served cytokinin signal transduction pathway influences cell cycle, leaf senescence, shoot initiation, and leaf patterning in different cell types at various developmental stages.

The completion of the *Arabidopsis* genome sequence has revealed 54 genes encoding putative AHKs, AHPs, ARRs, and related proteins, suggesting a substantial involvement of this signaling mechanism in many facets of plant cell regulation (*17*, *18*, *32*). The development of the *Arabidopsis* protoplast system has enabled a high-throughput functional genomic analysis of the two-component regulators (*6*). Because pronounced redundancy in the *Arabidopsis* genome is evident (*18*, *32*), cellular analyses of the two-component elements would complement the characterization of a large number of insertion mutants that may not display overt phenotypes. Genetic, genomic, and biochemical experiments will elucidate the details in cytokinin perception, protein-protein interactions, and target gene expression essential in cytokinin signaling.

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VIEWPOINT

Integrin Connections Map: To Infinity and Beyond

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Integrins are transmembrane proteins that serve as primary sensors of the extracellular matrix (ECM) environment. In response to interactions with the ECM, integrins initiate signaling pathways that regulate cell migration, growth, and survival. Advances in imaging have contributed to the understanding of the dynamic nature of these cell-ECM interactions and the complexes that form at these sites and have provided insights into their regulation and signal organizing functions.

Integrins are primary sensors of the extracellular matrix (ECM) environment and are thus essential for cell migration, growth, and survival. As transmembrane receptors, integrins recognize and bind to specific ECM ligands and transduce signals leading to the activation of intracellular signaling pathways and the assembly of actin-based adhesion structures that propagate cellular forces. Integrin research (described in more than 20,000 literature citations) has enumerated a large number of pathways and proteins thought to be important in integrin function. The Integrin Signaling Pathway (http://stke.sciencemag.org/cgi/cm/CMP_6880) in the STKE Connections Maps highlights the current state of knowledge of integrin function in nonlymphocytic cells and identifies key (but not all) pathways linked to these receptors (*1*).

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Integrins play a central role in organizing the actin cytoskeleton at sites of adhesion to the extracellular matrix (*2*). There has been a growing appreciation of the molecular heterogeneity and dynamic nature of integrin adhesion complexes (*3*, *4*). Although cells form functionally distinct adhesion complexes (for example, focal complexes, which are specific structures localized to the leading edge of migrating cells, and focal adhesions, which are beneath the cell body), a common feature of all adhesion complexes is their linkage to the actin cytoskeleton. Binding of proteins such as α-actinin and talin to integrin cytoplasmic tails, and the subsequent recruitment of the actin-binding protein vinculin and modulators of actin dynamics [such as vasodilator-stimulated phosphoprotein (VASP)], are important steps in linking adhesion complexes to the actin cytoskeleton (Fig. 1, orange). Integrins also regulate signaling pathways to members of the Rho family of small guanosine triphosphatases (GTPases) Cdc42, Rac, and Rho, which are molecular switches that control the dynamics and structure of actin-based processes, such as filopodia, lamellipodia, and stress fiber formation (Fig. 1, orange). Activation of Cdc42 and Rac contributes to the organization of actin networks at the leading edge of migrating cells. Activation of Rho is important for the organization of stress fibers and the regulation of acto-myosin contractility through myosin light chain kinase (MLCK) phosphorylation of myosin regulatory light chains (RLCs). Integrins also contribute to the dynamic turnover and remodeling of adhesion complexes by activating the focal adhesion kinase (FAK) and Src protein tyrosine kinase axis of signaling proteins, which includes the cytoskeletal regulator paxillin (Fig. 1, green). Inhibition or loss of components in this pathway (for example, FAK-null or paxillin-null fibroblasts) severely restricts adhesion complex turnover and inhibits integrin-dependent functions, such as cell migration (Fig. 1, red).

Integrin activation of members of the Ras family of GTPases (Ras, R-Ras, and Rap-1) appears to be important for the downstreamFn1 activation of serine-threonine kinases, such as extracellular signal-regulated kinase (ERK), p21-activated kinase (PAK), and c-Jun NH_2-terminal kinase (JNK), key regulators of gene expression and cell cycle progression (Fig. 1, purple). In addition, both ERK and PAK phosphor-

ylate MLCK, thus contributing to the regulation of acto-myosin contractility. The localization of ERK and PAK to adhesion complexes suggests that these kinases may directly phosphorylate components of adhesion complexes, possibly contributing to adhesion dynamics, the activation state of integrins themselves, or both of these processes (Fig. 1, green). Finally, integrins play an important role in governing the process of cell survival. Depriving epithelial or endothelial cells of contact with the ECM rapidly stimulates apoptosis, a process referred to as "anoikis." Integrin signaling to the phosphoinositide 3-kinase (PI3K)-Akt kinase pathway may be a central regulator of anoikis (Fig. 1, gray).

Twenty years of integrin research have yielded a first glimpse of the network of proteins involved in organizing and transmitting adhesion signals. However, the heterogeneity and dynamic nature of adhesion complexes portend a far greater molecular complexity than realized today. Armed with new approaches for isolation of adhesion complexes and their component molecules, and with sensitive techniques in mass spectrometry, new and interesting protein linkages undoubtedly will be unveiled. Where and when within the cell do integrins signal? The polarized structure of migrating cells (leading protrusive edges, retracting rear) clearly reflects very organized spatial and temporal signaling from integrins. New technologies are now in place to address questions of when and where integrin signals occur. For instance, the use of fluorescent tags [such as enhanced green fluorescent protein (EGFP) fusion proteins] to track proteins in living cells has broadened our appreciation for the dynamics of adhesion structures. These studies show that integrin-directed adhesion complexes assemble and disassemble rapidly in an extending lamellipodia, and the assembly of such complexes takes place in an ordered fashion, indicating a hierarchical assembly process (*5*). In addition, analysis of fluorescent proteins in "real time" demonstrates that small peripheral focal complexes—structures that appear to be distinct from focal adhesions—are, in fact, precursor complexes that have the potential to "mature" into the larger, Rho-dependent focal adhesions (*6*). As for the question of where individual proteins interact and are activated, the use of phospho-specific antibodies that recognize the phosphorylated (and often activated) form of proteins at discrete sites within the cell holds great promise. For instance, activated ERK, which functions in the nucleus to regulate gene expression, also localizes to focal adhesions where it likely catalyzes the phosphorylation of novel targets that regulate integrin signaling (*7*). Application of fluorescence resonance energy transfer (FRET) analysis to living cells allows the detection of activated states of proteins in vivo. This technique has localized activated Rac in membrane ruffles and activated MLCK in the lamellae of migrating cells (*8*, *9*). Finally, techniques such as chromophore-assisted laser inactivation (CALI) allow the local inactivation of proteins in living cells (*10*), providing the opportunity to dissect the contributions of specific adhesion complexes and components to the integrin-signaling pathway.

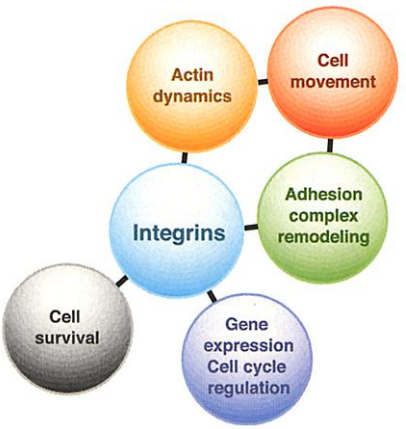

Fig. 1. Signal transduction pathways emanating from integrins (blue) regulate numerous cellular processes, including actin organization (orange) and adhesion complex remodeling (green), which together influence cell movement (red), as well as gene expression and cell cycle regulation (purple) and cell survival (gray). The pathways that regulate each process are outlined in the text and detailed in the STKE Connections Map (*1*).

Because integrin signaling is key to diverse cellular processes, aberrations in integrin signaling contribute to many different disease states from cancer to arthritis. Understanding the pathways that emanate from integrins is clearly an important challenge, as well as an increasingly achievable goal; however, much remains to be accomplished. In the words of the immortal Buzz Lightyear (of *Toy Story* fame), "To infinity and beyond!"

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VIEWPOINT

A Road Map for Those Who Don't Know JAK-STAT

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The Janus kinase–signal transducer and activator of transcription (JAK-STAT) pathway transmits information received from extracellular polypeptide signals, through transmembrane receptors, directly to target gene promoters in the nucleus, providing a mechanism for transcriptional regulation without second messengers. Evolutionarily conserved in eukaryotic organisms from slime molds to humans, JAK-STAT signaling appears to be an early adaptation to facilitate intercellular communication that has co-evolved with myriad cellular signaling events. This co-evolution has given rise to highly adapted, ligand-specific signaling pathways that control gene expression. In addition, the JAK-STAT signaling pathways are regulated by a vast array of intrinsic and environmental stimuli, which can add plasticity to the response of a cell or tissue.

Extracellular signaling polypeptides, such as growth factors or cytokines, are recognized by specific transmembrane receptors or receptor complexes on target cells. One consequence of this recognition is a rapid reprogramming or alteration in the pattern of expressed genes in the target cell. In many cases, the immediate responding genes (those that undergo increased transcription in the absence of new protein synthesis) are controlled by a family of transcription-regulating signaling proteins named signal transducer and activator of transcription (STAT). Intercellular signaling is critical for developmental regulation, growth control, and homeostasis in

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