BIOPHYSICS

Clutch Dynamics

A mechanical model describes how actin cytoskeletal dynamics and cell adhesion control mechanosensing and force generation.

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Growth cone filopodia are long, rodlike protrusions that extend from the leading cell edge of a migrating neuron and scan the local environment to sense and decode signals for growth cone advance (*1*). This exploratory function involves the transmission of both chemical cues and mechanical stimuli at filopodial tips. However, our knowledge of how filopodia sense and probe external mechanical cues is extremely limited. On page 1687 of this issue, Chan and Odde show that growth cone filopodia function as mechanosensitive organelles and propose a model in which the cell's internal architecture—the actin cytoskeleton—is harnessed for mechanosensing at sites where the growth cone is adherent to the extracellular matrix (*2*).

The dynamic filamentous actin (F-actin) cytoskeleton is crucial for cell shape change and directed cell migration. A striking feature of the F-actin cytoskeleton in adherent cells is that actin filaments move from the cell periphery toward the cell center in a process called "retrograde flow." Retrograde flow is ubiquitous and has been observed across many cell types, including fibroblasts (*3*), neurons (*4*), and epithelial cells (*5*). Near the cell's leading edge, rapid retrograde flow is driven by forces generated by the polymerization of F-actin against the cell membrane. Closer to the cell center, tensile forces generated by motor proteins (myosin II) drive retrograde flow. Although filopodia are composed of long, parallel actin bundles that protrude beyond the cell's leading edge, the base of each filopodial actin bundle is embedded within the myosin II–containing actin network. Consequently, retrograde flow in filopodia is at least partially myosin II–dependent (*6*).

The role and utility of F-actin retrograde flow in regulating cell adhesion and migration has been an outstanding question for several decades. A leading hypothesis is that the assembly of transmembrane proteins into complexes could create points of adhesion between the F-actin cytoskeleton and the immobile extracellular matrix to modulate its retrograde motion; thus, these cell-substrate adhesions may act as a "molecular clutch." Immobilization of the F-actin network by the clutch to the external matrix would allow new F-actin assembly to push forward the cell membrane and thus enable cell protrusion. Indeed, an inverse correlation between cellular protrusion and F-actin retrograde flow has been observed in fast-moving cells (*7*). This immobilization of F-actin also would be a means to efficiently transmit mechanical forces generated by the actin cytoskeleton to the extracellular matrix (*8*). Such cellular "traction forces" are essential in cell migration and remodeling of extracellular matrix. However, F-actin retrograde flow is not completely abrogated in adhesion sites (*9*, *10*), and changes in retrograde flow speed regulate cellular traction force on the extracellular matrix (*11*). Thus, it is clear that the dynamics of adhesion proteins are a necessary feature of any motor-clutch model (*12*).

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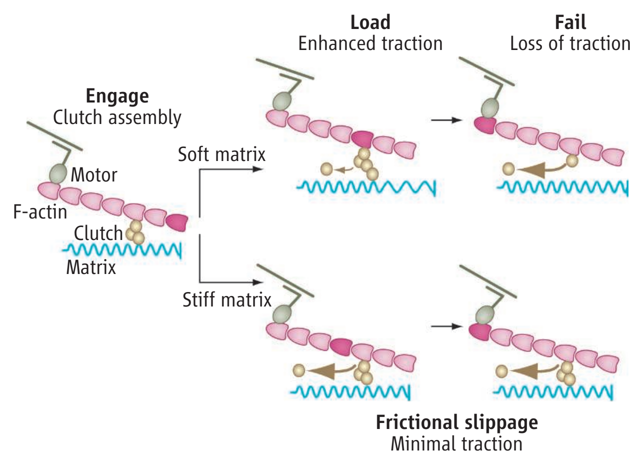

Harnessing actin for mechanosensing. Intracellular filamentous actin (F-actin) interacts with the extracellular matrix through a molecular assembly, or clutch. As the motor protein (anchored to an undefined intracellular substrate) generates tension on F-actin, tension builds in the bonds of the clutch and the extracellular matrix. On a soft matrix, the slow rate of tension buildup within clutch bonds maintains a low rate of bond dissociation (thin, brown arrow) and enhanced traction force on the extracellular matrix (stretching of the wavy line). Beyond a critical tension, the clutch bonds can no longer support the growth in tension and disengage from the extracellular matrix, resulting in loss of traction force and flux of F-actin (indicated by progression of the dark-colored chevron to the left). On a stiff matrix, rapid tension buildup in clutch bonds results in enhanced dissociation of adhesive bonds (thick, brown arrow), minimal traction force, and movement (slippage) of the F-actin at all times.

Chan and Odde have proposed a model of a dynamic motor clutch and find, surprisingly, that the clutch is also a mechanism by which cells can sense changes in the rigidity of their extracellular matrix. In their model, myosin II drives actin retrograde flow in a load-dependent manner, and the formation of clutch bonds that engage the F-actin to the extracellular matrix occurs at a characteristic rate. As tension builds within the extracellular matrix, tension also builds within bonds of the clutch, and bond dissociation increases exponentially until there is a failure of the clutch to engage the extracellular matrix with intracellular actin. The authors find that for soft matrices, the rate of tension buildup in the clutch is slow enough that a sufficient number of bonds remain engaged at early times of cell adhesion. At a critical time, the failure of a single bond results in catastrophic failure of all bonds. Thus, this regime is characterized by periods of adhesion formation and traction force buildup in the extracellular matrix that alternate with rapid adhesion failure and traction release (see the figure). On stiffer matrices, tension develops quickly within individual clutch bonds, and thus the failure of bonds limits the amount of traction force transmitted to the extracellular matrix at all times—a regime the authors call "frictional slippage."

Chan and Odde observed these distinct phenotypes in filopodia of neurons plated on matrices of two different stiffnesses. On the softer matrix, traction forces increase over several seconds before they rapidly fail; enhanced traction force occurs concomitantly with decreased retrograde F-actin flow speed. On the stiffer matrix, a consistently faster retrograde flow speed and lower traction forces are measured at all times. Interestingly, Chan and Odde found that the critical stiffness (the degree of matrix compliance predicted to lead to failure of all clutch bonds) sensed by filopodia was of the same order as measured in brain tissue (*13*). Previous studies have

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shown that branching morphogenesis of neuronal cells is only observed when cells are cultured on soft matrices (*13*); the present work suggests that the mechanosensing function of filopodia, with preferred adhesion to soft matrices, could guide branching morphogenesis at the cellular level. This has implications for the development of neuronal tissue in vivo.

Perhaps the most striking feature of this study is the simplicity of the motor clutch. A filopodial adhesion is modeled as a single, linear spring and the molecular details of the interface where bonds form, fail, and slip are not specified. In a filopodial adhesion, the "weakest link" of cell-substrate adhesion may occur at the level of transmembrane protein binding to the extracellular matrix. For example, integrin cell adhesion molecules translocate in filopodial tips (*14*). By contrast, adhesions that form proximal to the leading cell edge, called focal adhesions, undergo tension-dependent modification to their composition and size. Intracellular "shear slippage" has been observed at the interface of focal adhesion proteins (talin and vinculin) and the actin network (*9*, *10*). This suggests that changes in the composition of focal adhesions during their assembly and growth may regulate the interface of the weakest link in this molecular clutch. Indeed, variation in the flux of the protein zyxin at focal adhesions has been observed in response to externally applied mechanical perturbations (*15*). Unexpectedly, focal adhesions generate higher traction forces and strengthen in response to stiff matrices (*16*–*18*), whereas this study finds that filopodial adhesions generate higher traction on soft matrices. Further work is required to distinguish the roles of focal adhesions and filopodial adhesions in translating mechanical stimuli to the rest of the cell.

The work of Chan and Odde is an important step in building a quantitative framework to describe how actin cytoskeletal dynamics and focal adhesions can control cellular mechanosensing and force generation. This kind of mechanical model may be able to describe a range of cellular behaviors, as suggested by a similar model describing the saltatory motion of the pathogen *Listeria monocytogenes* (*19*). Thus, understanding the general mechanisms by which tension in actin cytoskeletal networks is built and released, at mesoscopic length scales, will elucidate a biophysical understanding of mechanosensing, force generation, shape change, and migration at the cellular level.

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BIOCHEMISTRY

Pressing Levers or Pulling Strings?

Linda A. Amos

The unique stalk of the molecular motor protein dynein may serve as a smart tether rather than as a lever during motion along microtubules.

Dynein is a molecular motor protein responsible for the movement of organelles, vesicles, and macromolecular complexes, for several steps in mitosis, and for flagellar and ciliary beating. It moves toward the minus ends of microtubules, the filamentous track used for long-distance transport in eukaryotic cytoplasm. On page 1691 of this issue, Vale and co-workers (*1*) report a crystal structure showing the stalk that protrudes from dynein's motor domain and interacts with a microtubule. The authors suggest how relative sliding between the antiparallel strands of the coiled-coil part of the stalk may change the structure of the globular domain at the tip of the stalk, enabling it to switch between strong and weak microtubule binding. They also elucidate the role of the stalk in determining the direction of movement along the microtubule and suggest a radical new model of the motility mechanism, in which the stalk is a smart tether rather than a lever for mechanical amplification.

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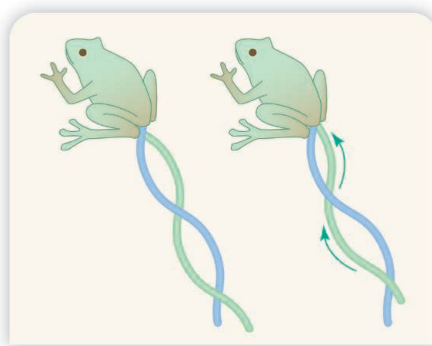

Interstrand sliding (*4*). A MTBD with a distinct polarity (shown as a frog) and an antiparallel coiled-coil stalk in two conformations; the blue and green strands are shown sliding over each other.

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Dynein is a molecular machine belonging to the family of ring-shaped adenosine triphosphatases (ATPases) associated with various cellular activities (referred to as AAA+). Most AAA+ rings are assembled from six identical subunits, but a dynein monomer contains six different AAA+ domains in tandem. Only four of these domains bind ATP; the other two seem to play a purely structural role (*2*). The heavy chain also forms a tail domain and a linker before the first AAA+ domain, and a C-terminal domain after the sixth AAA+ domain. Dyneins differ from other molecular motors such as kinesins and myosins in that the domain responsible for binding to the filamentous protein track—the microtubule-binding domain (MTBD) (*3*)—is distant from the ATPase domains, being situated at the end of the stalk, a 15-nm coiled-coil rod that folds up from an insertion between the fourth and fifth AAA+ domains (see the figures).

In earlier studies (*4*) aimed at understanding the means of communication between the

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