New and Notable

Three-Dimensional Forces for Two-Dimensional Motion

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When the cells move over a flat substrate, they generate forces both in and out of the substrate plane. Álvarez-González et al. (1) investigated three-dimensional traction forces during motion of the amoeboid *Dictyostelium* cells. Surprisingly, three-dimensional analysis helps us to understand why the cells move fast or slow, which was impossible to explain by looking in just two dimensions.

In order to move, the cells adhere and apply forces to their environment. These traction forces can be measured from the deformation of substrates with known mechanical properties (2,3). Until recently, traction forces were analyzed mostly in two dimensions. The main reasons are that two dimensions seem a reasonable approximation for the cells moving on a flat substrate, and that to track substrate deformation and extract forces in two dimensions is less technically challenging than in three dimensions. A common trend of the two-dimensional (2D) traction force patterns from various cell types is that the cell pulls on the substrate from the periphery to the center, i.e., backward at the front, and forward at the back. Traction forces are much stronger than what is minimally needed to set the cell in motion. This is because traction forces are balanced not by the viscous drag of the surrounding liquid media or inertial forces, but mostly by equally strong tractions from the opposite side of the cell. Thus, traction forces serve mostly to overcome the cell's own adhesion to the substrate. This sketch, however, does not take into account one detail: the cells are not 2D even if they move over a flat surface. Cellular force-generating machinery is not completely aligned with the substrate; consequently, the cell can pull or push the substrate in different directions. Everything becomes even more complex when the cells migrate within a 3D environment.

With the advance of the tools for 3D traction force analysis (4) it was discovered that even tightly adherent and well-spread cells on a flat substrate generate significant forces in the normal direction to the surface (5,6). In roundish amoeboid cells, normal forces are even more prominent: they are as strong as, or stronger than, forces parallel (tangential) to the substrate (7). Note that unlike tangential forces, normal forces on a flat surface cannot be of a pulling kind only, just as Baron Munchausen could not have pulled himself out of a swamp by his ponytail. Normal pulling forces are balanced by pushing, although pulling and pushing are spatially separated: the cell pushes down the center of its ventral surface to get leverage to pull at the periphery (7).

What are the mechanisms that generate normal and tangential forces? How do these forces affect cell locomotion? These questions are addressed by Álvarez-González et al. (1) in this issue of the *Biophysical Journal*. The authors investigated 3D traction forces and compared migration velocities in wild-type cells of *Dictyostelium discoideum* and eight different mutant strains with selective knockouts of the components of the cytoskeletal machinery. This allowed for partial isolation of the mechanisms behind tangential and normal forces. The authors observed that knockout of myosin II reduced tangential forces without affecting normal forces, while other cytoskeletal perturbations affected tangential forces more significantly than normal forces. In most strains, the tangential and normal pulling forces localized at the same sites, primarily at the front and back of the cell. These locations also coincided with actin foci that are thought to represent substrate adhesion sites in *Dictyostelium*. In contrast, pushing forces did not colocalize with actin foci, suggesting that they do not require adhesion. Colocalization of the two types of pulling forces was abolished by double knockout of myosins I A and IB (motor proteins connecting cytoskeleton to the membrane), accompanied by a reduction of traction force magnitude.

Based on these findings, the authors propose that tangential and normal forces are generated by two distinct, yet interconnected mechanisms: axial contractility and cortical tension. Axial contractility is a classical actin-myosin contraction mechanism responsible for traction in the direction of cell motion. In contrast, cortical tension is a new player in the traction force field. Cell cortex is a thin cytoskeletal layer underlying plasma membrane. The idea that normal forces originate somewhere near the plasma membrane comes from the similarity of the cellular normal force pattern to that of a liquid drop or a lipid vesicle in contact with a flat surface (7). Pulling forces at the perimeter of the vesicle-substrate interface originate from the surface tension of the vesicle. Surface tension is balanced by internal liquid pressure, which generates pushing forces in the interior of the contact zone. Álvarez-González et al. (1) demonstrated that the magnitudes of pressure that *Dictyostelium* strains applied to the substrate at the ventral surface match well with the values of cortical tension measured by an independent method, supporting cortical origin of the normal forces.

What are the roles of tangential and normal forces in cell motion? The authors propose that axial contractility

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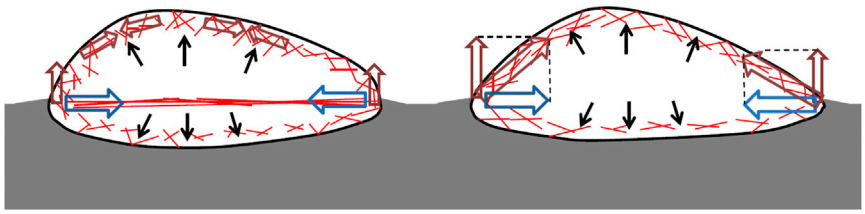

FIGURE 1 Possible force configurations in *Dictyostelium* cells. (*Left*) Axial contractile machinery generates forces parallel to the substrate, while cortex generates normal forces. (*Right*) Cortical contraction occurring obliquely to the substrate generates tangential and normal force components. (*Red lines*) Cortical and axial machinery; (*blue arrows*) forces that are parallel to the substrate; (*maroon arrows*) cortical tension and normal forces; (*black arrows*) cytoplasmic pressure.

helps to generate cell shape changes that are necessary for effective pseudopod formation and retraction at the back, while cortical tension resists these changes. Remarkably, comparison of all mutant strains revealed strong positive correlation between the migration velocity and the ratio of tangential to normal forces, while no correlation was apparent between migration velocity and either tangential or normal forces taken separately. Thus, in order to move efficiently the cell has to overcome not only substrate adhesion, but also its own cortical resistance, which may be one of the reasons for strong forces generated by migrating cells.

How universal are these findings? The authors are quick to point out that the conclusion about cell velocity is limited to amoeboid cells. Indeed, migration efficiency of strongly adherent cells, such as fibroblasts, was so far accounted for by the balance of adhesion and contractility, without excursion in the third dimension. Some of the rapidly migrating cells, e.g., fish epidermal keratocytes, do not change their shape during motion and therefore are unlikely to be slowed down by cortical tension. Another rapidly moving cell type, amoeboid nematode sperm cells, move faster when their membrane tension is elevated; it was proposed that tension aligns protrusive machinery in the direction of migration (8). Nevertheless, the relationship between cortical tension and traction forces is likely widely relevant.

Recently, two studies on strongly adherent cells considered force balance in relation to 3D shape (9,10). The idea that could be taken from these works is that the tangential and normal forces are somewhat artificial categories: tension from the same cytoskeletal element could be split into tangential and normal components, with relative strengths depending on the angle with the substrate. Intriguingly, structural identity of the axial contractile machinery in *Dictyostelium* is not clear: these cells do not display prominent actin fibers spanning the cell length. Is it possible that the same cortical network produces predominantly tangential or normal forces depending on its 3D organization, which, in turn, may be affected by motors and cross-linking proteins? Álvarez-González et al. (1) favor the idea of two distinct machineries linked through myosin I family proteins, but the possibility of single machinery with flexible organization could not be completely excluded (Fig. 1) and is supported by strong spatial and temporal correlation between tangential and normal pulling forces in most of the strains. Correlative force microscopy and high resolution 3D imaging of the cytoskeleton and cell shape may help to distinguish between these hypotheses.

Finally, what are the implications of this study for cell migration in three dimensions? The authors note that normal pushing forces due to cortical tension may be important for 3D migration. The impact of these forces in three dimensions or collective migration could be different from that in migration on the surface. A tight rounded belly that is difficult to deform may be an impediment to crawl, but it may help to open a door, or push others out of the way in a crowd. Migration efficiency may depend in a nontrivial way on the balance of cell deformability and contractility and the porosity and rigidity of the environment. Forthcoming traction force microscopy studies in controlled 3D environments will illuminate the role of cellular geometry and pushing and pulling forces in 3D migration.

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