Flux at Focal Adhesions: Slippage Clutch, Mechanical Gauge, or Signal Depot

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Flux at Focal Adhesions: Slippage Clutch, Mechanical Gauge, or Signal Depot

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Focal adhesions are plaque-like structures that form contact points between cultured adhesive cells and the underlying substrate (*1*). Their localization at these contacts suggests a number of possible functions, such as acting as "anchors" for maintaining cell shape and position, "feet" for cell migration, and "sensors" for physical communication with the environment. A large, complex inventory of proteins contributes to the performance of these roles (*1*). On the cytoplasmic side of the focal adhesion, actin filaments and associated proteins such as α-actinin maintain the integrity of the structure and transmit force to integrins, which span the membrane to engage the extracellular matrix (ECM). However, most intriguing are the components that link actin directly or indirectly to integrin, which include a growing list of enzymes involved in signal transduction, such as Src kinase and focal adhesion kinase (FAK) (*2*), kinase substrates such as p130Cas (*3*), and proteins such as talin and paxillin that may serve as scaffolds or adaptors (*4*). Although this complicated protein composition may seem consistent with the multitude of functions attributed to focal adhesions, it has long been a challenge to define a supramolecular "machine" that explains these functions. This task is complicated by the long list of potential interactions revealed by biochemical and domain analysis: Many focal adhesion proteins appear to have multiple binding partners, with their affinity for one over another being regulated by phosphorylation or other signals (*4*).

Recent papers by Hu *et al*. (*5*) and Brown *et al*. (*6*) add an important piece of information to this increasingly complex puzzle. Both studies used total internal reflection fluorescence (TIRF) microscopy (*7*) to capture high-resolution high-contrast images of focal adhesions in the lamellar region (just behind the leading edge) of migrating cells expressing various fluorescent focal adhesion proteins. Hu *et al*. used fluorescent speckle microscopy (*8*), which tracks the appearance, disappearance, and movement of individual speckles formed as a result of stochastic clustering of fluorescent probes, whereas Brown *et al*. used the related technique of spatial-temporal image correlation spectroscopy (*9*), which detects the directional drift of any nonhomogeneous distribution of fluorescent probes within a time-lapse sequence. These different approaches nevertheless led to very similar conclusions. Actin filaments underwent vigorous retrograde flux at or near focal adhesions in the lamella, whereas integrins in the same region remained largely stationary. Various focal adhesion proteins showed intermediate degrees of retrograde dynamics. Proteins that associate closely with actin filaments, such as α-actinin, migrated at a rate approaching that of actin filaments, whereas proteins thought to interact more closely with integrins, such as paxillin and FAK, moved at a lower rate and in a more disorganized manner.

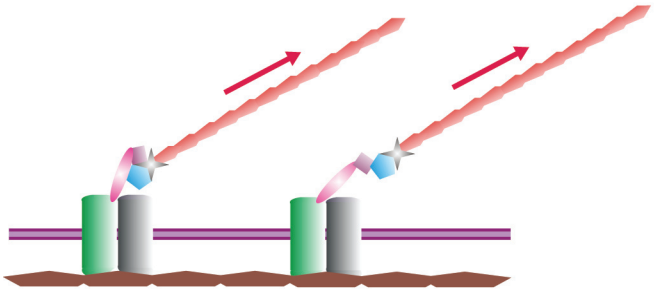

Fig. 1. Differential retrograde movements of focal adhesion proteins as a result of partial unraveling of a protein complex at focal adhesions. The complex serves as a connector between actin filaments (orange fiber) and integrins (double barrels), which bind to the ECM (brown tiles on the bottom). Contractile forces (red arrow) cause the complex to unravel and extend. Components located close to actin filaments (gray star) move for a longer distance than do components located close to integrins (pink oval).

The immediate question elicited by these results is, how do these focal adhesion proteins move? Most likely their movement involves dynamic associations between these proteins and actin filaments, which undergo continuous retrograde movement through a combination of de novo assembly at the leading edge (or at focal adhesions or both) and, as indicated by their sensitivity to inhibitors, myosin II–driven contractions regulated by the Rho-dependent kinase (ROK) (*6*). In this scenario, focal adhesion proteins that spend more time bound to actin filaments would move at a higher rate than those spending more time bound to integrins, which remain anchored to the ECM. Alternatively, their movement may involve partial dissociation and unraveling, as a result of myosin II–generated forces, of a multicomponent complex that connects actin filaments with integrins (Fig. 1). In this scenario, components close to integrins hardly move, whereas components near actin filaments move essentially in sync with actin. In either model, the differential movements of the focal adhesion proteins pose a further challenge to the notion of focal adhesions as a static machine. They instead emphasize the dynamic and potentially amorphous nature of these structures.

A more important question is, does the retrograde movement of focal adhesion proteins serve any useful functions? The movement appears to be related to traction forces: mechanical forces exerted by cells on the surrounding ECM. These forces are transmitted by the anterior focal adhesions in adherent cells, and, as is the case for the flux of focal adhesion proteins, are

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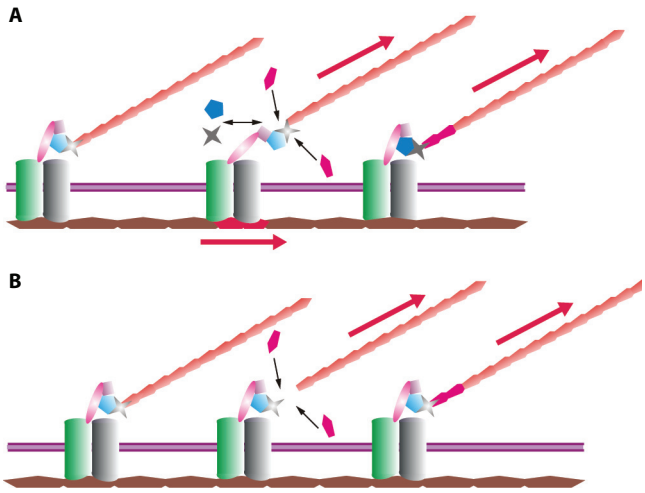

Fig. 2. Focal adhesion as a slippage clutch in its engaged (**A**) or disengaged (**B**) state. When the clutch is engaged (A), proteins in the focal adhesion bind firmly to both actin filaments and integrins (double barrels). The structure stretches as a result of the force of myosin contraction (slanted red arrow), which is transmitted from actin filaments (orange fiber) to the ECM (brown tiles on the bottom) to generate traction forces (horizontal red arrow). Recruitment of new actin subunits and turnover of focal adhesion proteins (black arrows) may allow the structure to return to the initial state and to repeat the cycle. When the clutch is disengaged (B), the protein complex interacts only weakly with actin filaments, causing the filament that undergoes continuous retrograde movement to "slip" at focal adhesions. This uncouples contractile forces from the ECM.

myosin II– and ROK-dependent (*11*). The correlated movement between focal adhesion proteins and actin filaments suggests that these proteins are involved in transmitting the contractile force of myosin II to the outside. Therefore, one possibility, as proposed by Hu *et al*., is that the differential movement of focal adhesion proteins may reflect the action of a "slippage clutch" (a linkage to allow force transmission when engaged but not when disengaged) for cell migration (*5*). In one scheme (Fig. 2), focal adhesion proteins bind the ends of actin filaments pulled continuously by myosin. Strong association causes these proteins to move along with actin, while the association with integrins allows part of the forces to reach the outside. Conversely, loose linkage to actin would cause these proteins to remain associated with integrins while the actin movement "slips" at focal adhesions, preventing the force of myosin contraction from reaching the ECM (Fig. 2). Therefore, the strength or stability of the binding of these proteins to actin filaments may allow the regulation of force transmission.

A second possibility is that the retrograde movement reflects the operation of a mechanical gauge for physical signals, such as mechanical forces and rigidity, carried by the ECM. Based on the observation that the retrograde movement of focal adhesion proteins increases with increasing fibronectin concentration, Brown *et al*. proposed that enhanced adhesion to the ECM promotes tighter coupling between focal adhesion proteins and actin filaments (*6*). Assuming that these proteins remain physically connected with integrins, increased coupling with moving actin filaments would increase the tension within focal adhesions, which could in turn promote downstream responses such as the stabilization of focal adhesions and assembly of large actin filament bundles, as well as the release of other signals (Fig. 3).

A third possibility is that protein transport may reflect the release of molecular signals from focal adhesions. Although it is well recognized that focal adhesions mediate profound downstream effects in response to anchorage to the ECM, exactly how these signals are released remains unclear. It is possible that some of the focal adhesion proteins serve as second messengers and that their retrograde movements along actin filaments reflect the directional transport of signals. This idea appears consistent with the release of zyxin from focal adhesions in response to mechanical stimulation (*12*, *13*). Its subsequent entry into the nucleus of smooth muscle cells is believed to play a role in gene regulation in response to stretching forces (*13*).

Clearly, much remains to be learned before one can fully understand the mechanism and function of the differential retrograde movements of focal adhesion proteins. To address the possibilities raised above, one must examine focal adhesion protein movement systematically relative to cell migration, ECM binding, and mechanical stimulation. In addition, focal adhesions are known to exist in different states of assembly, maturation, and disassembly, with distinct patterns of protein composition and phosphorylation (*14*). Furthermore, the general phenomenon of retrograde transport of actin encompasses the treadmilling of actin filaments in the lamellipodia (*15*), the retrograde transport of actin arcs in the lamella (*10*), and filament

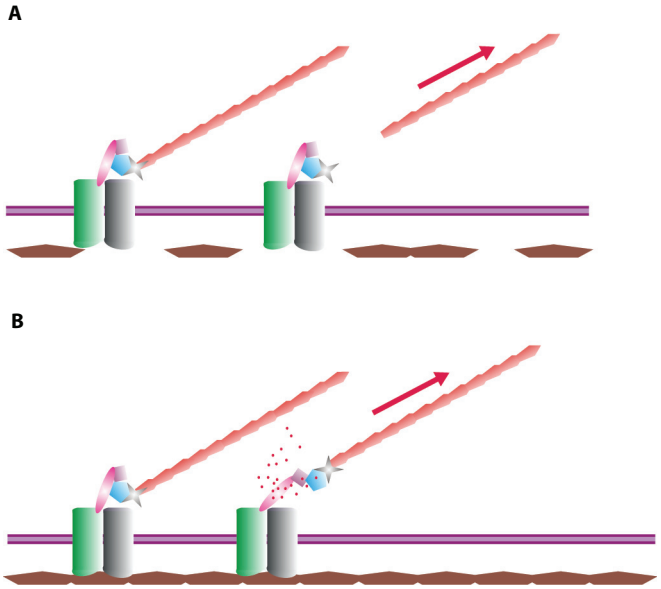

Fig. 3. Focal adhesion as a mechanical gauge in its deactivated (**A**) or activated (**B**) state. In the absence of physical signals from the ECM [(A), brown tiles on the bottom], proteins in the focal adhesion bind loosely to actin filaments (orange fiber) and remain associated with integrins (double barrels). With firm anchorage to the ECM (B), binding of focal adhesion proteins to actin filaments strengthens, which causes tension to build up within focal adhesions and activation of the complex, represented by the release of signals (small red dots).

assembly at focal adhesions in conjunction with bundling to form stress fibers (*16*). Future research should carefully distinguish among these focal adhesion states and transport processes, to determine both the requisite conditions and the consequences of retrograde movements of focal adhesion proteins. Additional insights may be gained by taking advantage of cells deficient in structural or signaling components at focal adhesions and using approaches that manipulate cell shape, migration, or mechanical responses. In addition, prominent focal adhesions are found primarily in cultured cells on conventional culture substrates such as plastic or glass surfaces. The extent of and setting in which retrograde transport of focal adhesion proteins takes place under more physiological conditions, such as within three-dimensional matrices or on flexible materials, may shed additional light on its function.

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