AKT1 when external potassium is low and the membrane potential is negative to −80 mV but is not hyperpolarized enough to drive K^+ into the cells. In fact, the voltage-dependent gating of the K^+ uptake channel of guard cells is also modulated by calcium, although K^+ movement is in the opposite direction.

Given the large number of possible pairs of CBL/CIPK proteins, many targets could be regulated to coordinate ion homeostasis and metabolism. In addition, it is possible that the same target, such as AKT1, is phosphorylated at different sites to independently affect voltage-dependent gating, conductance, and kinetics. Interestingly, recent studies have established that a PP2C-type phosphatase negatively regulates the Shaker-like channel AKT2, which is related to AKT1 (Cherel et al., 2002). Because PP2C-type phosphatases directly interact with CIPK-type kinases (reviewed in Batistic and Kudla, 2004), this suggests that preassembled PP2C-phosphatase/CIPK-kinase complexes could be the "on/off" switch for plant Shaker-like K+ channels. Moreover, in other plant cells, such as guard cells, more than one K^+ channel subunit is expressed (Szyroki et al., 2001). Depending on the cell type and the physiological conditions, CBL/CIPK regulation could turn on different sets of channel subunits and thereby adjust the properties of the subunit complex to meet the demands of diverse situations.

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Integrating Adhesion, Protrusion, and Contraction during Cell Migration

Martin A. Schwartz[1,*] and Alan Rick Horwitz[2]
[1]Departments of Microbiology and Biomedical Engineering and Robert M. Berne Cardiovascular Research Center
[2]Department of Cell Biology
University of Virginia School of Medicine, Charlottesville, VA 22908, USA
*Contact: maschwartz@virginia.edu
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Cell migration is fastest when the strength of the adhesion between the cell and the substrate is neither too strong nor too weak. In this issue of *Cell*, Gupton and Waterman-Storer (2006) reveal how adhesion and cytoskeletal dynamics are integrated to optimize migration speed.

Adhesion and protrusion are central features of cell migration (Lauffenburger and Horwitz, 1996). Although they were once thought to be largely independent processes, it is becoming increasingly clear that adhesion and protrusion are highly interrelated. Protrusion results primarily from actin polymerization at the leading edge of migrating cells, which is regulated by the Rho family GTPases Rac and Cdc42 through multiple effector pathways (Hall, 2005), whereas adhesion was predominantly thought to provide traction for force generation. In many cell types, migration speed has a biphasic response to the concen-

tration of adhesive ligand—meaning that the migration speed peaks at an intermediate concentration of ligand and is slower at both lower and higher ligand concentrations (Lauffenburger and Horwitz, 1996). This response has been shown to reflect differences, at least in part, in the strength of adhesion, as perturbations in receptor affinity or number changed the optimal concentration for migration with predictability (Palecek et al., 1997). This biphasic dependence of migration on adhesivity was also predicted by a physical model, which focused on spatial variation in adhesive strength and a balance of contractile force and adhesion (Lauffenburger and Horwitz, 1996). This model reinforced the notion that traction force is low under conditions of weak adhesion, whereas under conditions of high adhesion the release of adhesions is inhibited.

It is becoming clear, however, that the story is more complex. Rac and Cdc42 are activated by adhesion and also show a biphasic dependence on the concentration of the extracellular matrix protein fibronectin that parallels migration speed (Cox et al., 2001). Rho activity, by contrast, steadily increases with fibronectin concentration. Adhesion is also coupled to protrusion in other ways. In addition to preventing retraction at the rear of the cell, adhesion also increases the probability that the new protrusion will become stabilized. Furthermore, the molecular linkage between adhesion and actin inhibits retrograde flow and thus regulates the rate of protrusion by counterbalancing the forward movement of actin polymerization (Mitchison and Kirschner, 1988; Jay, 2000). The efficiency of this linkage would act like a molecular clutch by regulating the degree of coupling between actin and the immobile extracellular substratum and would thus serve to control rates of protrusion. Finally, adhesions in protrusions disassemble and release components to the leading edge (Nayal et al., 2006).

An emerging theme is that myosin II-mediated contraction couples adhesion, protrusion, and actin organization. Numerous reports have indicated that contraction is a key regulator of adhesion maturation and actin organization. Both of these processes are regulated in part by Rho through its effects on myosin II activity (Geiger and Bershadsky, 2001, Burridge and Chrzanowska-Wodnicka, 1996). Again, feedback loops are implicated: Adhesion activates Rho (Ren et al., 1999), leading to increased tension, which promotes further growth of adhesions. Contractility is also implicated in the disassembly of adhesions (Lauffenburger and Horwitz, 1996). The efficiency of the linkage between actin and adhesions not only regulates the rates of protrusion by controlling the balance between actin polymerization and retrograde flow but can also regulate the tension sensed by adhesions (Jay, 2000).

In this issue of *Cell*, Gupton and Waterman-Storer (2006) bring a new set of tools to bear upon the biphasic relationship between adhesion and migration speed. The paper describes in exquisite detail the cytoskeletal dynamics in PtK_1 epithelial cells migrating on low, medium, and high concentrations of fibronectin. Using new quantitative methods, they analyzed rates of actin polymerization and depolymerization and the flow of the actin network. They also quantified myosin distribution and the number, distribution, and dynamics of adhesions. What emerges from these measurements is that cells on low concentrations of fibronectin have relatively few but highly dynamic adhesions and less visibly organized patterns of actin polymerization and flow. In contrast, cells on high concentrations of fibronectin have more numerous but less dynamic adhesions that are associated with less dynamic actin. Cells on medium concentrations of fibronectin show the highest migration rates, have moderate numbers of adhesions with intermediate lifetimes, and have well-organized, dynamic patterns of actin flow. Importantly, myosin filaments in the cell periphery that drive movement of the actin networks are most pronounced at the optimal coating of fibronectin.

These observations prompted Gupton and Waterman-Storer (2006) to test the role of myosin. They found that on high fibronectin, increasing myosin phosphorylation by inhibiting phosphatases accelerated cell movement, whereas inhibiting myosin decreased migration speed. By contrast, on low fibronectin, phosphatase inhibition slowed migration, whereas inhibiting myosin increased migration speed. On intermediate fibronectin, both treatments were inhibitory, indicating that conditions are already optimal.

Gupton and Waterman-Storer (2006) interpret these data in terms of a beautifully simple mechanical model. The model draws on previous work showing that rates of both adhesion assembly and disassembly are dependent on myosin-generated tension. Their key observation is that myosin phosphorylation increased only slightly from low to high fibronectin, whereas adhesion number and total area increased more dramatically. Thus, force per focal adhesion should be highest on low fibronectin and should decrease as fibronectin increases. They propose that on low fibronectin, the high force/adhesion ratio mediates the high rates of both adhesion assembly and disassembly; on high fibronectin, lower force/adhesion ratios lead to slower assembly and turnover. These adhesion dynamics consequently mediate effects on actin dynamics and cell migration. The differential effects of myosin activation versus inhibition provide elegant support for the model.

The authors recognized that integrin signaling and regulatory pathways such as Rho family GTPases are likely also involved. Though not discussed, their data may fit well with available information on the activation of Rac, Rho, and Cdc42 by adhesion. All of these results can be reconciled by postulating that Rac activation is proportional to the total rate of new integrin ligation (binding to fibronectin)—that is,

proportional to the number of small, dynamic adhesions within the cell. The data from Gupton and Waterman-Storer (2006) suggest that low fibronectin is suboptimal for migration because there are too few adhesions even though the adhesions are dynamic. High fibronectin is suboptimal because the adhesions are too stable and do not signal. Cells on intermediate fibronectin have a high number of small, dynamic adhesions of the kind that are known to signal to Rac and Cdc42. Thus, the overall rates of both integrin ligation and adhesion assembly should be maximal, leading to high Rac and Cdc42 activity. These simple dynamics may be the key factor driving lamellipodial actin polymerization and flow.

The differential effects on Rho activity can also be understood if we consider integrin activation of Rho, which is temporally biphasic (Ren et al., 1999). Following new integrin ligation, Rho activity first decreases, followed by an increase at later times. Thus, young adhesions appear to inhibit Rho, whereas more mature adhesions activate it. As adhesion lifetimes increase in direct proportion to the concentration of fibronectin, these results may explain why Rho activity correlates with fibronectin.

This study has revealed some major insights and represents a large step in our understanding of cell migration. However, there are more chapters to be written. The actin-integrin linkage is a critical site that integrates adhesion, signaling, and protrusion; its regulation remains to be parsed. The "clutch" mechanisms by which myosin II-mediated tension regulates both adhesion assembly and disassembly may depend on the state of the adhesion, which remains to be defined. Finally, some highly motile cells do not show the highly organized adhesions and actin filaments seen in fibroblasts or epithelial cells migrating on fibronectin yet can show optima in migration speed that are dependent on substrate concentration. The factors that produce this relationship in these cells remain to be established.

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Promoter Cleavage: A TopoIIβ and PARP-1 Collaboration

John T. Lis[1,*] and W. Lee Kraus[1,*]

[1]Department of Molecular Biology & Genetics, Biotechnology Building, Cornell University, Ithaca, NY 14853, USA

*Contact: jtl10@cornell.edu (J.T.L.); wlk5@cornell.edu (W.L.K.)

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Hormones trigger dramatic changes in the structure and transcriptional activity of specific promoters that lead to exchange of repression complexes for activation complexes. Ju et al. (2006) now show that estrogen-dependent restructuring and transcription of the *pS2* promoter require the generation of a DNA double-strand break by a novel protein complex containing two enzymes, topoisomerase IIβ and poly(ADP-ribose) polymerase.

Higher eukaryotes invest considerable capital in carefully regulating thousands of genes. These genes are not only expressed at the correct time, place, and level, but many of these genes must respond rapidly and specifically to multiple developmental, nutritional, and environmental signals. To this end, sophisticated mechanisms of transcriptional regulation have evolved. One major class of factors that provides the selectivity of a gene's transcriptional regulation is sequence-specific DNA binding transcription factors. These in