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Review Article

Problems in biology with many scales of length: Cell–cell adhesion and cell jamming in collective cellular migration

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ABSTRACT

As do all things in biology, cell mechanosensation, adhesion and migration begin at the scale of the molecule. Collections of molecules assemble to comprise microscale objects such as adhesions, organelles and cells. And collections of cells in turn assemble to comprise macroscale tissues. From the points of view of mechanism and causality, events at the molecular scale are seen most often as being the most upstream and, therefore, the most fundamental and the most important. In certain collective systems, by contrast, events at many scales of length conspire to make contributions of equal importance, and even interact directly and strongly across disparate scales. Here we highlight recent examples in cellular mechanosensing and collective cellular migration where physics at some scale bigger than the cell but smaller than the tissue – the mesoscale – becomes the missing link that is required to tie together findings that might otherwise seem counterintuitive or even unpredictable. These examples, taken together, establish that the phenotypes and the underlying physics of collective cellular migration are far richer than previously anticipated.

Contents

1. Introduction . . . 1
2. Plithotaxis and kenotaxis . . . 2
3. The intercellular adhesome . . . 2
4. Cell sorting and differential adhesion . . . 2
5. Cell jamming and the case of the bronchial epithelium . . . 4
6. Conclusions . . . 5
Acknowledgments . . . 5
References . . . 5

1. Introduction

Understanding cell adhesion and associated mechanisms of mechanosensing remain critical challenges in explaining multiple facets of health and disease, including but not limited to development, wound healing, asthma, cardiovascular disease, and cancer. One finds in the literature two main contrasting approaches. Recent literature emphasizes the microscopic, bottom-up, granular approach in the context of specific molecules, their roles and their interdependencies [1–3]. This can range from identifying mechanically sensitive actin-linkers [4], to characterizing signaling pathways, [5] to finding downstream effectors of mechanotransduction such as YAP/TAZ [6]. The older literature, by contrast, emphasizes the macroscopic, coarse-grained, top-down approach in the context of mechanical forces, fields, and integrative physiological function. In addition to the classical work of Thompson [7], specific examples of the latter are Wolff's law [8] for adaptation of bone structure to the load that the bone supports, Murray's Law [9] for adaptation of blood vessel diameter to the flow that the vessel carries, and McMahon's [10–12] principle of elastic similarity to explain allometric variations of energy

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metabolism, muscle mass, and bone size with body mass.

If each were carried to its logical conclusions, one might expect that microscopic and macroscopic approaches, when taken together, would dovetail seamlessly to create a satisfying and comprehensive physical picture. The expectation, then, would be one of complementary parts combining, ultimately and inevitably, to form a complete logical framework that spans all pertinent scales of length. While such cases clearly exist, as when a single mutation affecting mechanosensing can be tied directly to macroscopic effects and disease [13], there is reason to believe that such an expectation might be illusory more generally in biology. At the intermediate scale – the mesoscale – pivotal phenomenon can and do emerge that are at once hidden at the macroscale but are not anticipated by or predictable from the microscale [14]. Nevertheless, at the level of integrated system behavior they become crucial. In the particular context of collective cellular migration, we provide here several such examples [15–20]. Because of its importance in wound healing, development, and cancer, collective cellular migration has been of interest for more than 100 years [21], but, as regards mechanosensing, it has been only in recent years that the mechanical stresses exerted between each cell and its substrate [22, 23], and between each cell and its immediate neighbors [24–26], have been measured and mapped.

2. Plithotaxis and kenotaxis

We begin with the example of collective cellular migration and the recently discovered mesoscopic phenomenon called plithotaxis. Epithelial cells comprising a confluent layer are known to move in cooperative streaks, strands, packs and clusters [27], but the intercellular mechanical stresses that drive these local cell motions were for a long time a matter of pure speculation. Tambe et al. [24, 25, 28] first measured these stresses within the confluent cell layer and showed that these stresses can fluctuate dramatically from cell to cell and from moment to moment; that is to say, intercellular stresses are typified by a dynamic heterogeneity [29] wherein fluctuations in space and time dominate. Moreover, Tambe et al. established that each individual cell within the layer can exhibit a strongly preferred stress orientation; that is to say, in addition to dynamic heterogeneity, the field of intercellular stress tends to be strongly anisotropic. When they examined the relationship between motions and stresses, they found that each cell within a cellular collective tends to move along a local orientation corresponding to that in which it pulls hardest upon its immediate neighbors; that stress is called the maximum principal stress, and that orientation is called the maximum principal orientation. Plithotaxis therefore implies the seemingly simple notion that the orientations of local cellular motions and local cellular stresses tend to coincide. However, in proximity to an island in which cells cannot adhere to the substrate, and therefore the monolayer has a cell-free boundary, plithotaxis breaks down altogether; the orientations of local cellular motions versus local cellular stresses tend to depart from one another dramatically and systematically [30]. Even as the cell migrates parallel to the boundary of the island, cellular tractions polarize so as to pull perpendicular to that boundary; this mesoscopic phenomenon of cells pulling toward a cell-free void is called kenotaxis.[31]

But whether near such a boundary or far from it, through what molecular processes does a cell within the cell cluster sense mechanical stresses exerted between itself and its immediate neighbors, and then use that information to coordinate its motion with that of the integrated cell cluster? In the mesoscopic process of plithotaxis, Das et al. [32] showed that the tumor suppressor merlin plays a key role. As intracellular stresses build up locally within a constituent cell of the layer, merlin disassociates from cortical cell–cell junctions and enters the cytoplasm. Merlin dissociation then leads to Rac1 activation and polarization, and, ultimately, to lamellipodium formation aligned along the direction of the maximal principal stress. Indeed, the orientation of Rac1 polarization matches stress alignment in the presence of merlin, whereas cells lacking merlin do not show alignment of Rac1 polarity and direction of maximal principal stress. Merlin is not required for cellular motion nor does it affect the development intercellular stress. Nevertheless, through this mechanism, merlin is shown to account for the long range cooperativity and alignment of cellular motions and intercellular stresses. Clearly, without measuring mechanical stress our understanding of merlin polarization would likely seem a perplexing process and, conversely, understanding cell alignment without merlin polarization would seem equally perplexing. But in the example of plithotaxis we now see how polarizations of local cellular motions, mechanical stresses, merlin and Rac1 link together across scales to provide an integrated physical picture [32].

3. The intercellular adhesome

The success of tying plithotaxis to a specific mechanotransduction pathway raises hope that similar meetings at the mesoscale can be found. In the epithelial cell sheet, for example, adhesion molecules associated with tight junctions, adherens junctions, desmosomes, and gap junctions have a role in the development of monolayer stresses that keep the layer continuous, intact and advancing to fill a wound or grow a tissue. Identification of distinct roles of specific molecules is complicated experimentally because knocking down one adhesion molecular could, in principle, cause overexpression of another by compensatory mechanisms. Taking that issue into account, Bazellieres et al. [33] identified distinct mechanical phenotypes that could be tied to groups of adhesion molecules, but in ways that proved to be most unexpected. For example, despite distinct loci in adherens junctions versus tight junctions, knocking down of P-cadherin versus occludin results in quite similar phenotypes, both being characterized by augmented migration speed together with reduced intracellular stress (top panels Fig. 1). And despite similar loci – both within tight junctions, knocking down of ZO-1 versus ZO-3 results in highly dissimilar phenotypes; knocking down of ZO-3 reduces intracellular stress as might have been anticipated, whereas knocking down of ZO-1 causes just the opposite, increasing intracellular stress even as it increases migration speed (bottom panels, Fig. 1). The knocking down of ZO-1 produces a distinct mechanical phenotype compared to all other adhesome proteins. This raises the natural question, could ZO-1 be directly tied to a mechanotransduction pathway in much the same way merlin is tied to plithotaxis?

Bazellieres et al. [33] were also able to highlight the unanticipated roles of some specific molecules, P-cadherin and E-cadherin in particular, in the development of monolayer stresses across these different phenotypes. Surprisingly, P-cadherin but not E-cadherin is linked to the magnitude of intercellular tensile stress, whereas E-cadherin is linked to the temporal build-up of intercellular stress. Although these roles extend across mechanical phenotypes, if E-cadherin is removed then P-cadherin assumes the role of E-cadherin in mechanotransduction.

4. Cell sorting and differential adhesion

Another meeting at the mesoscale involves organogenesis, cell sorting and the differential adhesion hypothesis (DAH) [34,35]. If distinct cell types are mixed *in vitro*, they segregate reproducibly

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