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PERSPECTIVES: CELL BIOLOGY

Cell Migration—Movin' On

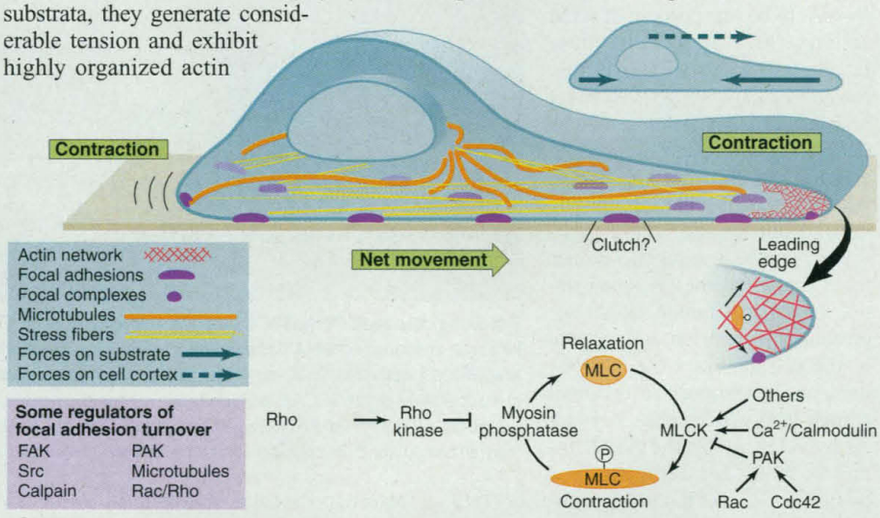
Alan Rick Horwitz and J. Thomas Parsons

Cell migration is crucial for embryonic development, the inflammatory immune response, wound repair, and tumor formation and metastasis (*1*). It begins with an initial protrusion or extension of the plasma membrane at the front (leading edge) of the cell (see the figure). The protrusions are driven by the polymerization of a network of cytoskeletal actin filaments and are stabilized through the formation of adhesive complexes. These adhesive complexes are regions of the plasma membrane where integrin receptors, actin filaments, and associated proteins cluster together. As the cells migrate, the small nascent adhesive complexes (focal complexes) at the front of the cell grow and strengthen into larger, more organized adhesive complexes (focal adhesions) that serve as points of traction over which the body of the cell moves. Finally, release of adhesions at the rear results in a net displacement of the cell. The mechanisms that regulate the formation of focal complexes at the cell's leading edge and the release of focal adhesions at the cell's rear remain unclear. Now, on page 1172 of this issue, Smilenov *et al.* (*2*) demonstrate that focal adhesions are highly motile in stationary fibroblasts yet stationary in migrating fibroblasts, suggesting the existence of a molecular clutch that couples traction and contractile forces. Other recent findings highlight the importance of tension, the actin and myosin filament network, the Rho/Rac family of signaling molecules, and microtubules in cell migration. Coordinating all of these complex processes is the challenge facing cells that are on the move.

Most adherent cells, although spread, are under tension. The mediators of tension are the integrin family of transmembrane adhesion receptors that link components of the extracellular matrix on the outside of the cell with the cell's cytoplasmic actin cytoskeleton (*1*, *3*). It is tension that causes cells to round up when integrin-directed adhesions are perturbed. In fibroblasts, at least, the degree of tension determines the strength of the adhesions and the organization of the actin cytoskeleton. For example, when cells adhere to firm, highly adhesive substrata, they generate considerable tension and exhibit highly organized actin bundles and adhesion complexes. When placed on a more pliable substrate, cells exhibit less organized actin and smaller, weaker focal adhesions. The response of the integrin receptors to the rigidity of the substrate is illustrated in experiments in which fibronectin-coated beads are placed on cells and held in position with a laser beam (laser tweezer) (*4*). The cells sense the strength of constraint imposed by holding the bead in place. They respond with a local, proportional strengthening of cytoskeletal attachments, as revealed by the increased force required to mechanically displace the bead from its cytoskeletal connections.

The distribution of forces over the surface of cells can be visualized by growing them on collagen-coated sheets in which fluorescently tagged beads are embedded (*5*). The contractile forces within the cell cause the substrate to move. Thus, the forces in migrating cells can be visualized by the movement of the fluorescent beads. These studies reveal a radial distribution of force from the cell perimeter toward a region near the cell nucleus (see the figure). In migrating cells, forces near the leading edge are strong and transient, whereas forces at the rear are weaker and more stable. These observations are consistent with cell movement being driven, at least in part, by contractile forces generated from behind the leading edge.

Smilenov and colleagues (*2*) labeled focal adhesions with a green fluorescent protein marker fused to the transmembrane and cytoplasmic domains of an integrin receptor. Because these marker proteins home to focal adhesions, the investigators were able to



Can you feel the force? Cell migration is regulated by a combination of different processes: forces generated by contraction of actin and myosin, G protein signaling, microtubule dynamics, and the turnover of focal adhesions. Migration is initiated by polymerization of an actin network at the cell's leading edge and is maintained by contraction of myosin. The formation of new focal complexes is controlled by the Rac signaling molecule, and their growth is determined by a Rho-dependent process. Contractile forces behind the leading edge drive movement of the cell body. The turnover of adhesive complexes is regulated by the combined activity of microtubules and regulators that reside in these complexes. The forces on the substratum and cell body are shown by solid and dotted arrows, respectively. In motile cells, traction on the substrate results in net forward movement.

CREDIT: K. SUTLIFF

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visualize the movement of focal adhesions in migrating cells. The authors found that in motile cells, the marker molecules—and hence the focal adhesions in which they reside—remain in place as the cell moves over them, confirming that these adhesions serve as points of traction (*1*). The observations in nonmotile cells, however, proved surprising. The adhesion complexes move toward the center of the cell at a rate that varies with the tension. The forces responsible for this tension are generated by contraction of actin-myosin networks, as indicated by inhibition of the movement of the adhesions with an inhibitor of myosin-induced contractility. Thus, adhesions are under tension in both motile and nonmotile cells. Presumably, in migrating cells, tension is transmitted to the substratum through focal adhesions, which impart the net forces that move the cell body forward and mediate retraction of the cell's rear. The movement of focal adhesions in nonmotile cells is intriguing. It suggests the existence of a molecular clutch that regulates traction. When engaged, contractile forces in the front of the cell pull the body of the cell forward; when not engaged, the forces on the adhesions result in their movement toward the cell's center.

How does tension arise, and what regulates it? Tension develops at the front of migrating or spreading cells as new integrin-dependent focal complexes form and stabilize (see the figure) (*6*). The production of protrusions, formation of new adhesions, or stabilization of existing adhesions are regulated by members of the Rho family of small guanosine triphosphatases (GTPases) (*7*). Two members of this family, Cdc42 and Rac, appear to be particularly important in regulating new protrusions and adhesions at the cell periphery. Activation of Cdc42 stimulates the polymerization of actin at the cell front to form long, thin extensions (filopodia), whereas activation of Rac leads to the formation of broader web-like extensions (lamellipodia). The formation of focal adhesions is tightly coupled to the polymerization and reorganization of actin at the leading edge and thus is linked to the activities of Cdc42 and Rac. These signaling molecules also appear to be important in regulating the contractile forces at the leading edge by modulating myosin light chain (MLC) phosphorylation (see the figure). In its active, GTP-bound state, Rac contributes directly to the regulation of cellular contraction, in part through its interactions with p21 activated kinase, PAK, which has been implicated in the control of MLC phosphorylation (*8*). Phosphorylation of MLCs by myosin light chain kinase promotes both their dimerization and their interactions with actin to drive contraction.

The small GTPase Rho regulates the further organization of actin into bundles and the production of the large, highly organized structures termed focal adhesions (see the figure). Rho also promotes tension through its action on MLC phosphorylation. However, in this case, Rho activates Rho kinase, which in turn inhibits the myosin phosphatase, thus maintaining MLCs in a highly phosphorylated (contractile) state. The resulting contractile forces organize the actin filaments and cluster the integrins, leading to tightly bundled actin and large, organized focal adhesions.

Recent experiments in neurons and fibroblasts show that the regulation of Rac and Rho activities is highly interconnected in a reciprocal fashion. Furthermore, these activities are likely to vary spatially within the cell (*9*). For example, inhibition of the Rho pathway with drugs that target Rho kinase causes loss of large focal adhesions, formation of smaller peripheral focal complexes, and protrusive activity. Moreover, introduction of activated Rac into cells causes a reduction in the size and integrity of existing focal adhesions and promotes the formation of focal complexes. A decrease in Rac activity induces the loss of focal complexes and the growth of focal adhesions. Interestingly, the formation of Rac-dependent focal complexes requires contraction of the actin-myosin networks but is not blocked by inhibitors of Rho kinase, indicating that Rac and Rho regulate myosin contractility by different pathways. Thus, a key element in cell migration is the reciprocal regulation of Rac, which appears active at the cell's leading edge where new protrusions and adhesions are forming, and Rho, which appears to generate tension and stabilize adhesions more centrally throughout the cell.

Recently, microtubules have emerged as regulators of focal adhesion and focal complex dynamics (*10, 11*). Depolymerization of microtubules in fibroblasts leads to a decrease in the turnover of focal complexes, which results in reduced cell spreading, large peripheral focal adhesions, increased tension, and reduced protrusive activity. Furthermore, upon restoration of the microtubular network, there is enhanced turnover of focal adhesions along with increased Rac activation and protrusive activity. Upon direct contact by microtubules, adhesions dissociate, and either the cell edge retracts or a new protrusion forms. Thus, microtubules appear to regulate the turnover of adhesions by targeting them directly and delivering relaxing signals to promote their turnover, initiating either protrusion or retraction. The nature of the relaxing signals is unclear, but they are likely to include regulators of the Rho family.

The regulation of focal adhesion and focal complex turnover is critical for the continued remodeling and reorganization of adhesion contacts during cell migration. At the cell rear, adhesions need to release, whereas at the front, the formation and maturation of adhesion complexes must be carefully controlled. Recently, migratory defects have been reported in cells lacking Src family kinases (*12*), focal adhesion kinase (FAK) (*13*), and calpain (*14*), all focal adhesion components. The defects appear to reflect an inhibition of focal adhesion turnover because in each case focal adhesion formation is not impaired. Curiously, cells deficient in Src, FAK, or calpain show inhibited cell spreading and strong focal adhesions that appear predominantly at the cell periphery. One view is that in the absence of remodeling near the leading edge, adhesion complexes continue to organize and strengthen. This prevents cells from forming new focal complexes at the leading edge by inhibiting reorganization or reuse of adhesive components. Evidence for this possibility is supported by two observations. First, when substrate-coated beads are placed on the surface of Src-deficient cells, strengthening of adhesive forces is enhanced relative to cells that express Src, consistent with Src being a negative regulator of adhesive strengthening. Second, reintroduction of FAK in FAK-deficient cells restores the ability of these cells to migrate. However, reintroduction of a mutant form of FAK that cannot transmit signals to Src or to Cas (an activator of the Rac pathway) fails to restore cell migration (*13*). These observations argue that the regulation of adhesion strengthening through remodeling or reorganization is a key element in the regulation of cell migration.

The multicomponent cytoskeleton, which traverses the entire cell, is emerging as a central integrator in migratory and signal transduction pathways. Key elements that regulate migration include the broad and important role of actomyosin-generated tension, the reciprocity of Rac and Rho activities, the role of microtubules as a delivery system for local messages, and the regulation of adhesion complex turnover. Understanding how these elements are integrated, both spatially and temporally, is the next big challenge.

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