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COMMENTARY

ARTICLE SERIES: CELL BIOLOGY AND DISEASE

Collective migration and cell jamming in asthma, cancer and development

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ABSTRACT

Collective cellular migration within the epithelial layer impacts upon development, wound healing and cancer invasion, but remains poorly understood. Prevailing conceptual frameworks tend to focus on the isolated role of each particular underlying factor – taken one at a time or at most a few at a time – and thus might not be tailored to describe a cellular collective that embodies a wide palette of physical and molecular interactions that are both strong and complex. To bridge this gap, we shift the spotlight to the emerging concept of cell jamming, which points to only a small set of parameters that govern when a cellular collective might jam and rigidify like a solid, or instead unjam and flow like a fluid. As gateways to cellular migration, the unjamming transition (UJT) and the epithelial-to-mesenchymal transition (EMT) share certain superficial similarities, but their congruence – or lack thereof – remains unclear. In this Commentary, we discuss aspects of cell jamming, its established role in human epithelial cell layers derived from the airways of non-asthmatic and asthmatic donors, and its speculative but emerging roles in development and cancer cell invasion.

KEY WORDS: Cell jamming, Cell unjamming, Cell shape, Collective cellular migration, Epithelial cell

Introduction

Open questions in cell biology concern how, when and why a cell comprising a part of a confluent epithelial layer remains quiescent and fixed in place, or instead might mobilize and migrate to great distances in cooperative and collective fashion – in multicellular packs, swirls, streaks and clusters. Such collective events are pivotal in physiological scenarios including wound repair, embryonic development, gastrulation, epiboly and morphogenesis, as well as in the pathophysiology of a carcinoma invading otherwise healthy tissue. Recent inroads have contributed to impressive advances in understanding (Fischer et al., 2015; Munjal et al., 2015; Pattabiraman et al., 2016; Zheng et al., 2015). Nevertheless, a comprehensive physical description that can explain these collective cellular events is currently lacking.

Epithelial cells line the inner and outer surfaces of all organs and body cavities, where they comprise a continuous barrier that serves to protect, separate, sense, transport, secrete and absorb. When quiescent, each constituent cell performs these physiological functions while fixed in its place, tightly anchored to its basement membrane and tightly adherent to its immediate neighbors. Even when an immediate neighbor tugs on it, the cell might jostle a bit in response, but, on net, move relatively little. However, that same cell can sometimes mobilize and then migrate to great distances, as in development. And when an epithelial cell that is part of a confluent layer does migrate, it tends to do so not as an individual cell but rather as a part of a coordinated and cooperative cellular collective, illustrating thereby the root of the term 'confluent', literally meaning flowing together (Arboleda-Estudillo et al., 2010; Das et al., 2015; Haeger et al., 2015; Holmes, 1914).

In these collective processes, the palette of underlying physical and molecular factors is diverse. And because the interactions among these factors are strong and complex, collective cellular migration remains poorly understood. Here, we explore the proposition that there is an overarching physical picture – or integrative framework – into which many of these individual factors might fit. If such a framework could be established, then the influence of each underlying factor might become more deeply appreciated, and the interactions among them better understood.

In particular, the epithelial layer is known to exhibit collective cellular behavior that is strongly reminiscent of the transition between fluid-like and solid-like phases of matter (Angelini et al., 2011; Farhadifar et al., 2007; Kim and Hilgenfeldt, 2015; Szabó et al., 2006; Vicsek and Zafeiris, 2012). Here, we focus on one particular kind of transition – the jamming and unjamming transitions (see Box 1 and Fig. 1). Just as coffee beans can flow in a chute in some circumstances but arrest, jam and rigidify in others, so too can the cellular collective. These circumstances and the distinctions between them are described in detail below. It has been further suggested that by systematically maintaining itself within striking distance of the transition between jammed and unjammed phases, the epithelial cellular collective might have evolved the capability for almost switch-like changes in physiological function – being in certain circumstances solid-like and virtually frozen, but in others fluid-like and highly mobile (Fredberg, 2014; Kim and Hilgenfeldt, 2015). The collective would thus attain by means of only modest changes in one or more of its physical properties an impressive range of adaptability and physiological scope (see Box 2). In this Commentary, we will highlight the emerging role of cell jamming in the contexts of development, asthma and cancer.

Fluid-like versus solid-like collective phases

Within the epithelial layer, how might a solid-like, jammed phase come about? If the layer contains no gaps or empty spaces, then each cell is surrounded by immediate neighbors. As such, each cell is imagined to be physically caged by those neighbors. The strength of any such cage has some finite limit, and this limit can be expressed in the form of an effective energy barrier. This barrier must be overcome if that cell is to escape its cage and, thereby, successfully complete a cellular rearrangement with its neighbors; the stronger the cage the greater is the energy barrier. If that barrier is so great that it can be overcome only rarely, then the cell will remain effectively

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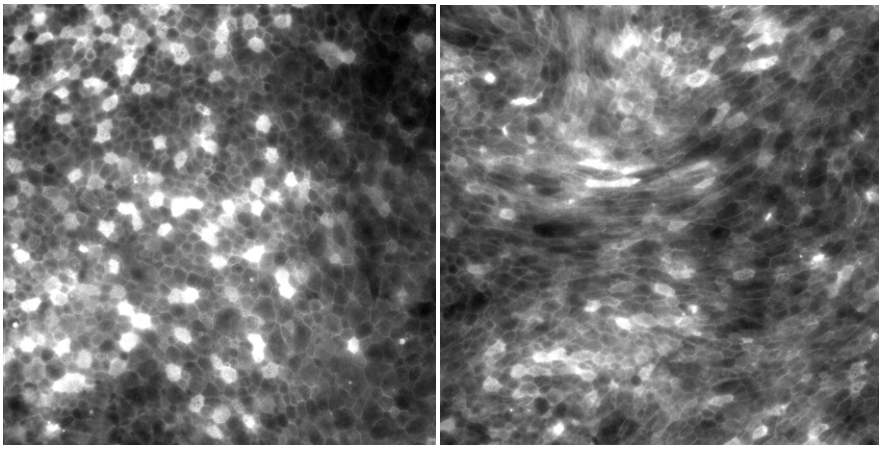
Box 1. Caging and disorder, glassiness and jamming



Appreciable experimental evidence now supports the idea that a confluent cellular collective can exhibit both a jammed, solid-like phase (left panel) and an unjammed, fluid-like phase (right panel) along with characteristic changes in cell shape, and underlying theoretical considerations have become well developed (Angelini et al., 2011; Bi et al., 2015, 2016; Garcia et al., 2015; Henkes et al., 2011; Nnetu et al., 2013, 2012; Park et al., 2015b; Pawlizak et al., 2015; Tambe et al., 2011; Trepat et al., 2009). In the familiar fluid-to-solid transition of liquid water freezing to solid ice, there is a spontaneous molecular ordering from the amorphous disorder that typifies a fluid to the long-range order that typifies a crystalline solid, whereas in the jamming transition of particulate matter no such spontaneous structural ordering occurs; disorder persists in fluid-like and solid-like phases alike, the latter of which is referred to as a glassy solid (Angelini et al., 2011; Garrahan, 2011). Shown are live actin staining of well-differentiated HBECs in control conditions (left, immobile and jammed) and after application of compressive mechanical stress simulating the effects of bronchospasm (right, rapidly migrating, unjammed). Note characteristic differences in cell shape.

locked in its place and trapped in its cage; it will escape its cage and thereby exchange places with an immediate neighbor only rarely. This results in a so-called glassy solid that has been reasoned to correspond to any stable tissue with homeostatic epithelial cell packing (Sadati et al., 2013). But if the energy barrier might somehow be overcome, say by enhanced cellular propulsive forces, or if the energy barrier itself might somehow be diminished – or even abolished altogether – as by the mechanisms described below, then the cell could escape its cage more readily; in a variety of such cellular collectives, the characteristic scales of time and length for such cellular rearrangements have been quantified (Angelini et al., 2011; Garcia et al., 2015; Nnetu et al., 2013; Park et al., 2015b). Such events would facilitate cellular rearrangements among neighbors, and ultimately the collective as a whole could therefore unjam and flow (Bi et al., 2015, 2016; Park et al., 2015b). Such unjamming might be the case in the advancing of a confluent cell layer into unfilled space to heal a wound, embryonic development, morphogenesis or invasion of cancer cells into a healthy tissue (Bazellières et al., 2015; Das et al., 2015; Fischer et al., 2015; Haeger et al., 2014; Serra-Picamal et al., 2012; Zheng et al., 2015). As shown below, cell jamming and unjamming are linked to cell shape and its changes.

Cell shape and the honeycomb conjecture

D'Arcy Thompson first noted the similarity between the structure of the epithelial cellular collective and a soap foam (Thompson, 1917), and this observation led to perhaps the simplest view of epithelial cell shape in a cellular collective, namely, the 'honeycomb conjecture'. Now proven, this conjecture holds that any partition of the plane into cells of equal area must have a net cellular perimeter that is at least that of a regular, hexagonal honeycomb tiling (Hales, 2001). All other configurations that can tile the plane into cells of equal areas – triangles, squares, parallelograms, etc. – necessarily have a greater net perimeter. Furthermore, if cell perimeter engenders some energy cost, then the honeycomb configuration would correspond to optimal packing and minimum cost. For such a configuration, the tiling of the cell plane is regular (i.e. ordered), the ratio of cell perimeter to the square root of cell area yields a non-dimensional index of cell shape that is close to 3.72, and any departures from this value are potentially instructive, an eventuality to which we now turn.

Cell shape and cell proliferation

Some epithelial sheets indeed approximate such a regular honeycomb configuration, such as the mature *Drosophila* imaginal disc. However, ongoing cellular divisions and apoptosis within the cell layer exert a strong disordering influence on cell shape and packing. These events systematically drive cellular arrangements away from honeycomb packing toward a polydisperse packing comprising irregular polygons that span four-sided to nine-sided cells but remains preferentially hexagonal (Farhadifar et al., 2007; Gibson et al., 2006). In that connection, Hertwig's rule holds that cell division tends to orient along the long axis of the interphase cell (Hertwig, 1893), and this propensity has been argued to facilitate both stress relaxation and isotropic growth with no need for cells to transduce local mechanical signals (Wyatt et al., 2015). Nevertheless, the tricellular junctions in *Drosophila* epithelium serve as a polarity cue for geometry and mechanical stress that acts through the dynein-associated protein Mud (Bosveld et al., 2016). Although our emphasis here is on events within the epithelial cell layer plane, it is important to recognize that not all pertinent epithelial events are necessarily restricted to lie within that plane. In the presumptive enveloping layer on the zebrafish embryonic surface, for example, cell divisions that are oriented in-plane versus out-of-plane contribute to variations in epithelial cell shape that are widely distributed and approximate a log-normal distribution

Journal of Cell Science

Journal of Cell Science (2016) 129, 3375-3383 doi:10.1242/jcs.187922

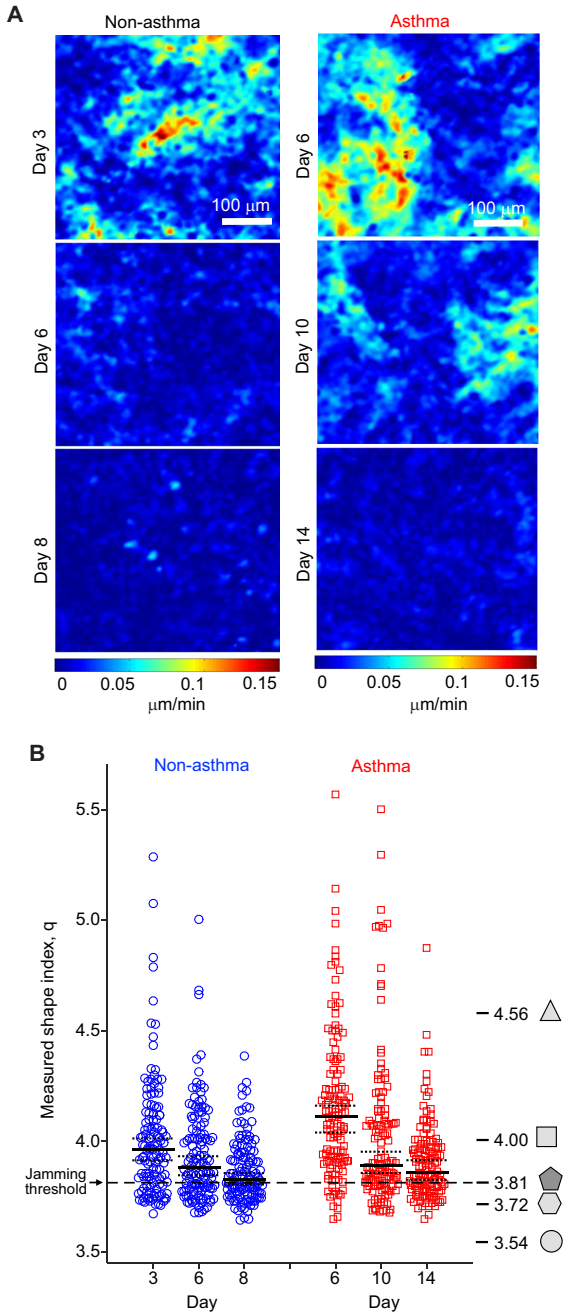
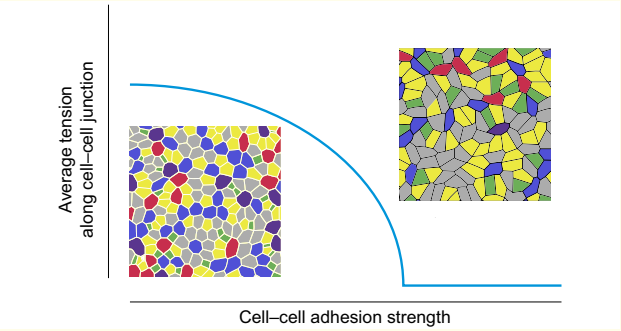


Fig. 1. Jamming in human bronchial epithelial cells. (A) As the HBEC layer matures and differentiates in the air–liquid interface (ALI) culture, speed maps of cellular motions indicate a transition from a fluid-like, mobile unjammed phase (red) toward a solid-like, immobile jammed phase (blue). Both in cells from non-asthmatic and asthmatic donors, the cell layers are mobile in the early days of ALI culture, but become jammed as they are mature. Cells from non-asthmatic donors (left panels) are unjammed on ALI day 3, but become jammed on ALI days 6 and 8, whereas cells from asthmatic donors (right panels) are unjammed on ALI days 6 and 10 and become jammed on ALI day 14. (B) The cell shape parameter (q) measured in snapshot phase images from time-lapse movies shown in A reveals that q approaches 3.81 as cells become jammed. In cells from non-asthmatic donors (blue circles), the median q (denoted by solid horizontal lines) approaches 3.81 on ALI day 8, as the layer jams, whereas in cells from asthmatic donors (red circles), it approaches 3.81 on ALI day 14, also as the layer jams. The dotted lines correspond to the 95% confidence intervals. The numbers on the right side of the graph represent the calculated cell shape parameter, which corresponds to each polygon indicated next to each number. Figures are adapted from Park et al. (2015b) with permission from Nature Publishing Group.

Box 2. Transition between fluid-like and solid-like epithelial phases



The vertex model of a confluent layer suggests that as cell–cell adhesion strength increases toward a critical value, the average cortical tension drops and then vanishes (Brodland, 2002; Farhadifar et al., 2007; Fletcher et al., 2014). Close to the boundary of this transition, small changes in cell–cell adhesion cause disproportionally large changes in cortical tension. These changes are paradoxical in sign but, nevertheless, are predicted by the theory of cell jamming. The figure insets show how changes in cell cortical tension are accompanied by characteristic changes of cell shape. In the left inset, the network is solid-like: when slowly deformed by a small imposed load, it will develop an elastic restoring force in response; when the load is removed it will return to its initial undeformed state. In the right inset, the network is fluid-like: when slowly deformed by a small imposed load it will not develop an elastic restoring force in response; when the load is removed, it remains in its deformed state. Colors denote number of nearest neighbors: green (4), yellow (5), gray (6), blue (7), red (8). The figure is adapted from Kim and Hilgenfeldt (2015) with permission from the Royal Society of Chemistry and from Farhadifar et al. (2007) with permission from Elsevier.

(Xiong et al., 2014). In the airway epithelium, similarly, overcrowding due to proliferation and migration induces Piezo1-dependent extrusion of live cells out of the cell plane (Eisenhoffer et al., 2012). But whether events are restricted to the epithelial cell plane or not, in tissues that undergo proliferation or apoptosis, one finds that polydispersity of cell shapes tends to be the rule and regular honeycomb packing the exception.

Cell shape, cell–cell adhesion and cell sorting

To account for collective cellular migration, as well as transitions between fluid-like and solid-like phases, the cell within the cellular layer has been modeled in a variety of ways, including as a self-propelled particle, a member of a flock and even as a locus on a fixed grid that interacts energetically with neighboring loci – the cellular Potts model (Basan et al., 2013; Garcia et al., 2015; Glazier and Graner, 1993; Graner and Glazier, 1992; Henkes et al., 2011; Vicsek and Zafeiris, 2012). With regard to the question of rearrangements of cells within a tissue, each of these approaches has afforded important insights; however, none of these approaches is equipped to address the physical determinants of cell shape and the relationship of cell shape to cell jamming.

It was first postulated in 1955 that morphogenetic movements, including invagination, evagination and layer spreading, might be attributable in part to differences in cell–cell adhesions (Townes and Holtfreter, 1955). Steinberg's differential adhesion hypothesis was subsequently elaborated to explain cell sorting during morphogenesis (Steinberg, 1970, 2007). To account for associated changes of cell shape, and even cellular rearrangements amongst

Journal of Cell Science (2016) 129, 3375-3383 doi:10.1242/jcs.187922

cell neighbors as a tissue reorganizes without leaving gaps, Sulsky et al. were the first to represent epithelial layer dynamics mathematically using a continuous and complete tiling of the cell layer plane (Sulsky et al., 1984). In that model, each polygonal cell is associated with its mutual neighbor–neighbor adhesion energies, which are expressed in the form of a mutual surface tension. Equilibrium is based upon force balance at each vertex – where cell–cell junctions meet – and these approaches are thus called vertex models (see Box 2). Sulsky et al. were also the first to call for a unified physical description of epithelial mechanics, that is, a theory linking physical forces within the cell layer to the cellular motions that they cause (Sulsky et al., 1984). Ever since then, such a unifying description has been sought but without success, in part because stresses within a cell layer could be postulated but could not be measured (Tambe et al., 2011). Cellular stresses within a contiguous cell layer and their distributions have since become well characterized experimentally, including the distribution of traction stresses exerted by each cell upon its substrate, and the distribution of both normal stresses and tangential stresses exerted by each cell upon its neighbors across their mutual cell–cell junctions (Bazellières et al., 2015; Kim et al., 2013; Tambe et al., 2011; Trepat and Fredberg, 2011; Trepat et al., 2009). Nevertheless, only limited systematic relationships between cellular stresses and cellular motions have thus far been identified. Far from any boundary, each cell in the collective tends to migrate along a trajectory in which shear stresses exerted upon neighboring cells across mutual cell–cell junctions are minimized – a phenomenon called plithotaxis – whereas for cells close to a cell-free void, each cell tends to exert upon its substrate a traction stress that is aligned toward the void – a phenomenon called kenotaxis (Kim et al., 2013; Tambe et al., 2011; Trepat and Fredberg, 2011).

Cell shape, cell jamming and the vertex model

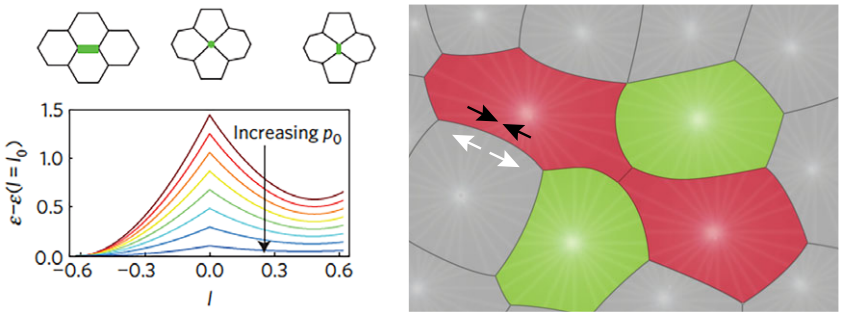
In the vertex model, each cell in the plane is regarded as possessing a preferred value of its cell–cell contact perimeter – a perimeter set point, P_0 – and departures from this set point are imagined to entail a spring-like energy penalty (Brodland, 2002; Farhadifar et al., 2007). Usually, although not universally (Maitre et al., 2012), investigators have found it useful, if not necessary, to separate this perimeter penalty into two competing contributions (Brodland, 2002; Farhadifar et al., 2007; Fletcher et al., 2014). First, contractile forces associated with the cell–cell junction act to decrease perimeter of the cell–cell junction and, second, adhesion between cells at their cell–cell junction acts to increase that perimeter (see Box 3). These effects are of opposite sign and are therefore competitive. The net effects on cell perimeter – junctional contraction versus junctional adhesion – can be expressed as an aggregate line tension that can be either positive or negative; if contraction is dominant, the net line tension is positive, but if adhesion is dominant, the net line tension is negative (Brodland, 2002; Farhadifar et al., 2007).

Each cell is also regarded as having a preferred value of its projected area – an area set point, A_0 – and departures from this set point are also imagined to entail a spring-like energy penalty; this penalty has traditionally been attributed to elastic deformations of the cell body (sometimes called a height elasticity) but is now understood to include changes in active cellular tension which can be sustained without viscoelastic relaxation over long scales of time (Vincent et al., 2015) and can increase with increasing cell density (Zimmermann et al., 2016). It has never previously been recognized, to our knowledge, that adhesion of the cell base to the cell substrate must come into play as well. For each cell within the continuous layer, all these contributions, taken together, yield a total energy that is given simply as:

$$E = K_A(A - A_0)^2 + K_P(P - P_0)^2. \quad (1)$$

Here, K_A and K_P are spring-like coefficients for area and perimeter changes, respectively, and A and P are the cell projected area and perimeter, respectively. In a theoretical analysis of such systems, fluid–solid transitions along with characteristic changes in cell shape have been reported (Farhadifar et al., 2007; Kim and Hilgenfeldt, 2015). However, Bi and colleagues were the first to perform a formal analysis that established the existence of critical behavior and an associated jamming transition between fluid-like and solid-like phases (Bi et al., 2015, 2016; Park et al., 2015b). They also identified three parameters that determine when such a

Box 3. Junctional contraction, adhesion and energy minimization



Contractile energy associated with the cell–cell boundary tends to minimize cell–cell contact perimeter (right panel, black arrows). However, adhesive energy associated with the cell–cell boundary tends to maximize that perimeter (white arrows). The sum of these competing effects is expressed as a net line tension that acts along the cell–cell junction, and can be positive or negative.

Local rearrangements and neighbor swapping among cells occur by a sequence of simpler transitions as shown on the left, so called T1 transitions. The figure is reproduced from Bi et al. (2015) with permission from Nature Publishing Group. The thick green edge represents a cell–cell interface that becomes contracted to a point and then extended in the perpendicular direction. If l is the length of that line during the transition, the energy barrier that must be overcome, ε, for such an event to occur depends on the ratio between cell–cell adhesive stress and cell cortical tension, denoted by p_0.

Journal of Cell Science

Journal of Cell Science (2016) 129, 3375-3383 doi:10.1242/jcs.187922

transition occurs, and depicted those parameters in a unified jamming phase diagram (see Box 4).

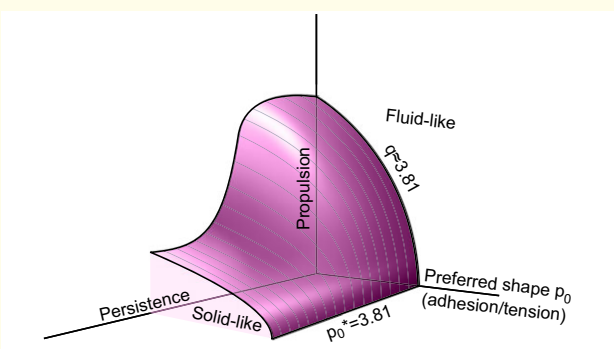
The first such parameter is $p_0=P_0/\sqrt{A_0}$, which is important for three reasons. First, p_0 is dimensionless and can be thought of as a simple index of the preferred cell shape. Second, p_0 is also a material property of the cell because it is set by the ratio between cell–cell adhesive stress and cell cortical tension (Bi et al., 2015; Farhadifar et al., 2007). Third, there is a special, or critical value, of p_0, denoted p_0*, which equals 3.81, at which there is a transition between the fluid-like phase and the solid-like phase of the cellular collective (Bi et al., 2015, 2016; Park et al., 2015b):

$$\begin{aligned} &p_0 > 3.81 \text{ fluid-like} \\ &p_0 = p_0^* \approx 3.81 \text{ critical point, jamming transition} \\ &p_0 < 3.81 \text{ solid-like.} \end{aligned} \tag{2}$$

In any given cell, p_0 might change owing to changes in cell–cell adhesion or cortical tension, or a combination of these two factors and their numerous underlying determinants. Nevertheless, the theory states that the transition is preserved under the simple condition of p_0 approaching the numerical value $p_0^*\approx 3.81$.

The cell shape that is preferred, p_0, compares with – and might differ from – the cell shape that is actually attained, as would be measured from microscopy. The attained cell shape is described by the shape index $q=P/\sqrt{A}$. The theory of Bi and co-workers predicts that whenever $p_0<3.81$, the cell layer becomes solid-like and jammed; each cell therefore is trapped in a shape that differs from its preferred shape, with $q\approx 3.81$. However, if $p_0>3.81$, the cell layer becomes fluid-like and unjammed; each cell is therefore free to assume its preferred shape, and $q\approx p_0$. That is to say, the solid-to-fluid transition occurs when cells 'wet' one another more than they pull on one another, and conversely.

Box 4. The jamming phase diagram



The transition between solid-like jammed phases and fluid-like unjammed phases defines a surface that depends on three parameters: preferred cell shape (p_0), cell propulsion and the persistence of that propulsion. The diagram is adapted from Bi et al. (2016) under the terms of the Creative Commons Attribution 3.0 License.

Preferred shape (p_0): factors that result in an increase in cortical tension cause p_0 to decrease and therefore drive the system toward jamming. Paradoxically, factors that result in increased cell–cell adhesion lead to an increase in p_0, driving the system toward unjamming.

Propulsion: self-propulsion, even when random and uncorrelated, can generate forces that are sufficiently large to surmount energy barriers and cause the jammed layer to unjam. When the layer cells does unjam in that way, it does so when cells attain a measured shape index q=3.81.

Persistence: self-propulsion forces are even more effective in unjamming the layer when they are persistent in time.

The second parameter on the jamming phase diagram concerns cellular propulsion (see Box 4). Propulsive forces are exerted by the cell layer upon its substrate; these propulsive forces fluctuate in space and in time, and are strongly cooperative (Angelini et al., 2011; Tambe et al., 2011; Trepat et al., 2009). To fill that gap in the theory, the so-called self-propelled Voronoi model has been developed and used to predict a motility-driven unjamming transition (Bi et al., 2016). This model postulates that propulsive forces can become sufficiently large to overcome energy barriers and thereby unjam the layer. In addition, this model shows that when motility-driven unjamming does occur, it does so when the measured shape factor q exceeds the same critical value close to 3.81.

Finally, the third parameter concerns persistence of propulsive forces, and how persistence amplifies their effects. Propulsive forces that tend to be directionally persistent have a greater effect than those that are directionally random. In summary, this model describes the jamming transition through three natural parameters – preferred cellular shape (p_0), propulsion and persistence – and organizes these parameters into a jamming phase diagram as shown in Box 4. Heterogeneity within the cell layer can have a variety of sources, is expected to make the predicted transition less sharp and leads to other interesting considerations (see Box 5).

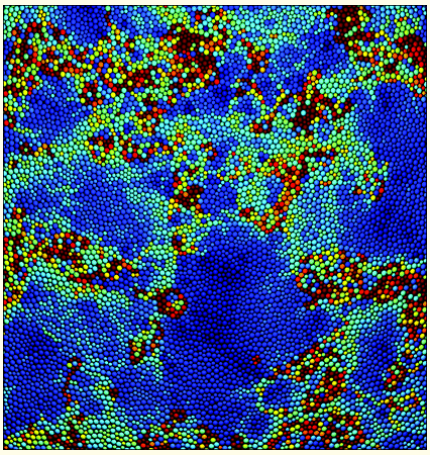
The perspective developed above has much supporting data in the case of the airway epithelium, as described below, but hardly reflects a consensus view. On the one hand, most investigators now concur that the cellular collective tends to evolve toward an amorphous solid-like glassy phase (Angelini et al., 2011; Garcia et al., 2015; Garrahan, 2011; Nnetu et al., 2013, 2012; Pawlizak et al., 2015; Tambe et al., 2011). But on the other hand, the mechanism or mechanisms through which this solid-like glassy phase comes about remains a matter of some dispute. In addition to the mechanism of cell jamming described above, others have argued in favor of contact-mediated inhibition of locomotion (Zimmermann et al., 2016), increased cell packing density (Henkes et al., 2011) or, independent of cell packing density, increased cell–cell frictional stresses as inferred from maturation of adhesive bonds (Garcia et al., 2015). Based upon good supporting experimental evidence, Garcia et al. suggest that within the maturing cellular layer, frictional stresses arise at the levels of cell–cell adhesion and cell–substrate adhesion, and that these frictional stresses couple to velocities in a manner that sets the velocity correlation length. Their theory rests on the notion of the forming and breaking of adhesive bond linkages, but does not incorporate non-frictional stresses or the competition between adhesive forces and cortical tension described above. As such, Garcia et al. provide a perspective into the mechanism underlying cellular jamming that is complementary to the one emphasized in this review, and the dominant mechanisms of collective cellular migration and the impact of cell jamming remain open questions.

The airway epithelium and its pivotal role in asthma

To illustrate these ideas in a specific context, we ask how this jamming framework and the jamming phase diagram impact our understanding of the pathogenesis of asthma. Asthma is associated with epithelial injury repair responses that are aberrant and dysregulated (Lambrecht and Hammad, 2012). These dysregulated processes include signaling pathways, such as those mediated by nuclear factor κB (NF-κB), sonic hedgehog (shh) and Wnt, as well as growth factors, including fibroblast growth factor

Journal of Cell Science (2016) 129, 3375-3383 doi:10.1242/jcs.187922

Box 5. Heterogeneity in jammed cellular systems



In an inert granular system approaching dynamic arrest, slow particles tend to cluster with other slow particles and faster particles with faster ones, thus creating large-scale highly-correlated swirls, streaks and clusters [image, particles are color-coded to depict overlap with their original positions; figure reproduced from the cover of PNAS, September 8, 2009; image courtesy of L. O. Hedges, University of California, Berkeley, CA) (Garrahan, 2011)]. This large scale dynamical heterogeneity emerges spontaneously even when the physical properties of constituent particles are identical and structure remains amorphous and uncorrelated. But just as dynamical heterogeneity can emerge from a system comprising identical inert constituents, so too dynamical heterogeneity can emerge in the living confluent epithelial layer (Angelini et al., 2011; Tambe et al., 2011; Trepat et al., 2009). Within the living cell layer, however, there exists, in addition, the innate biological heterogeneities associated with cell-to-cell variations in cell type, phenotype, size, adhesion, active propulsion, polarization and cell signaling (Garcia et al., 2015; Notbohm et al., 2016; Wilk et al., 2014). These two different sources of heterogeneity – dynamical heterogeneity and cell-to-cell biological heterogeneity – are distinct but are likely to be interactive and interdependent, thereby provoking the following unanswered questions. Does biological heterogeneity blur the transition between phases and perhaps impact upon the dynamics of the jamming transition in other important ways? Conversely, are dynamics of jamming transduced and responded to by cells? What controls cell pack size and why do packs arise at all? More broadly, might cell-to-cell heterogeneity in p_0, and resulting changes in cell shape, stand nevertheless as a structural signature differentiating motile and quiescent phases?

(FGF), epidermal growth factor (EGF) and tumor growth factor (TGF) (Holgate, 2008; Knight et al., 2004). During fetal lung development, all these factors are known to have roles in the activation of the epithelial–mesenchymal trophic unit (EMTU), which comprises opposing layers of epithelial and mesenchymal cells (Evans et al., 1999). In asthma, the EMTU becomes reactivated and is thought to be responsible, at least in part, for progressive deterioration of airway function and structural remodeling of the airway wall, which includes goblet-cell hyperplasia, basement membrane thickening, sub-epithelial angiogenesis, as well as airway smooth muscle hypertrophy and hyperplasia (Durrani et al., 2011; Homer and Elias, 2005; Huber and Koessler, 1922; Lazaar and Panettieri, 2003; Roche et al., 1989).

Dysregulation of these processes has been traditionally attributed to downstream effects of an upstream cascade of immune and inflammatory events, especially type 2 inflammation. For that reason, most current asthma therapies target type 2 inflammation, although recent clinical trials now demonstrate the failure of these therapies in patients who do not have the phenotype of type 2 inflammation, but nevertheless suffer from impaired lung function and frequent asthma exacerbations. To explain these negative findings, Fahy put forward the radical notion that although inflammation might be a major disease modifier in asthma, it might not be the core abnormality (Fahy, 2015). Rather, he suggested that the core abnormality must lie in some structural cell of the lung, such as the airway smooth muscle cell or the airway epithelial cell. In this context, Schuijs et al. demonstrated that chronic exposure to low doses of endotoxin protects against allergic asthma through the induction of the protein A20, which is expressed in airway epithelial cells, thus further supporting a fundamental role of the airway epithelium in asthma pathogenesis (Schuijs et al., 2015). Additionally, a role for the airway epithelium in aberrant airway remodeling has been implicated through the transduction of purely mechanical events; during bronchoconstriction, the airway epithelium becomes compressed and buckled into a rosette pattern. These compressive stresses have been shown to be sufficient to activate a cascade of mechanotransduction events that can drive airway remodeling even in the absence of additional airway inflammation (Grainge et al., 2011; Park et al., 2015a; Tschumperlin et al., 2004).

In the airway epithelial events described above, could cell jamming have been an unappreciated factor? And if so, the question arises as to whether cell jamming behavior differs in some innate or systematic ways between cell layers that are derived from non-asthmatic versus asthmatic donors. To answer this question, Park et al. assessed both the dynamic and structural signatures of the jamming transition in human bronchial epithelial cells (HBECs) that were derived from non-asthmatic and asthmatic donors (Park et al., 2015b). To recapitulate *in vitro* the differentiation and repair processes that occur in the maturing or injured airway epithelium *in vivo*, they used the well-established air–liquid interface (ALI) culture approach to recapitulate well-differentiated pseudostratified HBEC layers as *in vivo* airway epithelium (Dvorak et al., 2011; Whitcutt et al., 1988).

As the HBEC layer matures and differentiates, speed maps of regional cellular motions indicate that there is a transition from a fluid-like, mobile unjammed phase toward a solid-like, immobile jammed phase (Fig. 1A). In cells from non-asthmatic donors, the jamming transition occurs between days 6 and 8 of the ALI culture, whereas in cells from asthmatic donors, this transition is delayed until day 14. Furthermore, when the mature and fully jammed non-asthmatic HBEC layer is mechanically compressed to mimic the effects of bronchoconstriction, as occurs in asthma, the layer becomes unjammed immediately and then rejams only slowly, over the course of 36 to 48 h. Taken together, these findings show, first, that in cells derived from non-asthmatic and asthmatic donors, the jammed phase is associated with the mature and quiescent layer, and with correspondingly intact barrier function (Park et al., 2015b). Second, the unjammed phase is associated with the immature and active layer, as well as with the mature, but mechanically compressed layer, and, third, the transition to jamming is delayed in cells derived from asthmatic donors.

The vertex model predicts that the measured cell shape parameter q must approach $p_0^* \approx 3.81$ as the cell layer approaches jamming. In HBEC layers from non-asthmatic donors, the median q approaches p_0^* on day 8 of the ALI culture, just as the layer jams (Fig. 1B). And in asthmatic donors, it approaches p_0^* on day 14, again just as the layer jams. It is apparent from Fig. 1B that in addition to the

Journal of Cell Science

Journal of Cell Science (2016) 129, 3375-3383 doi:10.1242/jcs.187922

trajectories of the median q towards the jamming transition, there is also a substantial degree of variability in the q of the individual cells. The origin of this variability is unknown, but might reflect cell-to-cell biological variability as well as dynamical heterogeneity (see Box 5). Finally, in the jammed layer that becomes unjammed by compression, q increases substantially in response to compression, just as the layer unjams (Park et al., 2015b). These results therefore quantitatively confirm the prediction of a structural signature of layer jamming and the delay of that jamming onset in asthmatic cells.

Alternative gateways to cellular migration: EMT and UJT

EMT was originally described by Hay (1995), and is now understood to be a process by which the polarized epithelial cell within an epithelial cell layer undergoes a transition to a mesenchymal phenotype that is characterized by enhanced production of the extracellular matrix, resistance to apoptosis, enhanced migratory capacity and enhanced invasiveness. The inverse process, mesenchymal-to-epithelial transition (MET), has also been recognized (Kalluri and Weinberg, 2009). Well-known biological indices of EMT include enhanced activity of transcriptional factors, including those of the TWIST, SNAI and ZEB families, reduction of epithelial cell junction proteins, including E-cadherin, and induction of mesenchymal proteins such as vimentin (Thiery et al., 2009).

It has long been thought that the epithelial cell must acquire, at least in part, a mesenchymal phenotype in order to initiate migration and invasion. As such, EMT has been thought of as being indispensable for carcinoma cell invasion and metastatic disease (Pattabiraman et al., 2016). Data from recent studies now suggest, however, that the EMT is dispensable for carcinoma invasion and metastasis (Fischer et al., 2015; Zheng et al., 2015). In genetically engineered mouse models of pancreatic ductal adenocarcinoma, deficiency of either *Twist1* or *Snai1* does not alter cancer progression or the capacity for local invasion or metastasis to lung and liver (Zheng et al., 2015). Overexpression of miR200, which inhibits the expression of *Zeb1* and *Zeb2* and thereby locks cells into an epithelial phenotype, does not alter metastasis in spontaneous breast-to-lung murine models (Fischer et al., 2015). The results from both studies suggest that EMT enhances chemoresistance but does not affect cellular motility or metastatic potential. Moreover, overexpression of *Twist1* contributes to dissemination of breast cancer cells in a manner that requires the expression of E-cadherin and does not disrupt epithelial layer integrity or requires induction of the EMT (Shamir et al., 2014).

For tumor cells to initiate migratory dynamics and invasion, the EMT seems not to be necessary. This finding was unanticipated but, in retrospect, it is perhaps not so surprising. Starting from a quiescent, polarized, epithelial state, the EMT and the unjamming transition (UJT) are now understood to comprise alternative gateways to cellular migration. However, if morphological and molecular hurdles that are required to accomplish an EMT are high, then those required to accomplish an UJT are far less so. A transition between jammed and unjammed phases can be accomplished with slight but judicious modification of position on the jamming phase diagram (Box 4), for example, while an epithelial phenotype is retained throughout. The extent to which the UJT might be independent of, incidental to, or even necessary for the EMT or partial EMT (Tam and Weinberg, 2013) remain open questions, however.

Open questions

So, what does the concept of cell jamming and unjamming teach us, and what new challenges does it pose? With regard to asthma and the airway epithelium, the time scale required to jam the maturing HBEC layer *in vitro* – days to weeks – coincides roughly with the period of recovery from an asthmatic exacerbation *in vivo* (Jenkins et al., 1981; Petheram et al., 1979). However, a causal and mechanistic role for rejamming in the recovery process, or for unjamming or delayed jamming as precipitating factors in an asthmatic exacerbation, remain matters of speculation, but point nevertheless to open fundamental questions. First, are the jamming dynamics that are evident in HBECs *in vitro* (Park et al., 2015b) similarly present in the native bronchial epithelial layer in animal models or in humans? And if so, do the differences in jamming dynamics between HBECs in asthmatic versus non-asthmatic patients contribute to asthma pathogenesis – potentially comprising a 'core abnormality' in asthma (Fahy, 2015) – or are they merely an effect? However, whether cause or effect, the jammed phase corresponds to a quiescent, stable and homeostatic state of mature healthy epithelium in which the cell layer is solid-like and immobile, whereas the unjammed phase corresponds to a dynamic state of injured epithelium in which the layer is fluid-like and highly mobilized.

With regard to carcinomas, most metastases are now attributed to circulating multicellular tumor cell clusters (CTCs), many of which retain an epithelial phenotype throughout the metastatic process (Aceto et al., 2014; Fischer et al., 2015; Zheng et al., 2015). Cheung and Ewald have suggested, further, that successful transit of the CTC from the primary tumor to the distant metastatic site might require retention of an epithelial phenotype (Cheung and Ewald, 2016). If cells retain an epithelial phenotype throughout all stages of the metastatic process, then the idea of EMT as the sole or principal gateway to cellular migration does not pertain. But if not by EMT, then by what alternative physical process might migration become initiated and sustained? Insofar as cell jamming has been reported in murine cancer models *in vivo* (Haeger et al., 2014), a simple but untested hypothesis cannot be ruled out: (1) at the primary tumor site, migration of a leader cell, together with its followers, through surrounding solid tissues is triggered by the transition of those cells from a solid-like jammed phase to a fluid-like unjammed phase; (2) upon meeting the circulation, forming a CTC and becoming exposed to the destabilizing mechanical effects of blood-borne shear stresses, the CTC stabilizes itself in response by undergoing a transition back to a solid-like jammed phase; and, (3) upon reaching its ultimate metastatic site and unjamming once again (Au et al., 2016), these CTC cells initiate collective cellular invasion into non-cancerous solid tissue. The idea that metastasis requires EMT and its inverse, MET, thus compares with the alternative hypothesis that metastasis requires events that are by comparison substantially less drastic – transitions between solid-like (jammed) and fluid-like (unjammed) phases of the cell layer – during which strong collectivity and an epithelial phenotype is retained throughout. With regard to potential strategies to modulate these processes, an interesting but counterintuitive corollary is that increased cell–cell adhesion promotes unjamming, and vice versa.

In that context, each axis of the jamming phase diagram (see Box 4) is a determinant of the jamming transition, but at the same time is affected by multiple underlying molecular and physical factors, which, in addition, can have effects on the other axes. As a result of such a crosstalk and the associated pleiotropic effects, it is likely that there is no single molecule that could account for cell jamming or unjamming, and any experiment that attempts to vary only one axis while keeping the others fixed is likely to be challenging. Moreover, effects of cell–cell friction and contact inhibition of locomotion in a jamming phase diagram have yet to be

elaborated (Garcia et al., 2015; Zimmermann et al., 2016). Nevertheless, bioinformatics approaches might be helpful in examining the control of cell jamming in epithelial cell layers through the lens of network analysis, hub molecules, master mechano-regulators and key interaction modules.

Conclusions

To the extent that the quiescent epithelial layer is jammed, the unjamming of that layer and the consequent collective migration of its individual constituent cells are logical requirements for initiation of pattern formation, tissue remodeling and wound repair. In these physiological processes, the individual cell of the unjammed collective is guided in a cooperative and social fashion by chemical and mechanical cues from its neighbors. In pathophysiological processes, however, such as aberrant repair of the asthmatic airway wall or tumor invasion and metastasis in cancer, unjamming might be seen as a social failure, i.e. the breakdown of agreements that individual cells adopt when they first assemble into a multicellular collective during organogenesis (Apple, S., 2016, An old idea, revived: Starve cancer to death. In New York Times Magazine). From an evolutionary point of view, Newman has suggested, further, that such agreements must be not only ancient but also deeply rooted in generic physical processes expressed by inert soft matter; these generic physical processes, he argues, became bootstrapped by and harnessed within the very earliest multicellular living soft matter (Newman, 2012). Arguably, one striking and specific example of such a generic physical process is the jamming transition between fluid-like and solid-like phases of the cellular collective (Fredberg, 2014; Garrahan, 2011; Zhou et al., 2013). If cell jamming as a biological mechanism does have early evolutionary roots, then the dynamics of cell signaling and cell jamming might be intertwined so deeply that neither can be fully understood without consideration of the other.

Competing interests

The authors declare no competing or financial interests.

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