Powering morphogenesis: multiscale challenges at the interface of cell adhesion and the cytoskeleton

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ABSTRACT Among the defining features of the animal kingdom is the ability of cells to change shape and move. This underlies embryonic and postembryonic development, tissue homeostasis, and regeneration, and wound healing. Cell shape change and motility require linkage of the cell’s force-generating machinery to the plasma membrane at cell–cell and cell–extracellular matrix junctions. Connections of the actomyosin cytoskeleton to cell–cell adherens junctions need to be both resilient and dynamic, preventing tissue disruption during the dramatic events of embryonic morphogenesis. In the past decade, new insights radically altered the earlier simple paradigm that suggested simple linear linkage via the cadherin–catenin complex as the molecular mechanism of junction–cytoskeleton interaction. In this Perspective we provide a brief overview of our current state of knowledge and then focus on selected examples highlighting what we view as the major unanswered questions in our field and the approaches that offer exciting new insights at multiple scales from atomic structure to tissue mechanics.

AN EVOLVING VIEW OF JUNCTIONAL–CYTOSKELETAL CONNECTIONS

The discovery of cadherins and their catenin partners was a major advance. Cadherin–catenin complexes appeared to provide a simple, linear linkage between the cytoskeletons of neighboring cells: E-cadherin extracellular domains linked cells via homophilic adhesion, β-catenin bound to the cadherin cytoplasmic tail and to α-catenin, and α-catenin bound to actin. We probably should have been suspicious of this simplicity, given the much more complex picture of cell–matrix junctions. A pair of pivotal papers in 2005 shook our confidence in this simple picture, revealing that cadherin–catenin complexes have a very low affinity for actin, while α-catenin homodimers have a much higher affinity (Drees et al., 2005; Yamada et al., 2005), data hard to reconcile with in vivo studies (Hirano et al., 1992; Orsulic and Peifer, 1996; Desai et al., 2013). This revelation and others forced us to return to first principles, rebuilding the model of junction–cytoskeleton connections from the ground up.

We will not fully review the advances made in the past decade—for that we refer readers to the many excellent, more comprehensive reviews (e.g., Mege and Ishiyama, 2017; Yap et al., 2018; Angulo-Urarte et al., 2020; Arslan et al., 2021). In broad strokes, several recent advances paint a much more complex picture. First, many more proteins occupy the junctional space, casting doubt on any linear model of adherens junction (AJ)–cytoskeleton linkage. Second, the cadherin–catenin complex and the proteins it recruits act as mechanosensors, with applied force triggering structural changes mediating functional adaptation. Third, AJs are megadalton assemblies built by multivalent interactions, with at least some...
properties of phase-separated biomolecular condensates. Finally, AJs form connected networks, with tissue-level material properties influencing local interactions between the cytoskeleton and AJs. Rather than fully reviewing these developments, we will provide an admittedly idiosyncratic view of questions we are excited to see the field address, ranging across biological scales from atomic structures to tissue mechanics.

ARE AJs BIOMOLECULAR CONDENSATES EXHIBITING INTERNAL ARCHITECTURE (FIGURE 1B)?

The first electron microscopy (EM) images of AJs already suggested that they are layered structures of substantial size. In 2009 the Harris lab took this analysis further, counting the molecules present in “spot AJs,” the large punctate AJ complexes that form as cells polarize in early Drosophila embryos (Tepass and Hartenstein, 1994; McGill et al., 2009). This revealed underlying size and complexity: E-cadherin and β-catenin are present in stoichiometric amounts, with ∼1500 E-cadherins per spot AJ, while Par3 (Drosophila Bazooka—we will generally use mammalian nomenclature) is less abundant—approximately 400 proteins per spot AJ (McGill et al., 2009). While more mature AJs were initially viewed as continuous, superresolution microscopy reveals that they are also composed of discontinuous “spots,” albeit smaller and close enough to one another to give the appearance of continuity (Erami et al., 2015). However, other data suggest that the appearance of puncta may in fact reflect membrane convolutions (Li et al., 2021)—this will need to be resolved. Spot AJs may, in turn, be built up from smaller precursor complexes (McGill et al., 2009; Truong Quang et al., 2013; Wu et al., 2015). High-resolution light microscopy suggests an underlying structure, with proteins segregated to different “regions” of AJs (Bertocchi et al., 2017). This also is true in vivo—for example, Par3 segregates apically from E-cadherin as Drosophila embryonic AJ matures (Harris and Peifer, 2005), while early in junction formation Afadin enrichment extends more basally than E-cadherin at tricellular junctions (Bonello et al., 2018).

Evidence is accumulating that AJs have at least some attributes of phase-separated biomolecular condensates—non–membrane bound multiprotein complexes assembled by multivalent interactions (Banani et al., 2017). Phase-separated condensates often involve proteins with intrinsically disordered regions, which can mediate multivalent interactions (Posey et al., 2018). Intrinsically disordered regions are a feature of many AJ scaffolding proteins, including Afadin and ZO-1. Both ZO-1 and Par3 phase separate in vitro and in vivo (Schwayer et al., 2019; Liu et al., 2020). Intriguingly, while most or all AJ proteins exist in both stable and mobile pools, as assessed by fluorescence recovery after photobleaching (FRAP; e.g., Erami et al., 2015), like proteins in known condensates, different AJ proteins have different dynamics, with differences in both recovery rate and immobile fraction. For example, in Drosophila embryos Par3 has a slower entry rate and a lower immobile fraction than E-cadherin (McGill et al., 2009). This is also consistent with a more complex biomolecular condensate.

We are thus poised to define the underlying structure of AJs and determine how variations in AJ structure at different times and places help AJs adjust to tissues with different dynamics and force regimes. Superresolution microscopy (structured illumination microscopy [SIM], stimulated emission depletion [STED] microscopy, single molecule localization microscopy [SMLM], and other emerging approaches) provides the resolution to define junctional substructure (Gonschor et al., 2020). Initial efforts to examine this suggest that AJs have a layered architecture, with membrane-proximal cadherin tails segregated from the actin cytoskeleton by an interface containing vinculin, VASP, and zyxin (Oldenburg et al., 2015; Bertocchi et al., 2017). It will be interesting to contrast how AJ–cytoskeletal linkages differ on bicellular borders and tricellular junctions—the latter are subject to more molecular force (e.g., Choi et al., 2016). EM already revealed a layered cytoskeletal architecture present at bicellular borders in cultured mammalian cells. Arp2/3-generated branched actin networks are immediately adjacent to the cadherin tails, while linear actin arrays are found further into the cytoplasm (Efimova and Svitkina, 2018). The next frontier will be to bridge the gap between light and electron microscopy via technologies like correlated light and electron microscopy (CLEM; de Boer et al., 2015). Focused ion beam scanning electron microscopy (FIB-SEM; Xu et al., 2021), already applied to cell junctions in cardiac intercalated disc (Vanslembrouck et al., 2020), or electron tomography, already applied to desmosomal cadherins (He et al., 2003; Sikora et al., 2020), offer additional high-resolution three-dimen-sional information critical to understanding the nanoarchitecture of AJs. The idea of biomolecular condensates with internal structure is not a new one—for example, Drosophila germ granules, one of the first known biomolecular condensates, have internally segregated domains enriched for different mRNAs (Trecak et al., 2020). Defining the biophysics underlying layering is another important challenge.

In parallel, we need to explore the in vivo relevance of phase separation, using approaches bridging in vitro and in vivo protein behaviors and ensuring that they are done with sufficient rigor (Alberti et al., 2019). Fluorescence correlation spectroscopy (FCS) offers the chance to define protein concentrations and diffusion coefficients before and after assembly into AJs, key parameters for assessing AJs as biomolecular condensates—a recent application to β-catenin provided a glimpse of the possibilities (de Man et al., 2021). Quantitative data on the size and stoichiometry of the supramolecular AJ complex in vivo, by extending “molecule counting” approaches, will provide additional insight. Pioneering work on the cytokinetic ring revealed the potential of this approach (Wu and Pollard, 2005). Pairing this information with measurements of affinities of protein interactions may allow modeling of AJ assembly and stability. Finally, we need to determine whether applied force affects AJ condensation. The ultimate tests of the in vivo relevance of phase separation will involve using the knowledge gained about condensate composition, affinities, and basic biophysical parameters and pairing it with genetic analysis. For example, after defining the domains of ZO-1 and Par3 required for phase separation in vitro, the function of these domains can be tested in vivo during morphogenesis in Drosophila, looking for effects on AJ assembly, integrity, and function, including response to applied force. This pairing of biophysical and genetic studies should prove powerful.

WHAT MECHANISMS ENSURE AJ RESILIENCE AND TISSUE INTEGRITY (FIGURE 1A)?

The remarkable ability of epithelial cells to change shape and move while maintaining tissue integrity poses important questions: how can the linkage between AJs and the force-generating cytoskeleton be both resilient, resisting disruption when forces are applied, and dynamic, allowing cells to slide past one another and allowing junctions to rapidly stretch and shrink? Replacing the simple linear model of junction–cytoskeleton linkage made us rethink how protein interactions mediate this critical connection, revealing a more nuanced view of multivalent interactions and functional redundancy. Linkage is mediated by a network of proteins. Most are multidomain proteins interacting in multiple ways with network neighbors via multivalent linkages. Strikingly, different protein nodes in the network differ in their importance when assessed in vivo during
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Drosophila morphogenesis. Some, like the cadherin–catenin complex and Par3, are essential for adhesion itself, with epithelial integrity immediately lost in their absence. Others, like Afadin and ZO-1, are important for many morphogenetic movements, but not essential for cell adhesion itself. Finally, some, like Ajuba, vinculin, and Sidekick, are dispensable for viability, exhibiting tissue-level phenotypes in a subset of tissues, or when the system is stressed. This partial functional redundancy may not be that surprising: the ability of cells to change shape and move without disrupting tissues is fundamental to embryonic development, tissue homeostasis, and wound repair. Thus evolution has selected for a very robust AJ–cytoskeletal linkage that can accommodate a wide range of force regimes and cell junction dynamics at different times and places—small deficits in function that may be difficult to quantify in the lab may still provide grist for the mill of natural selection.

To fully assess the mechanisms underlying junctional resilience/robustness, we need to identify the full set of proteins in the AJ network and define mechanisms mediating assembly, adaptability, and robustness of this megadalton machine. This will require a multidisciplinary approach. First, we must identify immediate protein neighbors of proteins in the network. Proximity-dependent labeling approaches like BioID and APEX offer this ability (e.g., Van Itallie et al., 2013; Baskaran et al., 2021). A parallel methodology combining cross-linking mass spectroscopy with high-resolution microscopy recently demonstrated that Scribble and erbin form puncta separate from but adjacent to cadherin–catenin complexes (Troyanovsky et al., 2021). With the parts list in hand, structural biology offers the chance to define AJ protein structure and protein interactions at atomic resolution. X-ray crystallography has been supplemented by the cryo-EM revolution (Lyumkis, 2019), and this has begun to be applied to AJs. In parallel, artificial intelligence (AI)-driven approaches have revolutionized the ability to predict protein structure de novo (Baek et al., 2021; Jumper et al., 2021), and delivered databases of predicted structures of all proteins in multiple model animals including humans (Tunyasuvunakool et al., 2021). Even more exciting, machine learning allows prediction of the structures of multiprotein complexes (Humphreys et al., 2021)—the potential application to the AJ network is exceptionally promising.

However, we must not let gorgeous structures and structure predictions make us forget that AJs are dynamic, not static, and exciting.

**FIGURE 1**: Exploring multiscale challenges at the interface of cell adhesion and the cytoskeleton. Key questions and important approaches are presented at scales that range from atomic structure to tissue mechanics. (A) Questions at the atomic scale. Left image, structure of Canoe’s PDZ domain bound to the C-terminal peptide from its binding partner, the nectin Echinoid (image from Perez-Vale et al., 2021). Right image, Cryo-EM map of the actin–α-catenin actin-binding domain structure (image from Xu et al., 2020). (B) Questions about the idea of AJs as biomolecular condensates. Left image, Biomolecular condensate droplets formed in vitro from Whi3 protein and BNI1 RNA—picture provided by Erin Langdon and Amy Gladfelter. Right image, Illustration of how multivalent interactions can induce condensate formation. (Both images are derived from Schaefer and Peifer, 2019.) (C) Questions at the level of cell shape and subcellular localization. Left image, Localization of Afadin (Canoe) in a gastrulating Drosophila embryo: Afadin is enriched at tricellular junctions. Right image, β-catenin outlining cell junctions in a gastrulating Drosophila embryo in which Afadin lacks its Rap1-binding RA domains, leading to gaps in AJs under mechanical tension. (Both images are derived from Perez-Vale et al., 2021.) (D) Questions at the tissue level. Left image, A diagram representing cells as nodes and edges (image derived from Yu and Fernandez-Gonzalez, 2017). Right image, β-catenin outlining cell junctions in a Drosophila embryo in which the mesoderm is invaginating via apical constriction. (Image from Peifer lab.)
new approaches allow us to probe this. For example, combining negative stain EM or neutron spin-echo spectroscopy with small-angle x-ray scattering provided new insights into the structures of the cadherin–catenin complex and of α-catenin, emphasizing their inherent flexibility/dynamics (Nicholl et al., 2018; Bush et al., 2019). Similarly, high-speed atomic force microscopy imaging in solution revealed the inherent flexibility of cadherin extracellular domains (Nishiguchi and Oda, 2021). Biochemists can build on these data to define the rules underlying AJ assembly by reconstituting AJ complexes from the bottom up, beginning with purified proteins—recent work on large structures like the Wnt signaling regulatory complex provides a model (Ranes et al., 2021). Cryo-EM technology may then allow structural determination of in vitro constructed multiprotein assemblies, validating and extending predicted structures.

The ultimate tests of the mechanisms driving resiliency will involve experiments performed in vivo. CRISPR and other genetic tools now make it relatively straightforward to take apart multidomain proteins in vivo, allowing rapid testing of the importance of proteins or protein domains in multivalent network interactions (e.g., Rauskolb et al., 2019; Perez-Vale et al., 2021; Sheppard and Tepass, 2021). The field is beginning to use genetic approaches to explore the effects of removing individual protein–protein interactions on AJ stability during cell shape changes and rearrangements, often with surprising results. Some interactions are critical, while others are largely redundant, leading to minor or tissue-specific defects in AJs challenged by shape change or remodeling (e.g., Yu and Zallen, 2020; Perez-Vale et al., 2021; Sheppard and Tepass, 2021). Analyses like these will provide insights into one potential underlying principle explaining redundancy: that multivalent interactions between proteins in the network mean that disrupting one protein interaction leaves others intact, allowing retention of a functional AJ–cytoskeletal linkage. Another potential mechanism underlying redundancy involves the idea that the structure and composition of AJ biomolecular condensates may vary over space and time, with some proteins/linkages playing parallel roles in different locations. For example, Adfin and ZO-1 act in parallel to maintain epithelial integrity during Drosophila embryonic morphogenesis: data suggest that Adfin reinforces anterior–posterior cell borders while ZO-1 reinforces dorsal-ventral borders (Manning et al., 2019). Extending this analysis, proteomics will define prominent posttranslational modifications, the potential regulatory function of which can be tested by mutagenesis. Optogenetic tools offer exciting parallel approaches to modulate protein function, with virtually instantaneous temporal and spatial resolution (e.g., Ollech et al., 2020). Finally, AJs must also be disassembled during morphogenesis, and defining the underlying mechanisms, in all their complexity, will also be essential.

**HOW DO AJS SENSE AND ADAPT TO FORCE (FIGURE 1C)?**

The linear model portrayed cadherin–catenin complexes as molecular glue, linked to the cytoskeleton in a static, ON/OFF way. However, in both embryos and homeostatic tissues, cells are not glued together—AJ–cytoskeletal linkages must accommodate dramatic changes in cell shape and cell migration, both of which involve applying force to junctions. α-Catenin provided the first mechanistic clues (reviewed in Mege and Ishiyama, 2017). When force is applied to α-catenin, it triggers a conformational change, involving partial unfolding of its series of α-helical bundles. This shifts α-catenin into a state where its actin affinity is dramatically increased and simultaneously opens up binding sites for other AJ proteins, including vinculin, Aijuba, and Afdin, further reinforcing cytoskeletal linkage. Thus α-catenin is both mechanosensitive and mechanoresponsive. This reinforces AJs under elevated tension, like tricellular or planar-polarized bicellular junctions in Drosophila embryos, where contractile actomyosin cables drive junction shrinkage and thus cell intercalation (Kong et al., 2017). This catch-bond behavior of α-catenin, in which actin affinity switches upon applied force, provided a critical insight into the regulation of cell junction fluidity, and continuing explorations of this property and how it is controlled (e.g., Arbore et al., 2022) will help us further understand regulated adhesion.

Now the challenge is to extend this pioneering work, defining the multiple mechanisms mediating force sensing and responsiveness in different tissues and times. Once again, an interdisciplinary approach will be needed. Applying biophysical tools like laser traps to single molecules or purified biochemical complexes will allow extremely precise application and measurement of forces involved in conformational transitions—work on α-catenin has paved the way (Buckley et al., 2014). Dynamic conformational changes upon force application can be explored using thiol labeling to probe cysteine residue accessibility—this provided new insights into α-catenin (Terekhova et al., 2019). Combining this with the structural biology approaches described above and using mutational analysis to alter force-sensing abilities of individual proteins and test their function in vivo will help define the roles of force-responsive protein domains and protein interactions in mediating the response of AJs to applied force.

Some tension-dependent proteins, including VASP and zyxin, are recruited in response to mechanical stimuli independently of α-catenin (Oldenburg et al., 2015). LIM domain proteins like zyxin use an intriguing mechanism of mechanosensing, specifically recognizing mechanically stressed actin filaments (Anderson et al., 2021; Sala and Oakes, 2021). α-Catenin may also recognize specific actin filament conformations (Mei et al., 2020). Extending the analysis of these diverse mechanosensing mechanisms will prove powerful. We also need to explore the interlocking feedback loops that connect AJs and the cytoskeleton. For example, α-catenin binding alters actin structure (Hansen et al., 2013), something that should be explored for other actin-binding proteins in the AJ complex. We need to explore how force alters actin polymerization. Zyxin’s ability to recruit the actin polymerases Diaphanous and Ena/VASP to repair stress fibers under mechanical stress provides a model (Smith et al., 2010; Valencia et al., 2021). Intriguingly, Ena/VASP proteins localize to tricellular junctions in Drosophila and mammalian cells. Exploring how the cytoskeleton regulates cadherin clustering, both experimentally (Chen et al., 2015) and via modeling (Chen et al., 2021), also promises new insights—this can be extended to explore how patterned mechanical heterogeneity and cadherin clustering along a body axis help drive morphogenesis (Huebner et al., 2021). Finally, once these baseline interactions are defined, we can move on to explore the coordination of different tension-sensitive mechanisms: for example, coupling cadherin–cadherin catch bonds with α-catenin–actin catch bonds, or recruitment of reinforcing of the α-catenin–actin interaction with reinforcing of the cadherin–cadherin complex via, for example, stabilization by p120catenin.

**IS MYOSIN MOTOR ACTIVITY NECESSARY FOR THE CONTRACTION OF JUNCTIONAL ACTIN (FIGURE 1C)?**

Cell shape changes require the expansion and contraction of actin networks associated with AJs. This is assumed to be driven by the activity of the molecular motor nonmuscle myosin II, which promotes sliding of antiparallel actin filaments. During Drosophila germ band extension (Kong et al., 2017) or during the assembly of the AJ actomyosin network in cultured mammalian cells (Yu-Kemp et al. 2022),
the activity of myosin and the myosin activator Rho kinase are essential. However, junction-associated actin can also be organized in parallel filaments (Sanger and Sanger, 1980), and it is not clear how myosin motors would contract parallel filament networks. Mathematical modeling and in vitro data show that myosin-induced buckling of actin filaments favors filament severing and contraction of disorganized networks (Lenz et al., 2012; Lenz, 2014; Murrell and Gardel, 2012), providing a mechanism by which myosin could promote the contraction of parallel actin filaments in vivo. Other findings raise the possibility of contractility independence of myosin motor activity. Pioneering theoretical analysis suggested that actin disassembly, coupled with filament cross-linking (possibly by myosin, but also by other cross-linkers), could be sufficient to generate contractile forces (Zumdieck et al., 2007; Walcott and Sun, 2010). Both budding yeast (Mendes Pinto et al., 2012) and mammalian cells (Ma et al., 2012) can complete cell division in the absence of myosin motor activity. However, cytokinesis in Caenorhabditis elegans embryos cannot proceed without myosin motors (Osorio et al., 2019), and motor-dead myosin mutants that still bind actin disrupt AJ contractility during Drosophila axis elongation (Kasza et al., 2019). We need to define why the motor function of myosin is necessary in some systems and not others. In vitro, actin network architecture determines whether myosin motors can induce contraction (Reymann et al., 2012). Careful quantification of actin filament organization in contractile networks in vivo, for example, using cryo-EM (Huethn et al., 2018) or optical super-resolution microscopy to visualize the relative positions of the barbed and pointed ends (Xia et al., 2019), will reveal whether myosin is exclusively dispensable for contraction of disorganized actin networks or of networks largely formed by parallel filaments. It will also be important to determine whether motorless contraction is specific to subcellular actomyosin structures, or whether contraction of supracellular actomyosin networks, like those driving collective migration of neural crest (Shelliard et al., 2018) or metastatic cells (Hidalgo-Carcedo et al., 2011), can also proceed in the absence of motor activity.

What mechanisms drive motorless contraction? In vitro the actin cross-linker anillin can maximize actin filament overlap, regardless of filament orientation, effectively reducing actin bundle length (Kucera et al., 2021). Anillin uses its multiple actin-binding sites (Jananji et al., 2017), preferentially binding actin in regions of filament overlap and generating sliding forces inversely proportional to the degree of overlap (Kucera et al., 2021). Filament sliding velocity also decreases with filament overlap, possibly due to increasing friction between filaments. This could explain (at least in part) why in some systems, actomyosin network contraction slows as the network shrinks (Carvalho et al., 2009). Actin filament disassembly synergizes with anillin-based filament sliding to drive efficient contraction of actin bundles, at least in vitro (Kucera et al., 2021). However, this is not the sole mechanism of motorless contraction. For example, the actomyosin ring driving plasma membrane repair in Xenopus oocytes closes in the absence of myosin activity, through an elegant treadmilling mechanism involving a gradient of Rho GTPase activation toward the interior of the ring and inactivation toward the exterior, thus propelling the ring inward (Miller and Bement, 2009; Burkel et al., 2012). Whether other actin regulators can drive motorless contraction, and through which mechanisms, needs to be defined. Candidates identified via image-based, genome-wide screens using the powerful genetics of yeast and the simplicity of cytokinesis (Vizeacoumar et al., 2010) can then be tested in the context of cell shape change driven by junctional actomyosin contraction.

DO THE MATERIAL PROPERTIES OF TISSUES AFFECT AJ REMODELING (FIGURE 1D)?

We also need to move up in scale, to the tissue level. In epithelia, AJs form networks connected at tricellular vertices. A consequence of AJ connectivity is that when a cell–cell contact contracts it must overcome the resistance of the rest of the network. This is clear, for example, as cells disassemble their apical contacts during mesoderm invagination in Drosophila: junctional remodeling is facilitated when network connectivity is disrupted by reducing cell–cell adhesiveness (Martin et al., 2010). Thus, the material properties of the network affect the contraction of any given junction: if the network is soft or fluid, junctional contractility and neighbor exchange require lower energy than if the network is stiff or solid. However, we currently have a limited understanding of the effect of the material properties of a tissue on contraction/disassembly of cell–cell contacts.

Wound repair is an excellent model to investigate how tissue resistance affects junctional remodeling. In nonproliferating or slowly proliferating epithelial monolayers, the wound response is driven by the coordinated movement of the cells around the wound to cover the lesion (Hunter and Fernandez-Gonzalez, 2017). This coordination is mediated by polarization of the actomyosin cytoskeleton in the cells adjacent to the wound (Martin and Lewis, 1992; Bement et al., 1993). Actin and myosin accumulate at the interface with the wounded cells, forming a supracellular cable around the wound that generates mechanical forces to align cell movements. As the wound closes, the cable must contract against the increasing resistance to deformation of the rest of the epithelium (Abreu-Blanco et al., 2012; Kobb et al., 2017). Defining the mechanisms underlying wound repair will also shed light on similar molecular events that occur at tissue boundaries during development—for example, epiboly in zebrafish or the leading edge during Drosophila dorsal closure.

How does one balance contraction of the actomyosin cable and resistance of the tissue during wound healing? One possibility is for the cells around the wound to buffer the deformation of the rest of the tissue. In Drosophila, cells adjacent to embryonic wounds stretch dramatically into the lesion (Razzell et al., 2014). Recent data revealed that these cells might be doing more than simply stretching: cells adjacent to epidermal wounds in axolotl, zebrafish, and Drosophila embryos increase their volume in a process necessary for rapid wound healing (Tanner et al., 2009; Kennard and Theriot, 2020; Scepanovic et al., 2021). How this rapid (minutes) increase in volume occurs needs to be defined, but one possibility is that changes in osmotic pressure caused by wounding might be in part responsible (Gault et al., 2014). While several ion channels have been implicated in wound healing (Fuchigami et al., 2011; Xu and Chisholm, 2011; Holt et al., 2021; Scepanovic et al., 2021), the development of activity sensors to track the dynamics of channel activation during wound repair (Stewart et al., 2021), and novel optogenetic tools to manipulate channel activity in vivo, with ion specificity (Beck et al., 2018), will shed light on the role that different ion channels play in the cell volume changes associated with rapid wound healing.

There are other strategies to minimize the resistance of the tissue to contraction. For instance, during wound closure, cells can intercalate away from the wound edge as one segment of the actomyosin cable completes its contraction (Tetley et al., 2019). Cell intercalation at the wound edge occurs in Drosophila wing imaginal discs, but not in embryonic tissues, in which only cells away from the wound intercalate (Razzell et al., 2014). What drives this difference? Perhaps tissues that display active cell rearrangements (Classen et al., 2005; Aigouy et al., 2010) have intercalation naturally built into the wound-healing response, while others in which cell
Vertex models reveal that when the preferred shape index exceeds a certain critical value, cell rearrangements cost almost no energy and the system behaves like a fluid (Figure 2; Bi et al., 2015). In these fluid tissues, cells can “flow” as they exchange neighbors and move over long distances. In contrast, in solid networks cells are caged and display only small fluctuations around static positions (Bi et al., 2016). The development of better methods to probe tissue mechanics in vivo, including oil micropipette aspiration (von Dassow and Davidson, 2009), microdroplets (Campas et al., 2014), optical tweezers (Bambardekar et al., 2015), or magnetic tweezers (Doubrovinski et al., 2017), will help test the predictions of vertex models in developing animals. Furthermore, the dynamic distribution of junctional and cytoskeletal proteins within a cell in vivo, such as the planar polarization of AJ and actomyosin proteins to different cell borders during Drosophila gastrulation, could dictate the evolution of the preferred shape index over time. Models that integrate molecular dynamics with tissue mechanics (David et al., 2013; Siang et al., 2018) will be critical for determining the impact of changes in protein levels and polarity on the changes in material properties experienced by tissues in vivo.

In vivo, tissue architecture is often not simple. Until recently (Kim et al., 2021), vertex models were limited to studying confluent cell sheets with negligible extracellular volumes. In stark contrast with confluent tissues, in tissues with significant extracellular volumes,
where cells are not tightly zipped up to one another, such as the paraxial and presomitic mesoderm during body axis elongation in fish embryos, increasing cell–cell adhesion limits intracellular volume and stiffens the tissue (Mongera et al., 2018). Thus, increasing cell–cell adhesion does not fluidize tissues, but rather solidifies them by favoring cell attachment and limiting the actomyosin-based fluctuations that promote junctional remodeling. Similarly, in fish blastoderms, the central region of the animal undergoes transient fluidization associated with local reduction in cell–cell contact and the appearance of intercellular space (solid-to-fluid transition) as a consequence of mitotic rounding (Petridou et al., 2019, 2021). A local increase in cell–cell contact ensues, restoring tissue viscosity (fluid-to-solid transition). These results are consistent with a role of cell–cell contact in stiffening nonconfluent tissues. Therefore, extracellular volume is an additional, and often neglected, parameter that plays a fundamental role in determining the material properties of tissues and the ability of junctions to contract. This should inspire additional studies to quantify cell connectivity in two and three dimensions in other epithelial tissues, defining how cell connectivity evolves as junctions contract and tissues are sculpted.

Vertex models have their limitations. In these models, cell–cell contacts are either elastic—and can recover their original length after contractile forces are released—or viscous—and sustain permanent changes in the length with each contraction. Recent experimental work using optogenetic tools revealed that cell–cell contacts actually display viscoelastic behaviors, with a partial relaxation of junction length when contractile forces disappear (Staddon et al., 2019; Cavanaugh et al., 2020b). Only beyond a certain threshold is junctional contractility irreversible, due to endocytosis-mediated disassembly of AJs. Another limitation of vertex models is that they represent neighbor exchange events (T1 transitions) as instantaneous: once a junction is sufficiently short, it is immediately substituted by a new, perpendicular junction. However, in vivo, the four-cell vertices that form during T1 transitions have a finite, nonzero lifetime (Blankenship et al., 2006). Molecularly, the adhesion molecule Sidekick localizes to four-cell vertices during cell rearrangements in several Drosophila tissues, including the embryonic epidermis, the trachea, and the retina, and sidekick mutants display longer T1 transitions, suggesting that Sidekick promotes four-way vertex resolution (Finegan et al., 2019; Letizia et al., 2019). Thus, we must continue to refine vertex models, creating “second-generation” models that introduce irreversible junctional contractility and delayed T1 transitions (Staddon et al., 2019; Erdemci-Tandogan and Manning, 2021). Other potential extensions for vertex models include the integration of mechanical and molecular dynamics (Wang et al., 2012; Lan et al., 2015) beyond simple mass action and adding complex relationships such as catch bonds; spatiotemporal variation in fixed parameter values, such as preferred cell area, perimeter, or tissue viscosity; increased granularity in the definition of vertex forces, for instance, by having different energy terms to quantify the effects of cell adhesion and contraction; or explicit modeling of cell–substrate interactions, including the material properties of the substrate. It will be exciting to determine whether complex vertex models that better represent the experimental behavior of junctional networks make different predictions on the dynamics of junctional remodeling during animal development, or whether Occam’s razor is an appropriate approach for the application of vertex models in vivo.

**FINAL THOUGHTS**

It is an exceptional time to be working at the interface between cell and developmental biology. The past decade brought dramatic advances in our understanding of how cells change shape and move, to self-assemble the initial body plan and remodel and maintain it. As we outlined above, these advances have opened many exciting new questions and also unveiled new technologies that we could not have imagined a decade ago. We hope that this Perspective has given you a taste of the excitement we feel for our field, and we hope that some of you will join us in answering these questions.

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