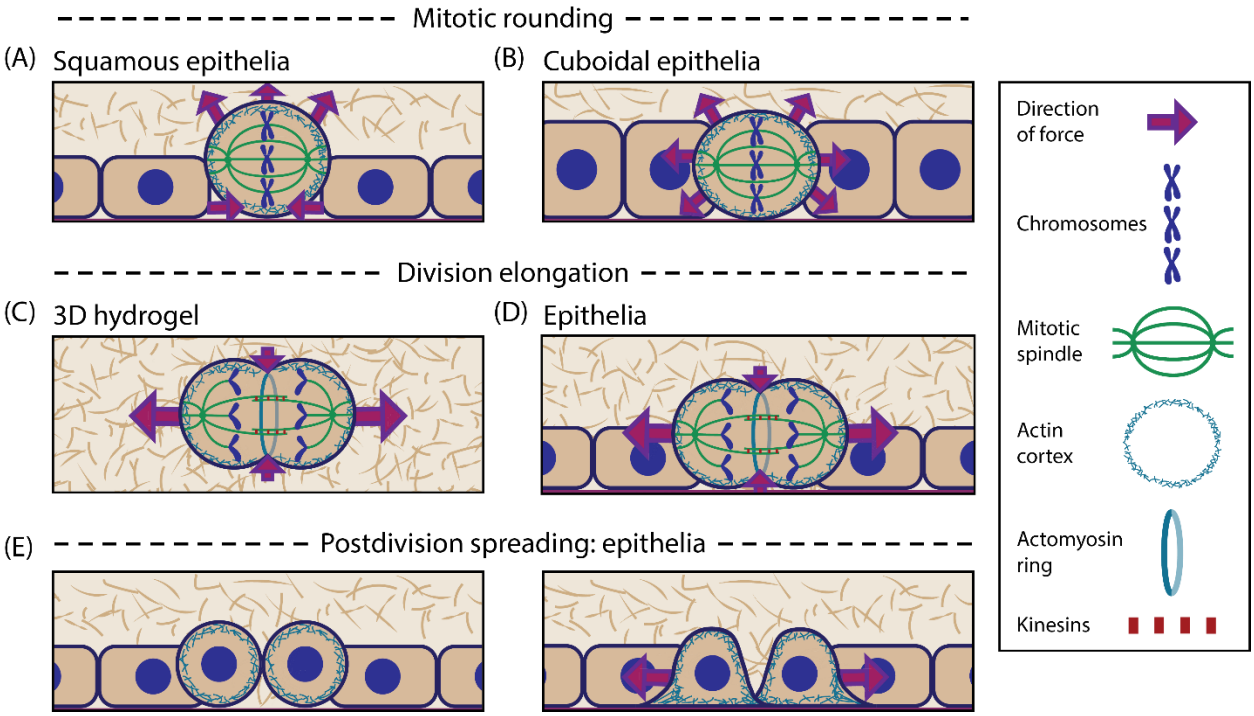


Figure 2. Extracellular Force Generation During Division. (A) During mitotic rounding, cells in flatter epithelia reduce their cross-sectional area within the monolayer plane, generating inward forces. (B) In more cuboidal epithelia, rounding leads to outward forces. (C, D) During elongation, cells generate outward forces through a combination of interpolar spindle elongation, and cytokinetic ring contraction due to conservation of cell volume; observed in 3D hydrogels (C) and within epithelia (D). (E) As newly divided daughter cells return to their native morphology, they may further exert forces. In epithelia, postdivision spreading leads to outward force generation.

Figure 3

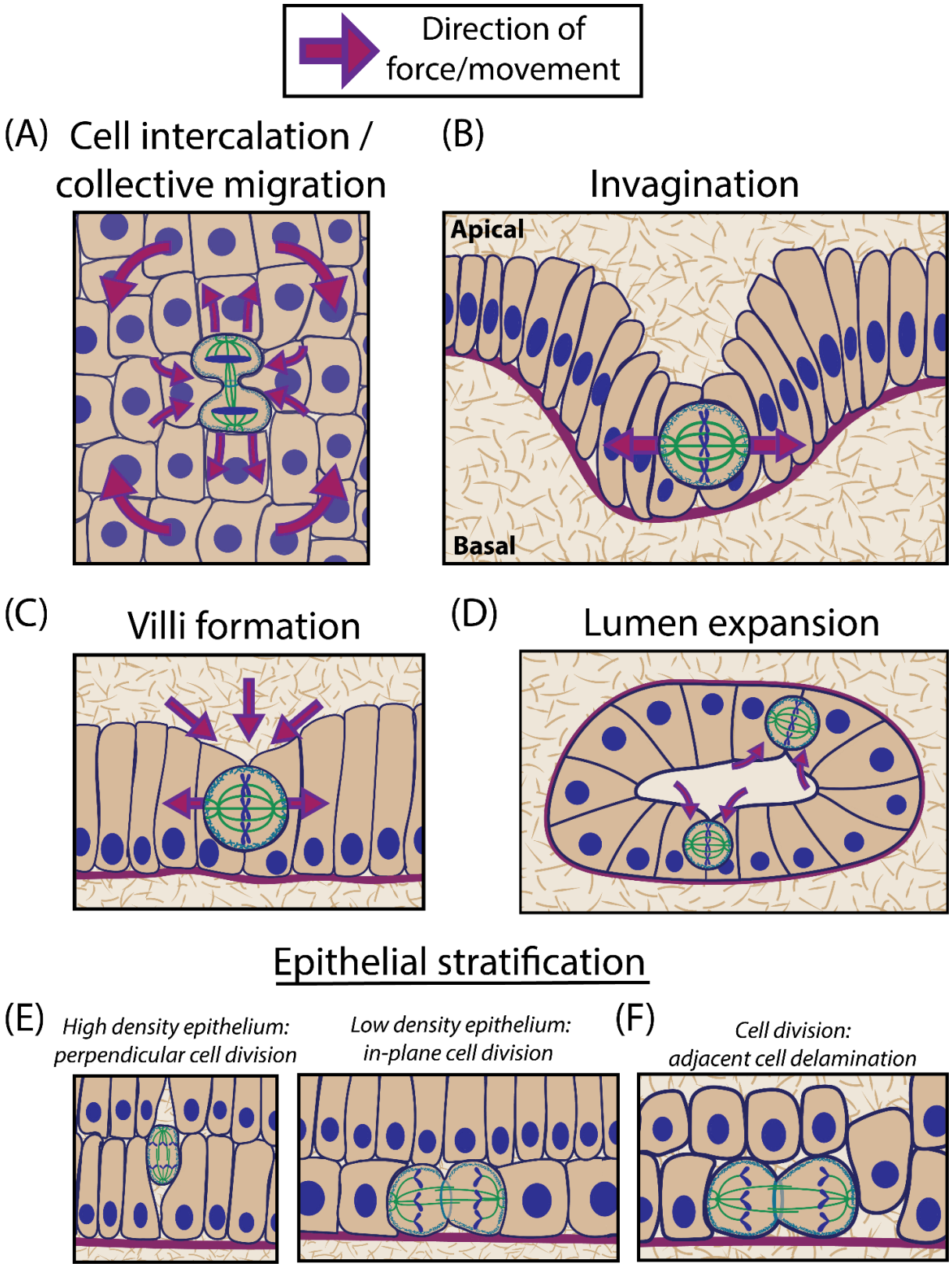

Direction of force/movement
(A) Cell intercalation / collective migration
(B) Invagination
Apical
Basal
(C) Villi formation
(D) Lumen expansion
Epithelial stratification
(E)
High density epithelium: perpendicular cell division
Low density epithelium: in-plane cell division
(F)
Cell division: adjacent cell delamination

Figure 3. Mechanical Forces Link Cell Division to Tissue-Scale Processes. (A) Cell division induces the intercalation of neighboring cells in between the newly formed daughter cells, as the daughter cells often migrate away from each other along the division axis. Larger-scale cell movements develop, pushing away from the division point along the division axis, and towards the division point along the perpendicular axes. (B) Outward forces generated during mitotic rounding can aid in epithelial buckling, leading to invagination [60]. (C) During mitotic rounding, pulling forces exerted along the apical-basal axis can lead to apical invagination, which precedes and aids in the formation of villi adjacent to these points [62]. (D) Pulling forces along the apical-basal axis during rounding can also create more room in developing lumens [63]. (E) In some contexts, dividing cells modify their orientation in response to shape cues determined by epithelial density, resulting in perpendicular divisions in a high density epithelium, and the formation/maintenance of stratified epithelia, or planar divisions in a low density epithelium, replenishing cells at the basal layer. (F) Division can also change the stress landscape in neighboring cells, inducing delamination [65]. Note: processes depicted are dependent on context and schematics are adapted from cited sources.

Reviewer #3: In the revised manuscript of Gupta and Chaudhuri the organization of the text is improved, which has helped with the clarity of the review. The authors have successfully addressed most of my original concerns, but I still have some remaining comments:

We thank the reviewer for their review of the manuscript and are pleased that we have addressed most of the original concerns.

- Although the revised section on 'mechanotransduction and cell cycle progression' is now clearer, at some places it still lacks a logical organization which makes it difficult to follow. In the section 'Cell spreading and contractility correlate with cell-cycle progression' it is both discussed how spreading/contractility are influenced by the cell cycle, as well as how spreading/contractility influence cell cycle progression. This could be better structured as the authors go back and forth between these topics (i.e. in this section first the role of spreading/contractility upstream of cell cycle progression is mentioned, but then the authors first discuss a role of spreading/contractility downstream of the cell cycle and only come back to an upstream role of spreading/contractility later).

We think this is a great point. We have re-ordered text within this paragraph to first discuss spreading/contractility trends upstream of cell-cycle progression, as this continues off the previous section, followed contractility trends downstream of cell-cycle control mechanisms.

I had a similar issue with the "Tissue-level compression inhibits cell-cycle progression" section, as part of this section is not necessarily on compression but instead on lack of tension (i.e. the part on Wee1 and YAP). This could be better connected to the earlier part on tension-dependent cell cycle regulation.

We have moved these two references to the tissue-level tension section.

- In one of the final sections 'Cell division guides tissue-scale processes' the authors indicate they will discuss how forces generated during cell division play a role in the 'cell movement, tissue elongation, stratification, etc. Despite the added text, for this reviewer the message of this section is still difficult to understand, and the different paragraphs read as very isolated parts. This is mainly because for some of the discussed processes it is unclear whether truly forces generated during cell division play a role. For instance, related to tissue elongation and stratification dependent on division orientation, it seems more the positioning of daughter cells instead of forces during division that are important. If the authors wish to maintain a discussion of processes in which forces generated during cell division are less clear, they may extend the introduction of this section a bit to introduce this more broadly.

For the tissue elongation and stratification sub-sections, we agree it is not definitively known whether forces generated during cell division play a role. We have specifically written what is known and which areas still require more research in the future. We have added additional text at the start of the tissue-scale processes section to clarify (1) we will discuss cases in which cell division forces *potentially* play a role in guiding these processes based on published work, (2) a lot is still unknown about the underlying mechanisms relating cell division to tissue-scale processes, and (3) cell division orientation and position (in addition to cell division forces) also play a role in guiding these processes.

- Throughout the manuscript, there are several key statements that lack a reference. In addition, occasionally several papers are cited after a sentence that makes a broader point, but when discussing

specific details it is not clear which paper(s) the authors are referring to. Without being exhaustive, examples are "Indeed, work suggests that α-catenin undergoes a conformational change under tension" and "in order to maintain epithelial barrier integrity, tension associated with rounding has shown to…". The authors should make sure citations are included when needed.

We thank the reviewer for pointing this out and have revised the text to cite all specific details.

- P6: I appreciate the authors clarifying the part on weakening of cell-cell junctions during mitotic rounding in Drosophila pseudostratified epithelia. However, the new sentence "Force generation patterns and strengthening of adhesions seems to vary in in other epithelial geometries" is confusing, as it suggests epithelial geometry determines whether junctions are weakened in mitosis (but this may be a specific event in Drosophila pseudostratified epithelia and not per se related to geometry).

We think this is an excellent point. We have revised “in other epithelial geometries” to “in other contexts”.

- I appreciate the authors including their definition of "extracellular forces" in the glossary. However, in the field this term is used to indicate forces that originate from outside a cell and can be exerted on a cell, and not forces generated by a cell on its environment. It would be good to introduce this term more properly when first using it (so it is clear that this is distinct from how this term is commonly used).

We thank the reviewer for bringing up this point. We have removed “extracellular forces” from the abstract, and first introduce the term in the introduction. In the introduction, we have added additional clarification in-text to make it clear extracellular forces are generated by cells and applied to the surrounding microenvironment.

- P3: "inhibitory dephosphorylation by Cdc25" is incorrect (it removes an inhibitory phosphorylation

We have revised the sentence.

- P3: "Additionally, cyclin-CDK complexes are regulated by inhibitory phosphorylation by Wee1, and inhibitory dephosphorylation by Cdc25, at different binding domains" - this should be rephrased, as only Cdk1 is regulated by Wee1/Cdk1 and not all cyclin-Cdk complexes as suggested by this sentence.

We have revised the sentence.

- Figure 1: I appreciate the added text to the legend of this Figure, but this should include everything that is shown in the figure (some mechanisms shown in the figure are not indicated in the legend).

Given that a large part of the figure is text, we intend the caption to simply orient the reader to how the figure is organized, with the figure being largely self-explanatory. We think it would be redundant to replicate all of the information shown in the figure within the caption as well.

Figure 1

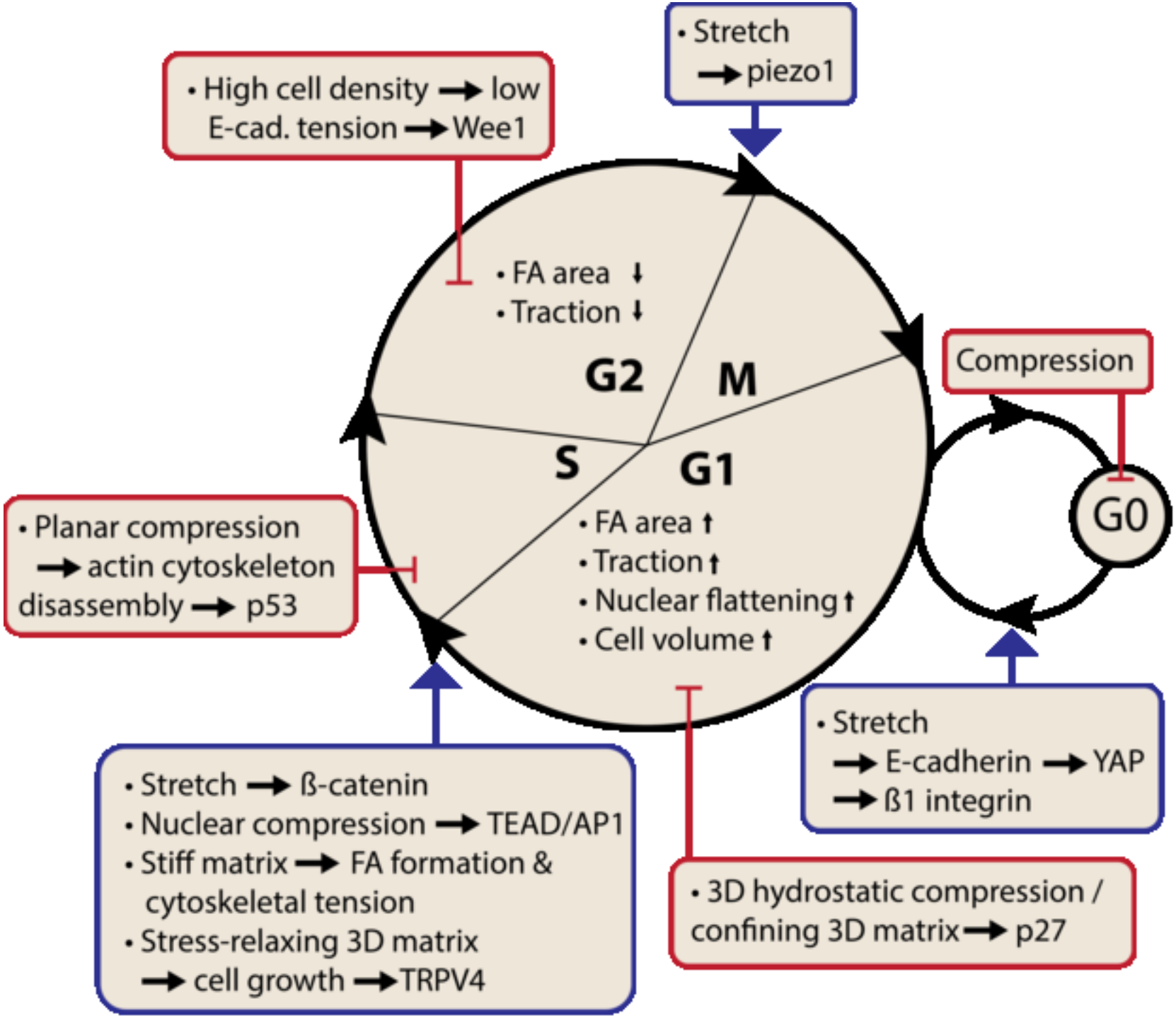


Figure 2

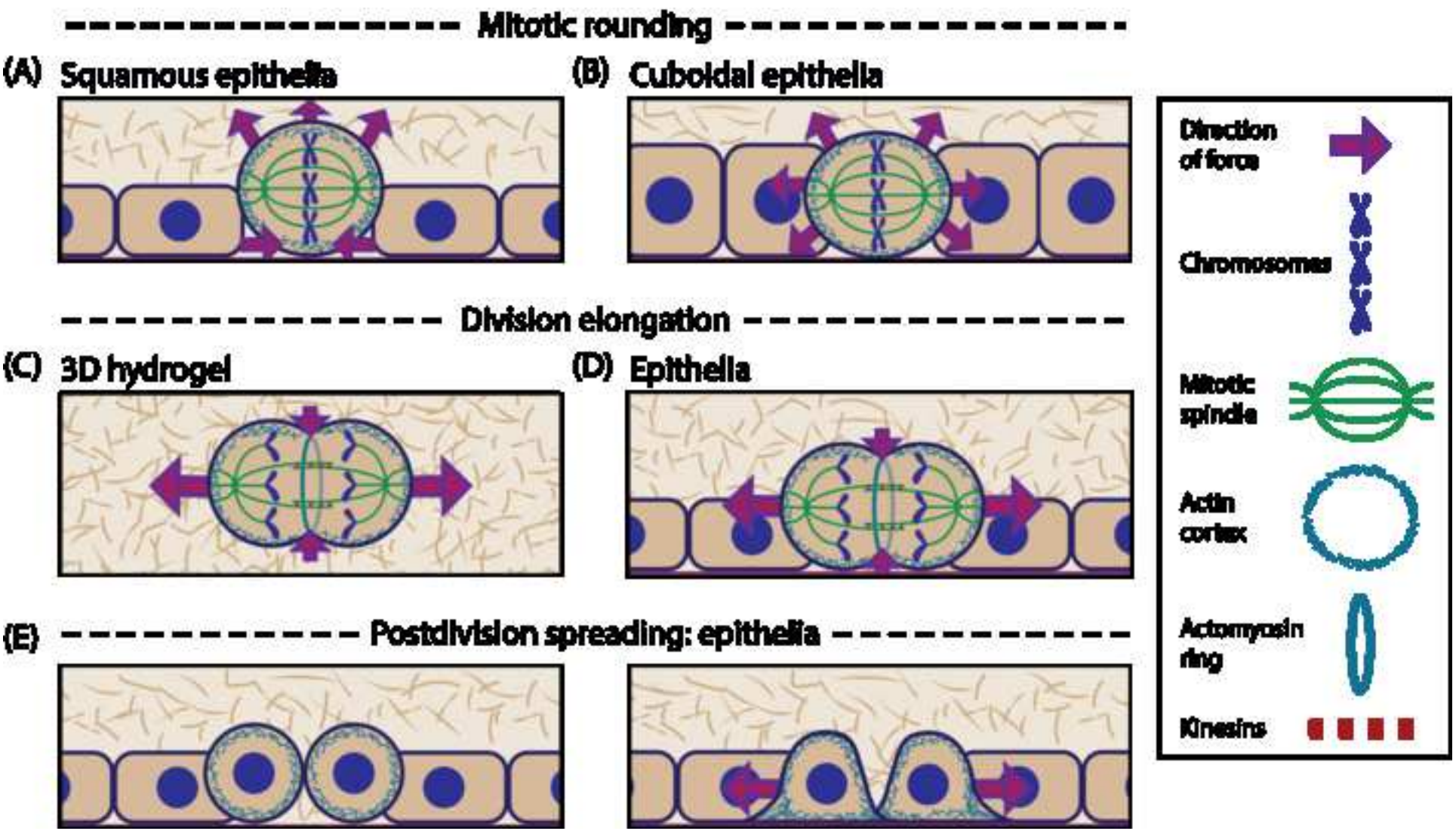


Figure 3

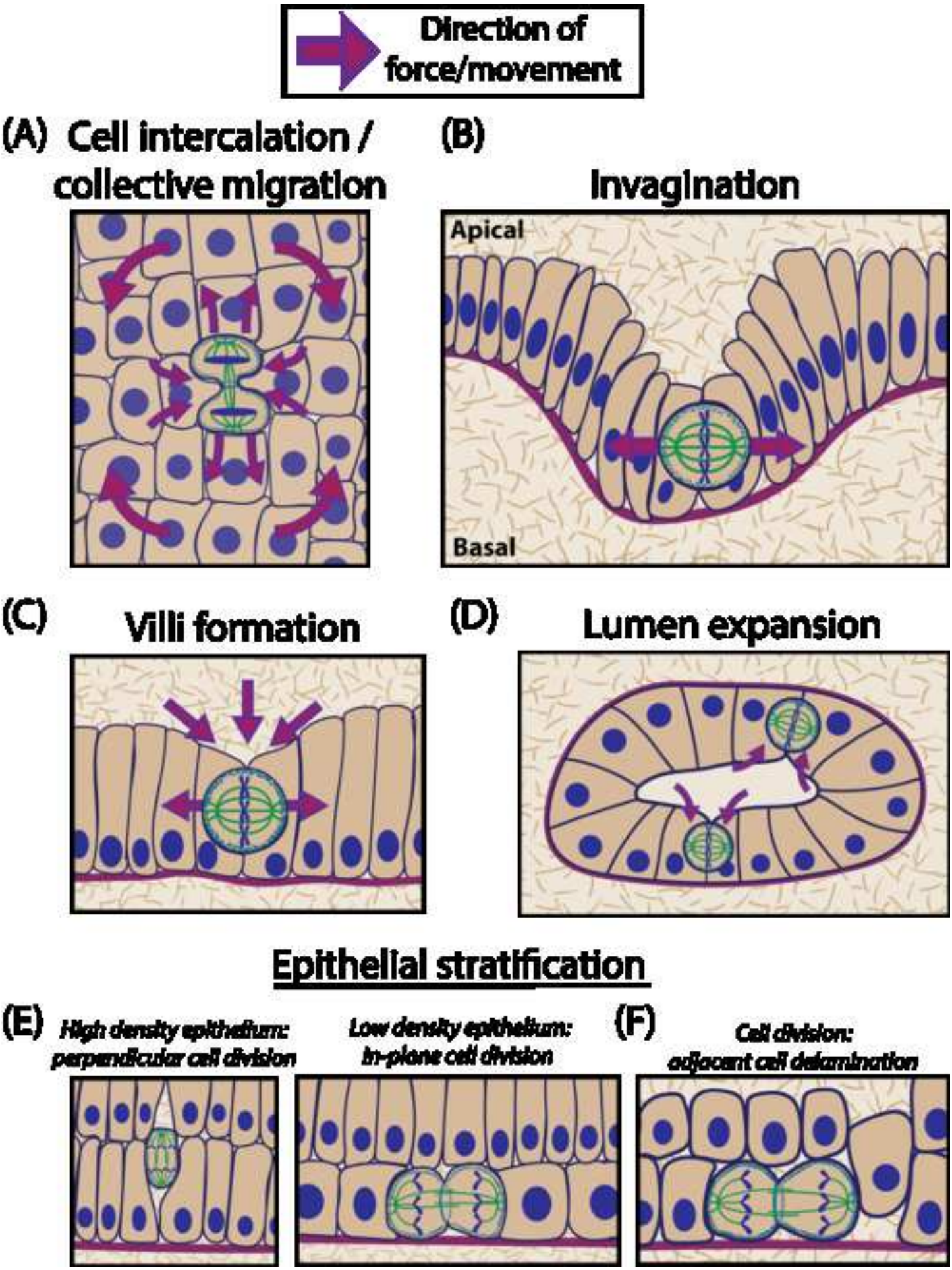
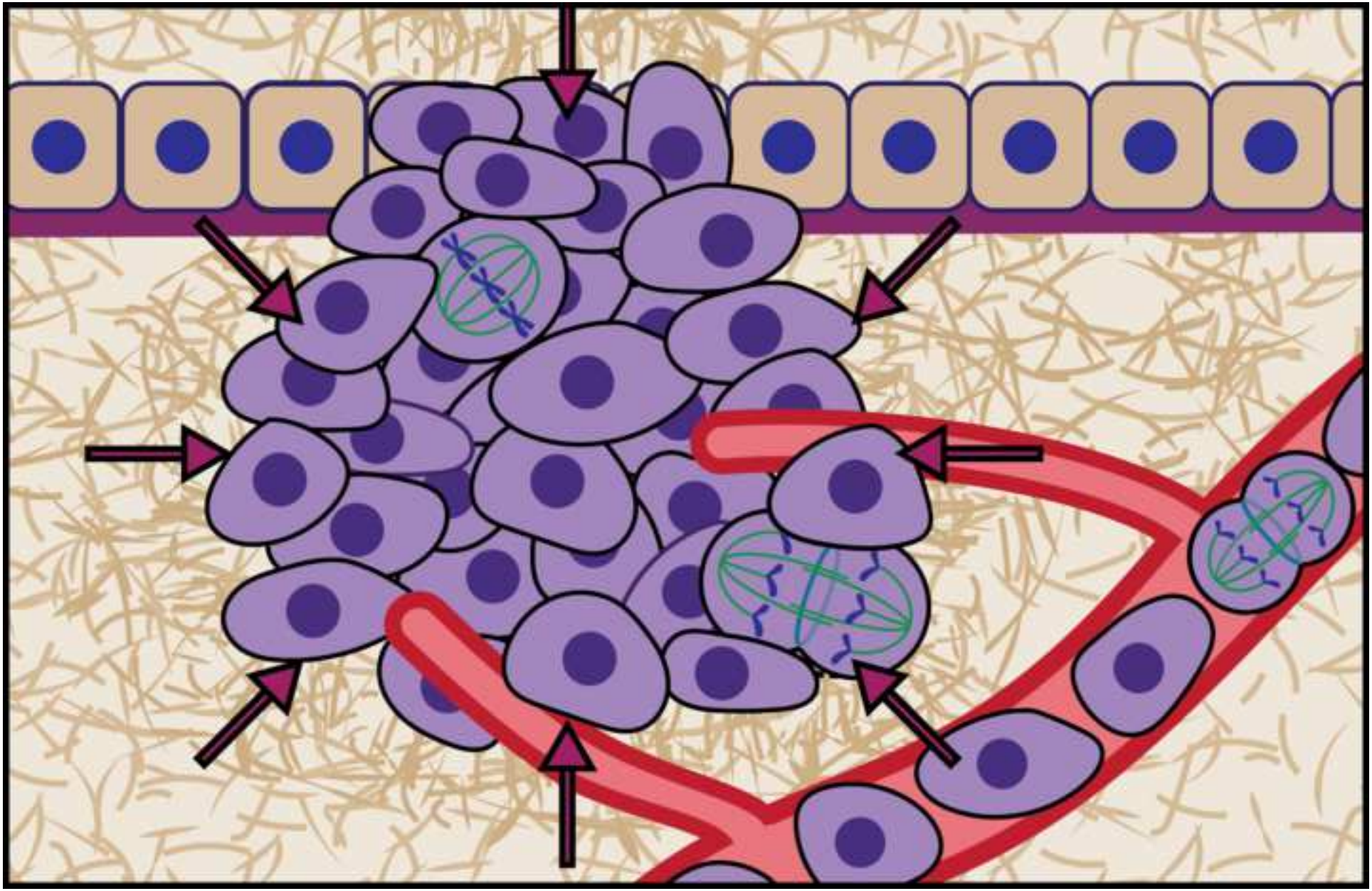


Figure I (Box I)

Click here to access/download;Figure;FigureI.png



Highlights

- Tissue-level tension, cytoskeletal tension, and stiff substrates for 2D culture generally promote cell cycle progression and division, whereas confining environments or compression inhibit cell growth, leading to delayed or halted cell cycle progression and division.
- Cells dividing in confining environments generate extracellular forces to drive major morphological changes which are necessary for proper division completion, during mitotic rounding, division elongation, and postdivision spreading.
- Extracellular forces generated during cell division contribute to cell migration and tissue-scale processes important in development, including tissue growth, invagination, luminogenesis, and epithelial stratification.
- Cancer cells are able to undergo cell cycle progression and cell division within the confining tumor microenvironment.

Outstanding questions

- How does cell division lead to longer-length scale collective cell migration, and what role does this play in development and disease?
- What role do forces generated during division elongation and post-division spreading play in tissue scale processes during development or maintenance of homeostasis?
- Do cancer cells generate greater forces to grow during the cell cycle, undergo division elongation, or spread after division, in more confining tumor microenvironments?
- Do changes in the mechanical properties of the ECM lead to DNA damage or cell division errors?