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.

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VIEWPOINT

The Phosphoinositide 3-Kinase Pathway

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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-

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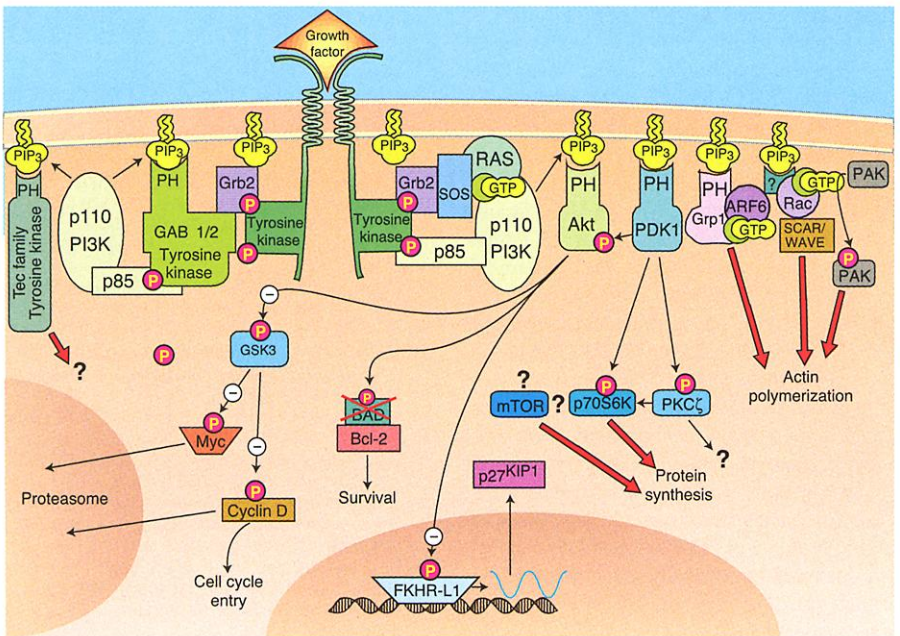

Fig. 1. Signaling pathways downstream of phosphoinositide 3-kinase (PI3K) affect cell growth, cell survival, and cell movement. Activation of growth factor receptor protein tyrosine kinases results in autophosphorylation on tyrosine residues and transphosphorylation of adaptor proteins, such as GAB-1 on tyrosine. PI3K can also be stimulated by integrin-dependent cell adhesion and by G protein-coupled receptors (not shown). PI3K is brought to the membrane and activated by directly binding to phosphotyrosine residues of growth factor receptors or adaptors. The lipid product of PI3K, phosphatidylinositol-3,4,5-trisphosphate (PIP_3), recruits a subset of signaling proteins with pleckstrin homology (PH) domains to the membrane, where they are activated. These proteins include protein serine-threonine kinases (Akt and PDK1), protein tyrosine kinases (Tec family), exchange factors for GTP-binding proteins (Grp1 and Rac exchange factors), and adaptor proteins (GAB-1). Ultimately, these proteins initiate complex sets of events that control protein synthesis, actin polymerization, cell survival, and cell cycle entry. See the text and (*2*) for details.

cated in the cytosol of unstimulated cells but, in response to lipid phosphorylation, accumulate at the plasma membrane because of their ability to associate with the newly formed phosphoinositides. At the membrane, these proteins become activated and initiate various local responses, including polymerization of actin, assembly of signaling complexes, and priming of protein kinase cascades. Hyperactivation of this pathway contributes to human cancers and defects in the pathway contribute to type II diabetes; thus, further studies of phosphoinositide signaling are likely to reveal new targets for drugs to combat these diseases.

Although multiple forms of phosphoinositide 3-kinases (PI3Ks) exist in higher eukaryotes, the class Ia enzymes are primarily responsible for production of D-3 phosphoinositides in response to growth factors (*1*). Class Ia enzymes are heterodimers of regulatory and catalytic subunits [see the PI3K Pathway (http://stke.sciencemag.org/cm/CMP_6557) (*2*)]. The regulatory subunit maintains the p110 catalytic subunit in a low-activity state in quiescent cells and mediates its activation by direct interaction with phosphotyrosine residues of activated growth factor receptors or adaptor proteins. Direct binding of p110 to activated Ras protein (also induced by growth factor stimulation) further stimulates PI3K activity. The activated PI3K converts the plasma membrane lipid phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$] to phosphatidylinositol-3,4,5-trisphosphate [$PI(3,4,5)P_3$].

Signaling proteins with pleckstrin-homology (PH) domains accumulate at sites of PI3K activation by directly binding to $PI(3,4,5)P_3$. Of particular interest are the protein serine-threonine kinases Akt [also called protein kinase B (PKB)] and phosphoinositide-dependent kinase 1 (PDK1). Association with $PI(3,4,5)P_3$ at the membrane brings these proteins into proximity and facilitates phosphorylation of Akt by PDK1 (*3*). This phosphorylation stimulates the catalytic activity of Akt, resulting in the phosphorylation of a host of other proteins that affect cell growth, cell cycle entry, and cell survival. Most of the known protein targets of Akt become inhibited by the phosphorylation event. For example, phosphorylation of the Forkhead-related transcription factor 1 (FKHR-L1) by Akt creates a binding site for the 14-3-3 family of proteins (*4*). The complex of FKHR-L1 and 14-3-3 is retained in the cytosol, blocking transcription of genes normally stimulated by FKHR-L1. Similarly, Akt phosphorylation of the apoptosis-inducing protein Bad creates a binding site for 14-3-3 proteins and prevents Bad from binding to Bcl-2 family members Bcl-2 and Bcl-X_L, thus releasing them for a cell survival response (*4*). The pathway from insulin receptor type protein-tyrosine kinase to PI3K to Akt and FKHR-L1 is conserved from *Caenhorhabditis elegans* to mammals (*5*). Regulation of survival through phosphorylation of BAD appears to have evolved more recently.

A third target of Akt is glycogen synthase kinase 3 (GSK3). This protein kinase is constitutively active in unstimulated cells and phosphorylates many proteins (including glycogen synthase, c-Myc, and cyclin D) to keep them in inactive states or promote their degradation. Phosphorylation of GSK3 (both alpha and beta isoforms) by Akt turns off the catalytic activity of this enzyme, resulting in the activation of pathways that are normally repressed by GSK3.

PDK1 phosphorylates and activates other protein kinases, including p70 S6-kinase, cytokine-independent survival kinase (CISK), and protein kinase C ζ (PKCζ). p70 S6-kinase contributes to cell growth by activating translation of specific messages, CISK, like its close relative Akt, mediates cell survival (*6*), and PKCζ is implicated in many cellular responses.

Other PH domain-containing proteins that are activated by $PI(3,4,5)P_3$ include guanosine disphosphate (GDP)-GTP exchange factors for Rac and for adenosine diphosphate (ADP)-ribosylating factor 6 (ARF6) and protein tyrosine kinases of the Bruton's tyrosine kinase (Btk) and Tec family. Activation of Rac (and perhaps also ARF6) by local production of $PI(3,4,5)P_3$ plays a major role in remodeling the actin cytoskeleton for directional motility in response to chemotactic agents. Tec family members regulate both acute events (changes in cytosolic calcium concentrations) and longterm events (changes in gene expression).

The termination of PI3K signaling by degradation of $PI(3,4,5)P_3$ can be mediated by at least two different types of phosphatases. The Src-homology 2 (SH2)-containing phosphatases (SHIP1 and SHIP2) dephosphorylate the 5 position of the inositol ring to produce $PI(3,4)P_2$. Although this dephosphorylation impairs some signaling downstream of PI3K, $PI(3,4)P_2$ can also mediate PI3K-dependent responses and may mediate events independent of those stimulated by $PI(3,4,5)P_3$. Loss of SHIP2 causes a dramatic increase in insulin sensitivity, suggesting that this phosphatase critically regulates PI3K signaling downstream of insulin (*7*). In contrast, the

phosphatase PTEN dephosphorylates the 3 position of $PI(3,4,5)P_3$ to produce $PI(4,5)P_2$. Loss of PTEN protein or function has been found in a large fraction of advanced human cancers, indicating that uncontrolled signaling through PI3K contributes to metastatic cancers (*8*).

In summary, lipid products of PI3K provide an anchor for assembling signaling proteins at specific locations in the membrane in response to cell stimulation. These signaling proteins coordinate complex events leading to changes in cell metabolism, cell growth, cell movement, and cell survival. Further studies of this pathway are likely to lead to new drug targets for diseases such as diabetes and cancer.

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