Protein:protein interactions involved in intracellular signal transduction

Joan S Brugge

ARIAD Pharmaceuticals, Inc., 26 Landsdowne Street, Cambridge, MA 02139 USA.

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Cell behavior is regulated by cues taken from the extracellular environment in the form of soluble factors (such as hormones, cytokines, neurotransmitters), or cell surface molecules (such as adhesion molecules and antigen-presenting molecules). These extracellular factors interact with cell surface receptors, resulting in the activation of intracellular pathways that cause pleiotropic changes in cell behavior. In traditional models of cellular pathways, such as metabolic pathways, a series of successive catalytic interactions leads to the formation of a metabolic product. In these models, the enzymes are usually compartmentalized in a common subcellular location and are therefore in close proximity. In contrast, the complex pathways activated by extracellular factors are initiated at the plasma membrane, transduced throughout the cytoplasm, and propagated into the nucleus. Many of the responses triggered by these pathways take place very soon after receptor activation. Such rapid dissemination of signals requires mechanisms that recruit and organize specific cellular proteins to the site of receptor interaction.

As shown in Fig. 1, many cellular proteins involved in signal transduction contain small modular domains that mediate highly specific non-catalytic binding interactions between cellular proteins. These domains serve as molecular 'traps' that localize signalling proteins to specific sites within the cell, recruit substrates to catalytic domains, or regulate protein function through intramolecular interactions (see [1–4] for reviews). The binding interactions can be either constitutive, organizing proteins into pre-existing complexes and positioning proteins within close proximity, or inducible, being regulated by covalent protein modifications (such as phosphorylation), or by the binding of small molecules (such as GTP, ATP, or lipids) or ions (such as calcium). Related binding domains are shared by many proteins (Fig. 1). Two particularly good examples are the Src-homology 2 and 3 domains (SH2 and SH3), which are found within protein tyrosine kinases, phospholipases and lipid kinases, and which recognise short contiguous amino acid sequence motifs containing phosphotyrosine (P-Tyr) or proline, respectively [1–4]. Certain proteins, like Grb-2, are composed solely of such binding domains and appear to function as adaptor proteins, coupling other cellular proteins together.

The binding of a growth factor causes conformational changes in the cytoplasmic region of the receptor and induces receptor dimerization, activating the receptor's protein tyrosine kinase activity to phosphorylate multiple tyrosine residues within the tails of the dimer pair. Many of the P-Tyr residues can then bind to proteins containing SH2 domains. Each SH2 domain shows specific preferences for the amino acids surrounding the P-Tyr; thus, proteins that have different SH2 domains bind to different P-Tyr-containing motifs. Once bound to the membrane receptor, the signalling molecules themselves can be activated or repressed by phosphorylation. Each of these primary receptor interactions activate distinct pathways that involve cascades of additional binding and catalytic interactions.

The importance of protein:protein interactions in signalling is illustrated by the Shc pathway [3,5]. Phosphorylation of Shc on tyrosine after receptor activation creates a ligand binding site for the SH2 domain of Grb-2, which is bound constitutively to the guanine nucleotide exchange protein SOS via two SH3 domains. Grb-2 thus carries SOS to the plasma membrane, where it can interact with its substrate, Ras, leading to GTP-loading on Ras. Ras-GTP then activates the protein serine/threonine kinase Raf to phosphorylate and activate MAP kinase. MAP kinase has multiple substrates, including nuclear transcription factors (which activate or repress transcription) and phospholipase A2 (which leads to the physiological events mediated by prostaglandins and leukotrienes).

The protein:protein interactions described here suggest new targets for therapeutic invention in a variety of human diseases. By designing small molecules that mimic the short contiguous amino acid sequences that serve as binding ligands for many of these interactions, one could effectively short-circuit the pathways that are involved in the progress of disease. The precise molecular defects responsible for many human diseases have been elucidated through advances in molecular and cellular biology over the last decade. This information, coupled with classic drug-screening approaches and structure-based drug design, should allow the creation of a new generation of highly specific, small molecule drugs for the treatment of many human diseases.

References

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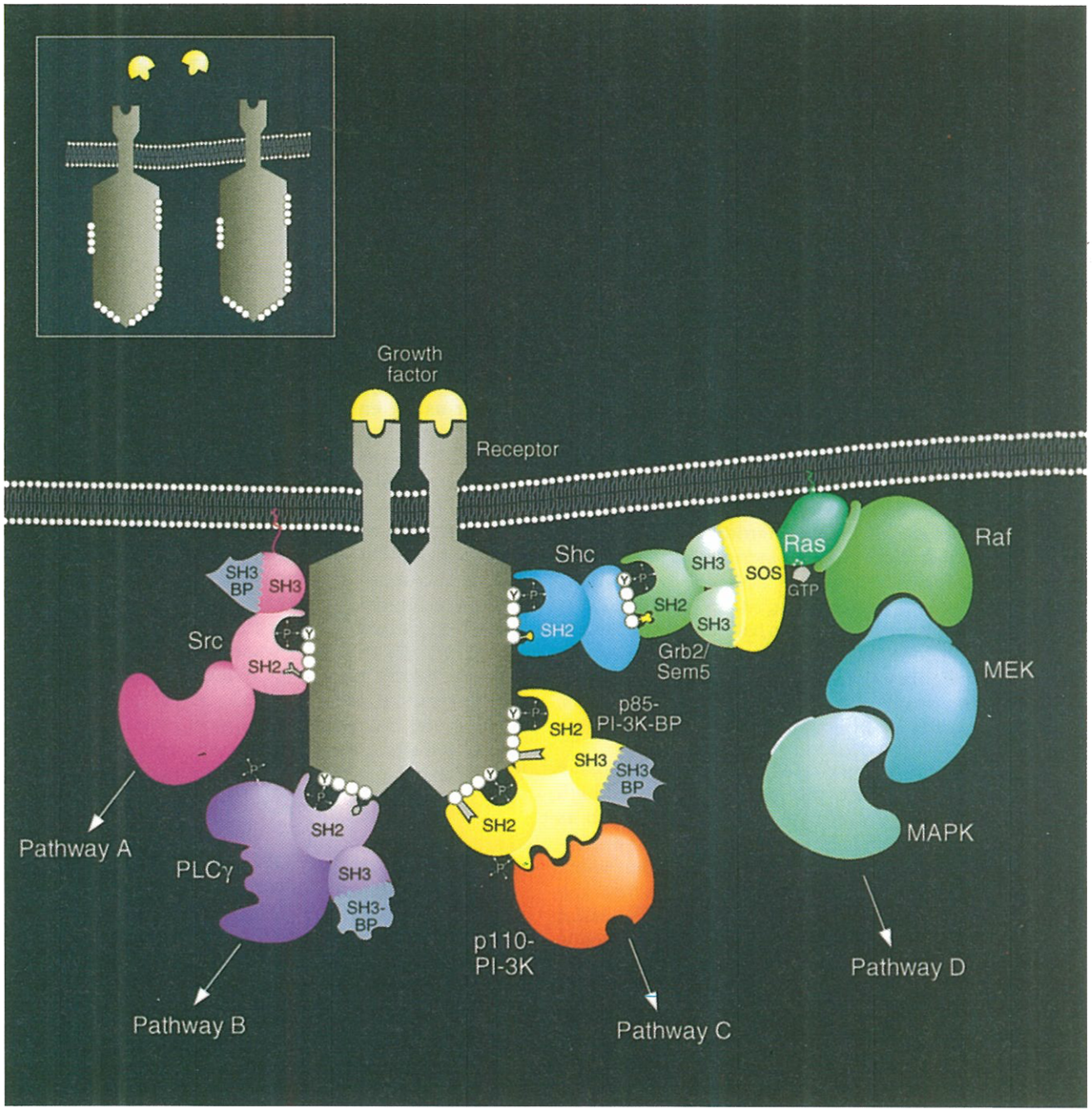

Fig. 1. Protein:protein interactions involved in growth receptor signal transduction. The upper left panel shows a growth factor receptor before interaction with a growth factor (such as epidermal growth factor, platelet derived growth factor, or colony stimulating factor). The main figure illustrates representative protein:protein interactions that are triggered by growth factor-induced receptor dimerization. Dimerization activates the protein tyrosine kinase activity of the receptor, resulting in auto-phosphorylation of multiple tyrosine residues in the cytoplasmic domain of the receptor. These tyrosine phosphorylated residues and surrounding amino acids serve as binding sites for the SH2 domains of several proteins such as Shc (a proline-rich adaptor protein), the p85 subunit of phosphatidylinositol 3′ kinase (PI-3K), phospholipase C (PLC), and Src (a protein tyrosine kinase). The specific substrate proteins that are phosphorylated by the Src protein tyrosine kinase in pathway A are not known; however, this pathway appears to be important for growth factor-induced DNA synthesis. Pathway B leads to hydrolysis of phosphatidylinositol giving diacylglycerol (which activates protein kinase C) and inositol trisphosphate (which causes intracellular calcium mobilization). Pathway C, involving the p85/p110 phosphatidylinositol 3′ kinase complex, causes the formation of 3′ phosphorylated phosphatidylinositol products which are involved in stimulation of DNA synthesis, receptor internalization and vesicle transport in several systems. Pathway D, involving Ras and mitogen-activated kinase (MAPK), regulates multiple intracellular pathways involving transcriptional activation, cell proliferation, and cytoskeletal rearrangements. This pathway is initiated by the interaction of Shc with the activated growth factor receptor. The site of tyrosine phosphorylation on Shc serves as a binding site for the interaction with proline-rich binding motifs on the SOS GTP exchange protein, which leads to activation of Ras through GDP to GTP exhange. The GTP bound form of Ras binds to the Raf protein, which phosphorylates and activates the serine/threonine kinase MEK. Activated MEK phosphorylates and activates MAPK. Each SH3-BP represents an unidentified SH3 domain binding proteins. (The sizes and shapes of these molecules are not drawn to scale or based on precise structural information, but see the cover of this issue for a structure of SH3 bound to a peptide ligand.)