15.4.2. Ras, Another Class of Signaling G Protein

We now turn our attention to another important family of signal proteins, the *small G proteins*, or small GTPases. This large superfamily of proteins—grouped into subfamilies called Ras, Rho, Arf, Rab, and Ran—plays a major role in a host of cell functions including growth, differentiation, cell motility, cytokinesis, and transport of materials throughout the cell (Table 15.3). Like their relatives the heterotrimeric G proteins (Section 15.1.2), the small G proteins cycle between an active GTP-bound form and an inactive GDP-bound form. They differ from the heterotrimeric G proteins in being smaller (20–25 kd versus 30–35 kd) and monomeric. Nonetheless, the two families are related by divergent evolution, and small G proteins have many key mechanistic and structural motifs in common with the G_{α} subunit of the heterotrimeric G proteins.

In their activated GTP-bound form, small G proteins such as Ras stimulate cell growth and differentiation. Recall that Sos is the immediate upstream link to Ras in the circuit conveying the EGF signal. How does Sos activate Ras? Sos binds to Ras, reaches into the nucleotide-binding pocket, and opens it up, allowing GDP to escape and GTP to enter in its place (Figure 15.33). This process is presumably analogous to the stimulation of nucleotide exchange in heterotrimeric G proteins by activated 7TM receptors, a process for which structural details are not yet available. Sos is referred to as a *guaninenucleotide exchange factor (GEF)*. Thus, the binding of EGF to the EGF receptor leads to the conversion of Ras into its GTP form through the intermediacy of Grb-2 and Sos (Figure 15.34).

Like the G_{α} protein, Ras possesses an intrinsic GTPase activity, which serves to terminate the signal and return the system to the inactive state. This activity is slow but is augmented by helper proteins termed *GTPase-activating proteins (GAPs)*. The guanine-nucleotide exchange factors and the GTPase-activating proteins allow the G-protein cycle to proceed with rates appropriate for a balanced level of downstream signaling.

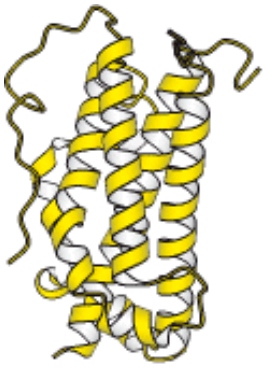

Figure 15.24. Human Growth Hormone Structure. Human growth hormone forms a four-helix bundle.

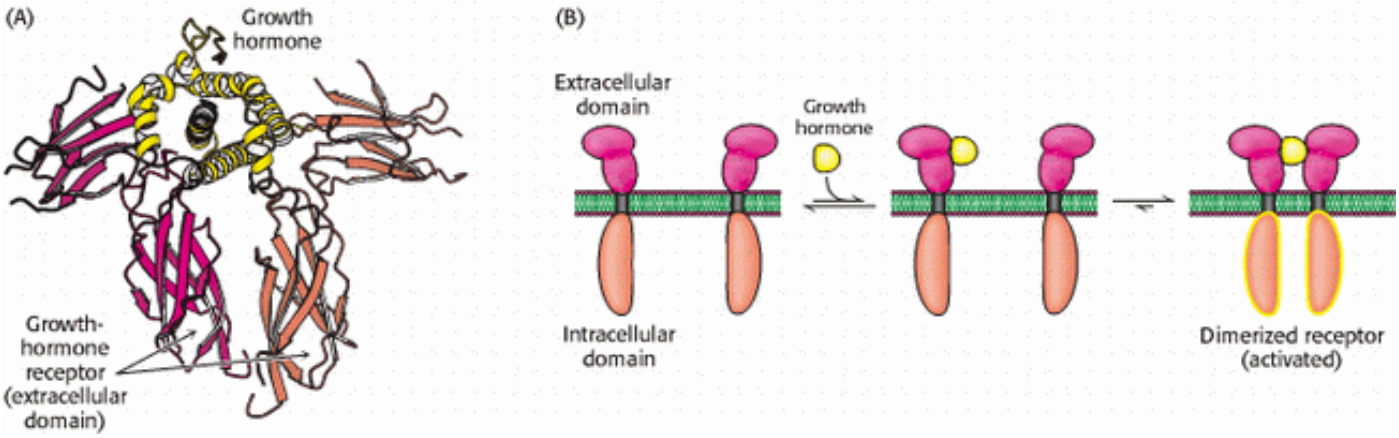

Figure 15.25. Binding of Growth Hormone Leads to Receptor Dimerization. (A) A single growth-hormone molecule (yellow) interacts with the extracellular domain of two receptors (red and orange). (B) The binding of one hormone molecule to two receptors leads to the formation of a receptor dimer. Dimerization is a key step in this signal-transduction pathway.

Figure 15.26. Janus Kinase Domain Structure. A Janus kinase (JAK) includes four recognized domains: an ERM domain that favors interactions with membranes, an SH2 domain that binds phosphotyrosine-containing peptides, and two domains homologous to protein kinases. Only the second protein kinase domain appears to be enzymatically functional.

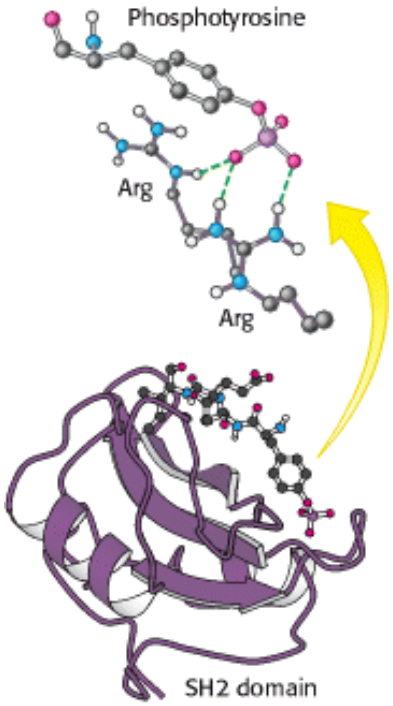

Figure 15.27. Recognition of Phosphotyrosine by SH2 Domains. The structure of an SH2 domain (purple) bound to a phosphotyrosine-containing peptide. The hydrogen-bonding interactions between the phosphotyrosine residue and two arginine residues are shown; interactions with other residues are omitted for clarity.

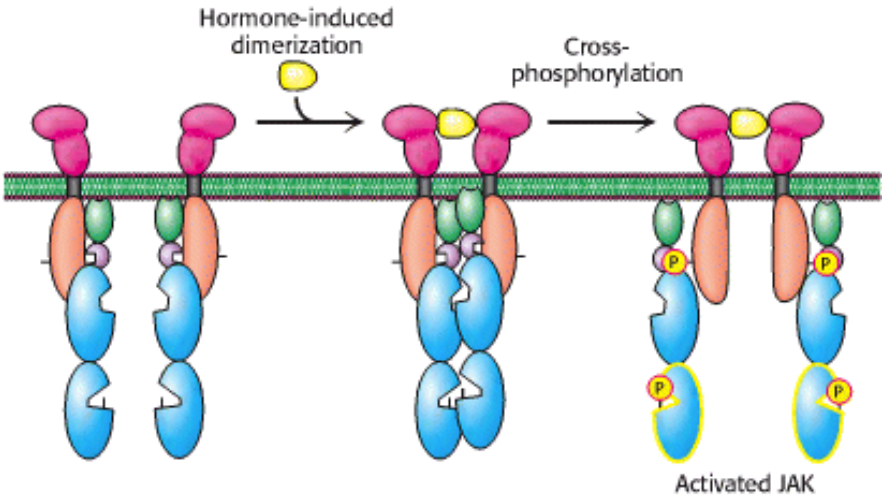

Figure 15.28. Cross-Phosphorylation of Jaks Induced by Receptor Dimerization. The binding of growth hormone leads to receptor dimerization, which brings two JAKs together in such a way that each phosphorylates key residues on the other. The activated JAKs remain bound to the receptor.

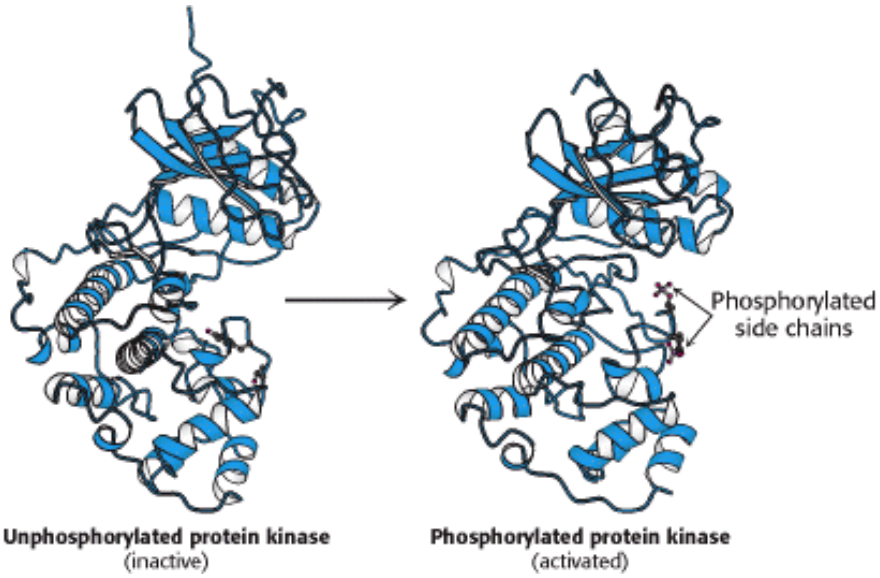

Figure 15.29. Activation of a Protein Kinase by Phosphorylation. In the unphosphorylated state, a key loop is in a conformation unsuitable for catalysis. Phosphorylation (at two sites in the case shown) stabilizes an active conformation.

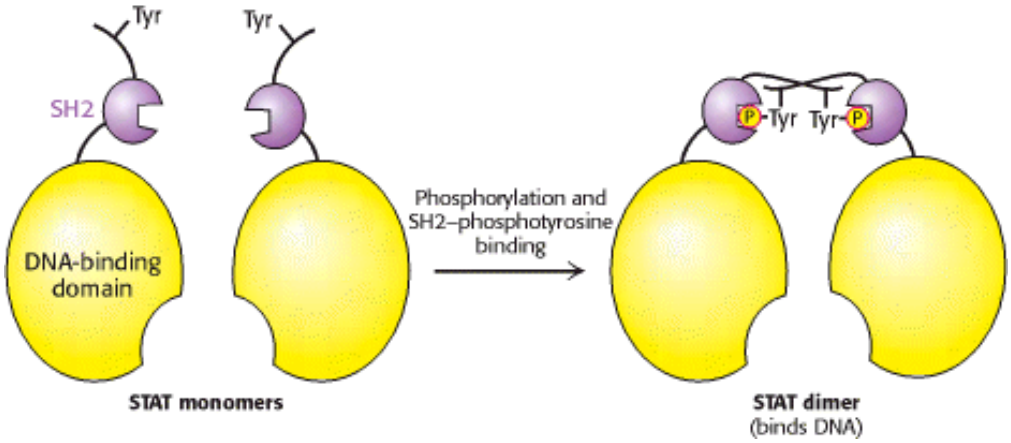

Figure 15.30. Phosphorylation-Induced Dimerization of STAT Proteins. The phosphorylation of a key tyrosine residue on each STAT protein leads to an interaction between the phosphotyrosine and an SH2 domain on another STAT monomer. The STAT dimer produced by these reciprocal interactions has a high affinity for specific DNA sequences and is able to alter gene expression after binding to DNA.

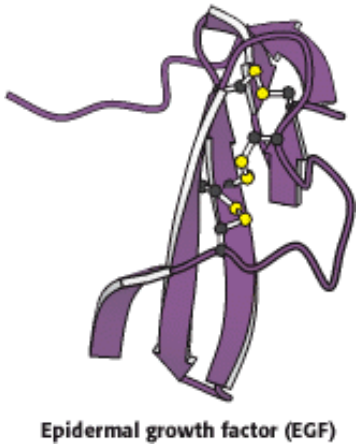

Figure 15.31. Structure of Epidermal Growth Factor. This protein growth factor is stabilized by three disulfide bonds.

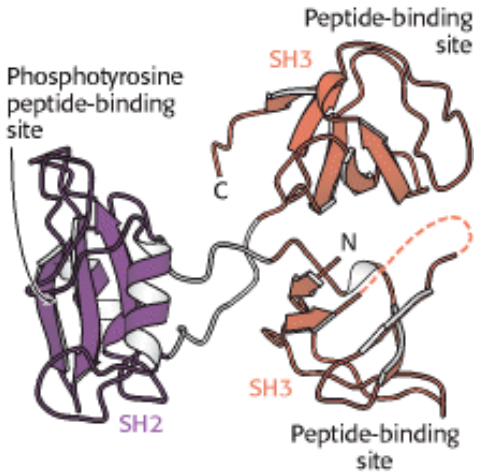

Figure 15.32. Structure of Grb-2, an Adaptor Protein. Grb-2 consists of two SH3 domains and a central SH2 domain. The SH2 domain binds to phosphotyrosine resides on an activated receptor while the SH3 domains bind proline-rich regions on other proteins such as Sos.

Table 15.3. Ras superfamily of GTPases

Subfamily	Function
Ras	Regulates cell growth through serine-threonine protein kinases
Rho	Reorganizes cytoskeleton through serine-threonine protein kinases
Arf	Activates the ADP-ribosyltransferase of the cholera toxin A subunit; regulates vesicular trafficking pathways; activates phospholipase D
Rab	Plays a key role in secretory and endocytotic pathways
Ran	Functions in the transport of RNA and protein into and out of the nucleus

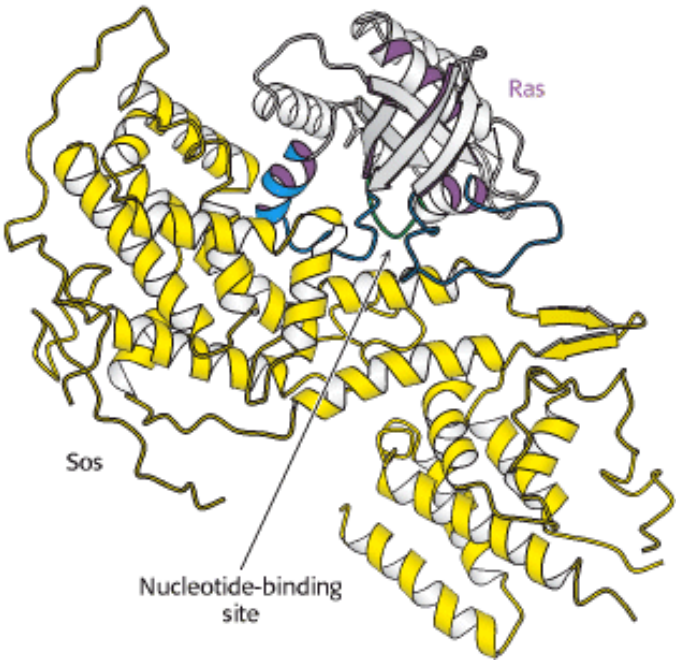

Figure 15.33. Structure of Sos, a Guanine-Nucleotide Exchange Factor. Sos (yellow) binds to Ras and opens up its nucleotide-binding site, allowing GDP to escape and GTP to bind. In the GTP-bound form, Ras can bind to and activate other proteins, including protein kinases.

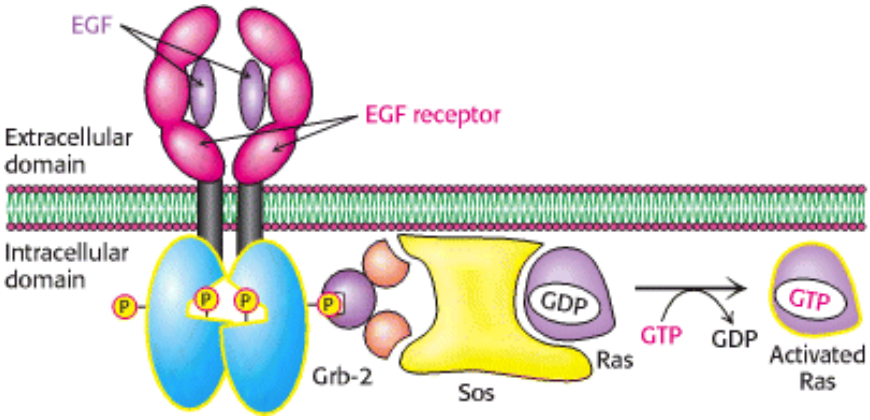

Figure 15.34. Egf Signaling Pathway. The binding of epidermal growth factor (EGF) to its receptor leads to cross-phosphorylation of the receptor. The phosphorylated receptor binds Grb-2, which, in turn, binds Sos. Sos stimulates the exchange of GTP for GDP in Ras. Activated Ras binds to and stimulates protein kinases (not shown).

15.5. Defects in Signaling Pathways Can Lead to Cancer and Other Diseases

In light of their complexity, it comes as no surprise that signal-transduction pathways occasionally fail, leading to pathological or disease states. Cancer, a set of diseases characterized by uncontrolled or inappropriate cell growth, is strongly associated with defects in signal-transduction proteins. Indeed, the study of cancer, particularly cancer caused by certain viruses, has contributed greatly to our understanding of signal-transduction proteins and pathways.

For example, Rous sarcoma virus is a retrovirus that causes sarcoma (a cancer of tissues of mesodermal origin such as muscle or connective tissue) in chickens. In addition to the genes necessary for viral replication, this virus carries a gene termed v-*src*. The v-*src* gene is an *oncogene*; it leads to the transformation of susceptible cell types. The protein encoded by v-*src* is a protein tyrosine kinase that includes SH2 and SH3 domains (Figure 15.35). Indeed, the names of these domains derive from the fact that they are *S*rc *h*omology domains. The v-Src protein is similar in amino acid sequence to a protein normally found in chicken muscle cells referred to as c-Src (for cellular Src). The c-*src* gene does not induce cell transformation and is termed a *proto-oncogene.* The protein that it encodes is a signal-transduction protein that regulates cell growth. As we shall see, small differences in the amino acid sequences between the proteins encoded by the proto-oncogene and the oncogene are responsible for the oncogene product being trapped in the "on" position.

An examination of the structure of c-Src in an inactive conformation reveals an intricate relation between the three major domains. The SH3 domain lies nearest the amino terminus, followed by the SH2 domain and then the kinase domain. There is also an extended carboxyl-terminal stretch that includes a phosphotyrosine residue. The phosphotyrosine residue is bound within the SH2 domain, whereas the linker between the SH2 domain and the kinase domain is bound by the SH3 domain. These interactions hold the kinase domain in an inactive conformation. The Src protein in this form can be activated by three distinct processes (Figure 15.36): (1) the phosphotyrosine residue bound in the SH2 pocket can be displaced by another phosphotyrosine-containing polypeptide with a higher affinity for this SH2 domain, (2) the phosphoryl group on the tyrosine residue can be removed by a phosphatase, and (3) the linker can be displaced from the SH3 domain by a polypeptide with a higher affinity for this SH3 domain. Thus, Src responds to the presence of one of a set of distinct signals. The amino acid sequence of the viral oncogene is more than 90% identical with its cellular counterpart. Why does it have such a different biological activity? The C-terminal 19 amino acids of c-Src are replaced by a completely different stretch of 11 amino acids, and this region lacks the key tyrosine residue that is phosphorylated in inactive c-Src. Since the discovery of Src, many other mutated protein kinases have been identified as oncogenes.

How did the Rous sarcoma virus acquire the mutated version of *src*? In an infection, a viral genome may pick up a gene from its host in such a way that the region encoding the last few amino acids is missing. Such a modified gene may have given the Rous sarcoma virus a selective advantage because it will have favored viral growth when introduced with the virus into a host cell.

Impaired GTPase activity in a regulatory protein also can lead to cancer. Indeed, *ras* is one of the genes most commonly mutated in human tumors. Mammalian cells contain three 21-kd Ras proteins (H-, K-, and N-Ras) that cycle between GTP and GDP forms. The most common mutations in tumors lead to a loss of the ability to hydrolyze GTP. Thus, the Ras protein is trapped in the "on" position and continues to stimulate cell growth.

15.5.1. Protein Kinase Inhibitors May Be Effective Anticancer Drugs

The widespread occurrence of over active protein kinases in cancer cells suggests that molecules that inhibit these enzymes might act as antitumor agents. Recent results have dramatically supported this concept. More than 90% of patients with chronic myologenous leukemia (CML) show a specific chromosomal defect in affected cells (Figure 15.37). The translocation of genetic material between chromosomes 9 and 22 causes the c-*abl* gene, which encodes a tyrosine kinase, to be inserted into the *bcr* gene on chromosome 22. The result is the production of a fusion protein called