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.

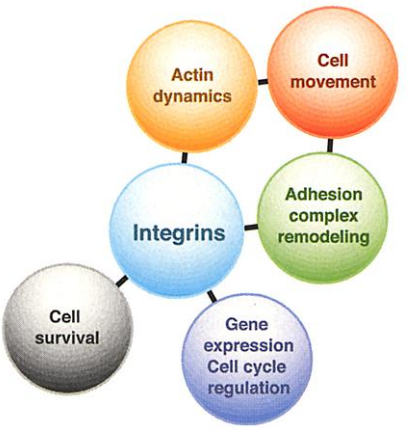

Fig. 1. Signal transduction pathways emanating from integrins (blue) regulate numerous cellular processes, including actin organization (orange) and adhesion complex remodeling (green), which together influence cell movement (red), as well as gene expression and cell cycle regulation (purple) and cell 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!"

References

1. S. A. Boerner, *et al.*, Integrin Signaling Pathway. *Science's STKE* (Connections Map, as seen May 2002), http://stke.sciencemag.org/cgi/cm/CMP_6880.
2. R. O. Hynes, *Cell* **69**, 11 (1992).
3. B. Geiger, *Science* **294**, 1661 (2001).
4. A. R. Horwitz, J. T. Parsons, *Science* **286**, 1102 (1999).
5. C. M. Laukaitis *et al.*, *J. Cell Biol.* **153**, 1427 (2001).
6. K. Rottner *et al.*, *Curr. Biol.* **9**, 640 (1999).
7. V. J. Fincham, M. James, M. C. Frame, S. J. Winder, *EMBO J.* **19**, 2911 (2000).
8. V. S. Kraynov *et al.*, *Science* **290**, 333 (2000).
9. T. L. Chew *et al.*, *J. Cell Biol.* **156**, 543 (2002).
10. Z. Rajfur, P. Roy, C. Otey, L. Romer, K. Jacobson, *Nature Cell Biol.* **4**, 286 (2002).

VIEWPOINT

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

David S. Aaronson[1] and Curt M. Horvath[2]*

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

[1]Department of Pharmacology and Biological Chemistry, [2]Immunobiology Center, Mount Sinai School of Medicine, New York, NY 10029 USA.

*To whom correspondence should be addressed. E-mail: curt.horvath@mssm.edu

multicellular organisms, and STAT pathways have been found in slime molds, worms, flies, and vertebrates but are absent from fungi and plants (*1*). In mammals, there are seven STAT genes, *STAT1*, *STAT2*, *STAT3*, *STAT4*, *STAT5A*, *STAT5B*, and *STAT6*. There is sufficient diversity in the STAT amino acid sequences and their tissue-specific distributions to account for their diverse roles in responses to extracellular signaling proteins. STAT proteins are inactive as transcription factors in the absence of specific receptor stimulation and are localized in the cytoplasm of unstimulated target cells. They are activated rapidly in response to receptor-ligand coupling and are recruited to the intracellular domain of the receptor through specific binding between STAT Src-homology 2 (SH2) domains and receptor phosphotyrosine residues. These SH2-phosphotyrosine interactions are highly specific and are a critical step in determining the specificity of receptor-mediated STAT activation.

Many growth factor receptors have intrinsic tyrosine kinase activity, but most STAT-activating cytokine receptors do not. Instead, the required tyrosine kinase activity is provided by receptor-associated cytoplasmic proteins from the Janus kinase (JAK) family (*2*). JAKs are also evolutionarily conserved, and there are four JAK proteins in mammalian cells, JAK1, JAK2, JAK3, and TYK2. The fundamental role of JAKs in cytokine signaling is evidenced by the inherited immunodeficiencies caused by mutations that block receptor-JAK interactions or the kinase activity of the JAKs. JAKs bind specifically to intracellular domains of cytokine receptor signaling chains and catalyze ligand-induced phosphorylation of themselves and of intracellular tyrosine residues on the receptor, creating STAT docking sites. Phosphorylation of STATs on activating tyrosine residues leads to STAT homo- and heterodimerization. STAT dimers are rapidly transported from the cytoplasm to the nucleus and are competent for DNA binding. Most STAT dimers recognize an 8– to 10–base pair inverted repeat DNA element with a consensus sequence of 5′-TT(N_{4-6})AA-3′. Differential binding affinity of a particular activated STAT dimer for a single target DNA sequence is determined by variations in the exact nucleotide sequence (*3*). This consensus DNA element is usually referred to as a GAS element, reflecting its initial characterization as a γ-interferon activation sequence recognized by STAT1 homodimers (*4*). The affinity of a STAT-DNA complex for a natural target gene promoter is also determined by cooperative dimer-dimer interactions mediated by NH_2-terminal amino acids (*5*, *6*).

Once the activated STAT dimer recognizes a target promoter, the transcription rate from this promoter is dramatically increased. The ability to induce transcription of target genes is an intrinsic property of the STAT dimers, reflecting the ability of STAT transcriptional activation domains to recruit nuclear co-activators that mediate chromatin modifications and communication with the core promoters. STAT-binding elements appear in the context of additional promoter-bound proteins that vary from one gene to another and are required for optimal gene-specific regulation. Examples of complex enhancers in which STATs are critical activating components include the γ-interferon-responsive cell cycle regulator $p21^{WAF1}$ (*7*, *8*) and the interleukin 6 (IL6)-responsive acute phase response protein α-2 macroglobulin (*9*–*11*). These examples are certain to represent a large number of STAT-dependent cooperative transcriptional mechanisms. Although the STATs are generally associated with transcriptional activation, examples of STAT-dependent transcriptional repression have also been reported (*12*–*14*)

The JAK-STAT signaling pathways do not usually function autonomously; rather, they are regulated by a vast array of intrinsic and environmental stimuli. These complex means of regulation can add plasticity to the transcriptional output in a specific cell or tissue. Diverse protein kinases, including several mitogen-activated protein kinases (MAPKs), phosphorylate STATs on serine residues, allowing additional cellular signaling pathways to potentiate the primary STAT-activating stimulus (*15*). Similarly, it is possible that additional sites of regulated serine phosphorylation or other posttranslational modifications may regulate attenuation of STAT activity (*16*, *17*).

Negative regulation of the JAK-STAT pathway is accomplished by such common mechanisms as receptor internalization to endocytic vesicles and subsequent receptor degradation. More specific inhibition signals come from protein tyrosine phosphatases that can act at the level of the membrane-associated receptor-kinase complex (*18*–*20*) or, in the nucleus, by dephosphorylation of activated STAT dimers and recycling of the latent STAT monomer to the cytoplasm (*21*, *22*). The JAKs have their own inhibitors, called suppressor of cytokine signaling (SOCS) proteins, which directly bind to and inactivate the kinases (*23*). Expression of SOCS genes can be stimulated by the same cytokines that enhance STAT activation, so the SOCS proteins can act in classic feedback inhibition loops. Protein inhibitors of activated STATs (PIAS) bind to phosphorylated STAT dimers, preventing DNA recognition (*24*). The steady-state and signal-inducible concentrations of all the positive and negative regulators determine the intensity and duration of the signal response in a particular cell type.

The Connections Maps we have constructed for the Signal Transduction Knowledge Environment (STKE) include a generic, or canonical, JAK-STAT pathway (*25*) and three specific examples of JAK-STAT pathways (Fig. 1), the

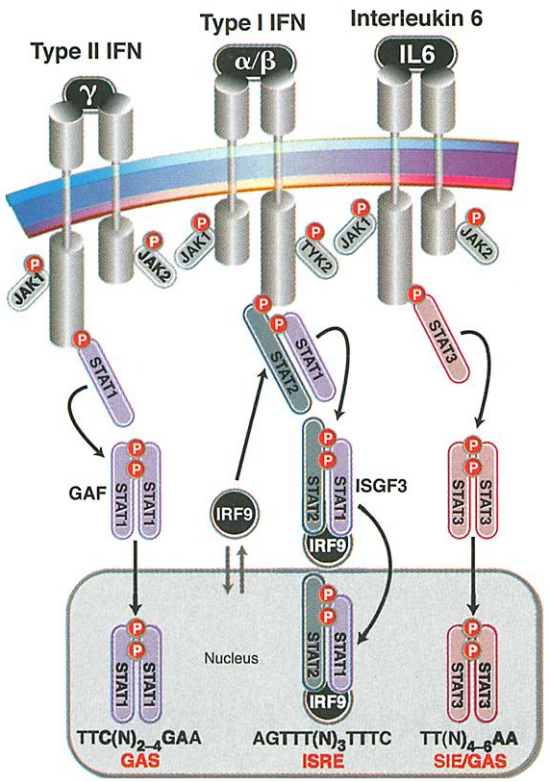

Fig. 1. Three examples of signaling in the JAK-STAT pathway. Specific ligand-receptor interactions generate active transcription complexes composed of distinct STAT proteins. *Left*: Type II IFN (IFNγ) binding induces receptor tyrosine phosphorylation (P) by JAK1 and JAK2 proteins, producing a recruitment site for STAT1. STAT1 dimers form the IFNγ-activated factor (GAF), which translocates to the nucleus and activates transcription from IFNγ target gene promoters containing GAS elements. *Center*: Type I IFNs (IFNα or IFNβ) stimulate the activity of JAK1 and TYK2 proteins, leading to STAT2 tyrosine phosphorylation. The STAT2 phosphotyrosine is a docking site for latent STAT1. The activated factor ISGF3 is a heterotrimer of STAT1 and STAT2 in association with IRF9, which alone can enter the nucleus, but is retained in the cytoplasm by interactions with STAT2. *Right*: IL6 activates JAK1 and JAK2, producing a phosphotyrosine docking site for STAT3. STAT3 dimers translocate to the nucleus and activate transcription from target gene promoters containing a GAS-like element, sometimes referred to as the sis-inducible element (SIE). IL6 also activates STAT1, leading to homo- and heterodimers of STAT1 and STAT3 (not illustrated).

type I interferon (IFNα/β) pathway that uses STAT1 and STAT2 (*26*), the type II interferon (IFNγ) pathway that uses STAT1 (*27*), and the STAT3 pathway that is widely activated in a variety of cellular contexts (*28*).

The type I interferon (IFNα/β) pathway is mechanistically distinct from the majority of STAT pathways. In this pathway, the endpoint transcription factor is not a simple STAT dimer, but a heterotrimer consisting of a STAT1-STAT2 dimer and an obligatory DNA binding subunit from a member of the interferon regulatory factor family, IRF9. The association of the STATs with IRF9 results in recognition of a distinct DNA response element, the IFN-stimulated response element (ISRE), with the sequence 5′-AGTTTN$_3$TTTCC-3′. The trimeric factor ISGF3 (interferon-stimulated gene factor 3) is the primary signaling mechanism leading to expression of target genes required for innate antiviral immunity in higher organisms. The importance of STAT1 and STAT2 in establishing an initial line of defense is underscored by the finding that these proteins are targeted by virus immune evasion strategies (*29*, *30*).

The type II IFN (IFNγ) pathway is a paradigm for most aspects of JAK-STAT signaling that result in dimeric STAT transcription factors. IFNγ induces the activation of STAT1 homodimers that recognize the GAS element in the promoter of target genes involved in innate and adaptive immunity and is important for shaping antitumor immune responses (*31*, *32*). Germline mutations in STAT1 lead to defective antimicrobial immunity (*33*).

The third specific pathway focuses on STAT3, which is broadly studied because of its many functions in animal cell growth regulation, inflammation, and early embryonic development resulting from diverse stimuli [reviewed in (*34*, *35*)]. STAT3 is activated by many cytokines that use signaling receptor subunits similar to gp130. Activation of STAT3 occurs in many solid and hematologic tumors and is correlated with growth stimulation and anti-apoptotic effects in malignancies. Many routes lead to STAT3 activation other than cytokine stimuli, including growth factors, such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), that use tyrosine kinase receptors. Several oncogenic non-receptor tyrosine kinases can activate STAT3, which is required for their ability to malignantly transform cells in culture. In addition, STAT3 is activated in response to oncogenic heterotrimeric guanine nucleotide-binding protein (G protein) subunits through the activation of a cellular non-receptor tyrosine kinase, c-Src, a striking example of cross talk and interconnections between functionally and conceptually distinct signaling pathways through a cellular proto-oncogene (*36*).

It is anticipated that further connections between the JAK-STAT pathways and the other signal transduction systems illustrated by the STKE Connections Maps will be unveiled, providing diversions along the roads that lead to gene regulation.

References and Notes

1. J. E. Darnell Jr., *Proc. Natl. Acad. Sci. U.S.A.* **94**, 11767 (1997).
2. W. J. Leonard, *Int. J. Hematol.* **73**, 271 (2001).
3. G. B. Ehret *et al.*, *J. Biol. Chem.* **276**, 6675 (2001).
4. T. Decker, D. J. Lew, J. Mirkovitch, J. E. Darnell Jr., *EMBO J.* **10**, 927 (1991).
5. X. Xu, Y. L. Sun, T. Hoey, *Science* **273**, 794 (1996).
6. U. Vinkemeier *et al.*, *EMBO J.* **15**, 5616 (1996).
7. Y. E. Chin *et al.*, *Science* **272**, 719 (1996).
8. T. Ouchi, S. W. Lee, M. Ouchi, S. A. Aaronson, C. M. Horvath, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 5208 (2000).
9. X. Zhang, J. E. Darnell Jr., *J. Biol. Chem.* **276**, 33576 (2001).
10. J. Y. Yoo, W. Wang, S. Desiderio, D. Nathans, *J. Biol. Chem.* **276**, 26421 (2001).
11. X. Zhang, M. H. Wrzeszczynska, C. M. Horvath, J. E. Darnell Jr., *Mol. Cell. Biol.* **19**, 7138 (1999).
12. C. V. Ramana *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 6674 (2001).
13. M. P. Gil *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 6680 (2001).
14. V. N. Ivanov *et al.*, *Mol. Cell* **7**, 517 (2001).
15. T. Decker, P. Kovarik, *Oncogene* **19**, 2628 (2000).
16. K. A. Mowen *et al.*, *Cell* **104**, 731 (2001).
17. S. T. Ahmed, L. B. Ivashkiv, *J. Immunol* **165**, 5227 (2000).
18. M. David, H. E. Chen, S. Goelz, A. C. Larner, B. G. Neel, *Mol. Cell. Biol.* **15**, 7050 (1995).
19. M. P. Myers *et al.*, *J. Biol. Chem.* **276**, 47771 (2001).
20. J. Irie-Sasaki *et al.*, *Nature* **409**, 349 (2001).
21. R. L. Haspel, M. Salditt-Georgieff, J. E. Darnell Jr., *EMBO J.* **15**, 6262 (1996).
22. M. David, P. M. Grimley, D. S. Finbloom, A. C. Larner, *Mol. Cell. Biol.* **13**, 7515 (1993).
23. D. L. Krebs, D. J. Hilton, *Stem Cells* **19**, 378 (2001).
24. K. Shuai, *Oncogene* **19**, 2638 (2000).
25. D. S. Aaronson, C. M. Horvath, Jak-STAT Pathway, *Science's STKE* (Connections Map, as seen May 2002), http://stke.sciencemag.org/cgi/cm/CMP_8301.
26. D. S. Aaronson, C. M. Horvath, Type I Interferon (Alpha/Beta IFN) Pathway, *Science's STKE* (Connections Map, as seen May 2002), http://stke.sciencemag.org/cgi/cm/CMP_8390.
27. D. S. Aaronson, C. M. Horvath, Interferon Gamma Pathway, *Science's STKE* (Connections Map, as seen May 2002), http://stke.sciencemag.org/cgi/cm/CMP_9590.
28. D. S. Aaronson, C. M. Horvath, STAT3 Pathway, *Science's STKE* (Connections Map, as seen May 2002), http://stke.sciencemag.org/cgi/cm/CMP_9229.
29. J.-P. Parisien, J. F. Lau, J. J. Rodriguez, C. M. Ulane, C. M. Horvath, *J. Virol.* **76**, 4190 (2002).
30. S. Goodbourn, L. Didcock, R. E. Randall, *J. Gen. Virol.* **81**, 2341 (2000).
31. D. H. Kaplan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 7556 (1998).
32. V. Shankaran *et al.*, *Nature* **410**, 1107 (2001).
33. S. Dupuis *et al.*, *Science* **293**, 300 (2001).
34. T. Hirano, K. Ishihara, M. Hibi, *Oncogene* **19**, 2548 (2000).
35. S. Akira, *Oncogene* **19**, 2607 (2000).
36. P. T. Ram, R. Iyengar, *Oncogene* **20**, 1601 (2001).

37. Research in the Horvath laboratory is supported by grants from the New York City Council Speaker's Fund for Biomedical Research, the American Cancer Society, and the NIH (AI-48722 and AI-50707). D.S.A. is supported by a Howard Hughes Medical Institute Research Training Fellowship for Medical Students.

VIEWPOINT

The Phosphoinositide 3-Kinase Pathway

Lewis C. Cantley*

Phosphorylated lipids are produced at cellular membranes during signaling events and contribute to the recruitment and activation of various signaling components. The role of phosphoinositide 3-kinase (PI3K), which catalyzes the production of phosphatidylinositol-3,4,5-trisphosphate, in cell survival pathways; the regulation of gene expression and cell metabolism; and cytoskeletal rearrangements are highlighted. The PI3K pathway is implicated in human diseases including diabetes and cancer, and understanding the intricacies of this pathway may provide new avenues for therapeutic intervention.

The acute phosphorylation of phosphatidylinositol lipids at the D-3 position of the inositol ring in response to cell stimulation by growth factors and hormones sets in motion a coordinated set of events leading to cell growth, cell cycle entry, cell migration, and cell survival. How does lipid phosphorylation coordinate such complex behavior? Various signaling proteins, including protein serine-threonine kinases, protein tyrosine kinases, and exchange factors that regulate heterotrimeric guanosine triphosphate (GTP)–binding proteins (G proteins), have domains that specifically bind to D-3 phosphorylated phosphoinositides. These proteins are lo-

Department of Cell Biology, Harvard Medical School and Division of Signal Transduction, Beth Israel Deaconess Medical Center, Boston, MA 02115–5713, USA.

*To whom correspondence should be addressed. E-mail: cantley@helix.mgh.harvard.edu