Signaling Through enzyme-coupled receptors

SIGNALING THROUGH ENZYME-COUPLED RECEPTORS

- Like GPCRs, **enzyme-coupled receptors** are transmembrane proteins with their ligand-binding domain on the outer surface of the plasma membrane.

- Instead of having a cytosolic domain that associates with a trimeric G protein, however, their cytosolic domain either has **intrinsic enzyme activity** or **associates directly with an enzyme**.

- Whereas a GPCR has seven transmembrane segments, each subunit of an enzyme-coupled receptor typically has only one.

- GPCRs and enzyme-coupled receptors often activate some of the same signaling pathways.

- In this section, we describe some of the important features of signaling by enzyme-coupled receptors, with an emphasis on the most common class of these proteins, the receptor tyrosine kinases.

SIGNALING THROUGH ENZYME-COUPLED RECEPTORS

Many extracellular signal proteins act through receptor tyrosine kinases (RTKs).
These include many secreted and cell-surface-bound proteins that control cell behavior in developing and adult animals.

TABLE 15–4 Some Signal Proteins That Act Via RTKs

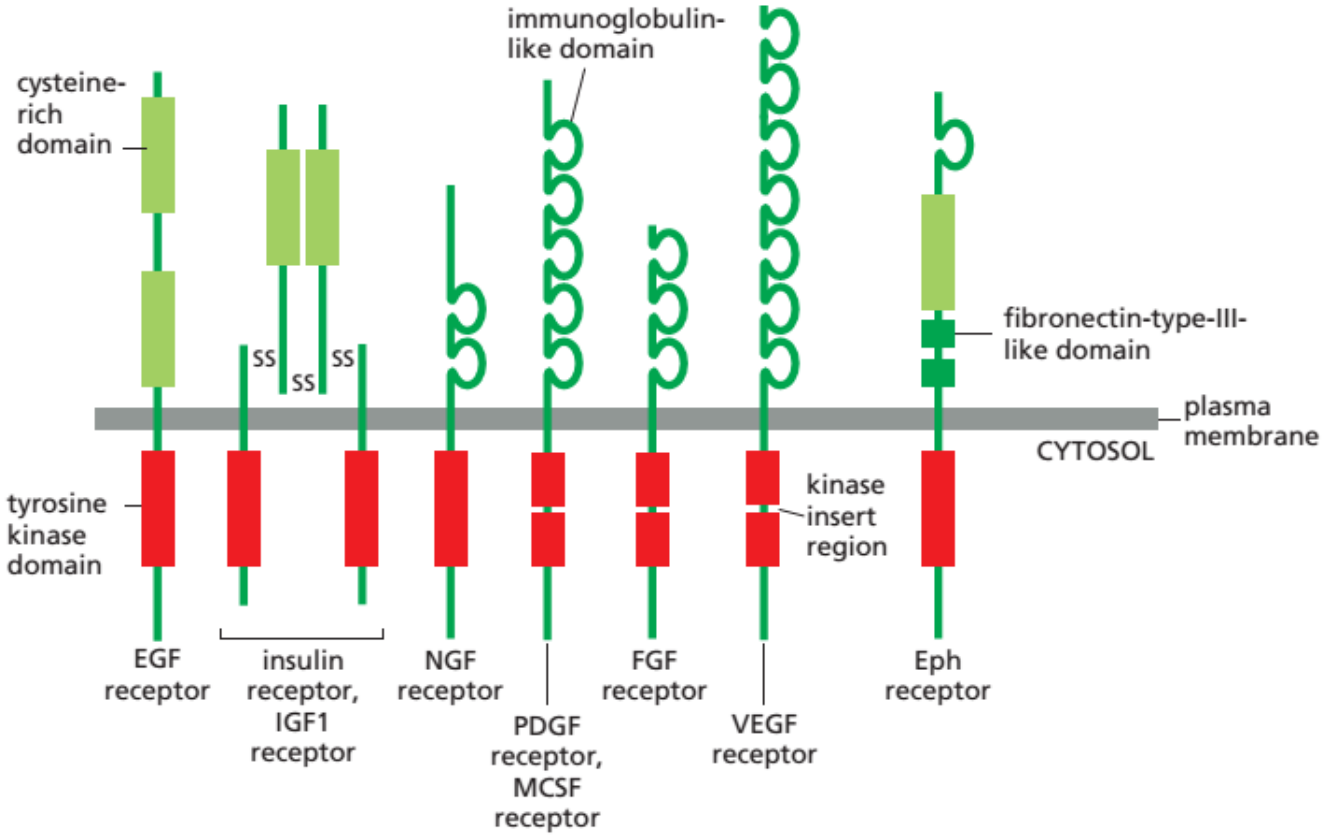
Signal protein family	Receptor family	Some representative responses
Epidermal growth factor (EGF)	EGF receptors	Stimulates cell survival, growth, proliferation, or differentiation of various cell types; acts as inductive signal in development
Insulin	Insulin receptor	Stimulates carbohydrate utilization and protein synthesis
Insulin-like growth factor (IGF1)	IGF receptor-1	Stimulates cell growth and survival in many cell types
Nerve growth factor (NGF)	Trk receptors	Stimulates survival and growth of some neurons
Platelet-derived growth factor (PDGF)	PDGF receptors	Stimulates survival, growth, proliferation, and migration of various cell types
Macrophage-colony-stimulating factor (MCSF)	MCSF receptor	Stimulates monocyte/macrophage proliferation and differentiation
Fibroblast growth factor (FGF)	FGF receptors	Stimulates proliferation of various cell types; inhibits differentiation of some precursor cells; acts as inductive signal in development
Vascular endothelial growth factor (VEGF)	VEGF receptors	Stimulates angiogenesis
Ephrin	Eph receptors	Stimulates angiogenesis; guides cell and axon migration

SIGNALING THROUGH ENZYME-COUPLED RECEPTORS

- There are about **60 human RTKs**, which can be classified into about 20 structural subfamilies, each dedicated to its complementary family of protein ligands.

- In all cases, the binding of the signal protein to the ligand-binding domain on the extracellular side of the receptor activates the tyrosine kinase domain on the cytosolic side.

- This leads to **phosphorylation of tyrosine side chains** on the cytosolic part of the receptor, creating **phosphotyrosine docking sites** for various intracellular signaling proteins that relay the signal.



SIGNALING THROUGH ENZYME-COUPLED RECEPTORS

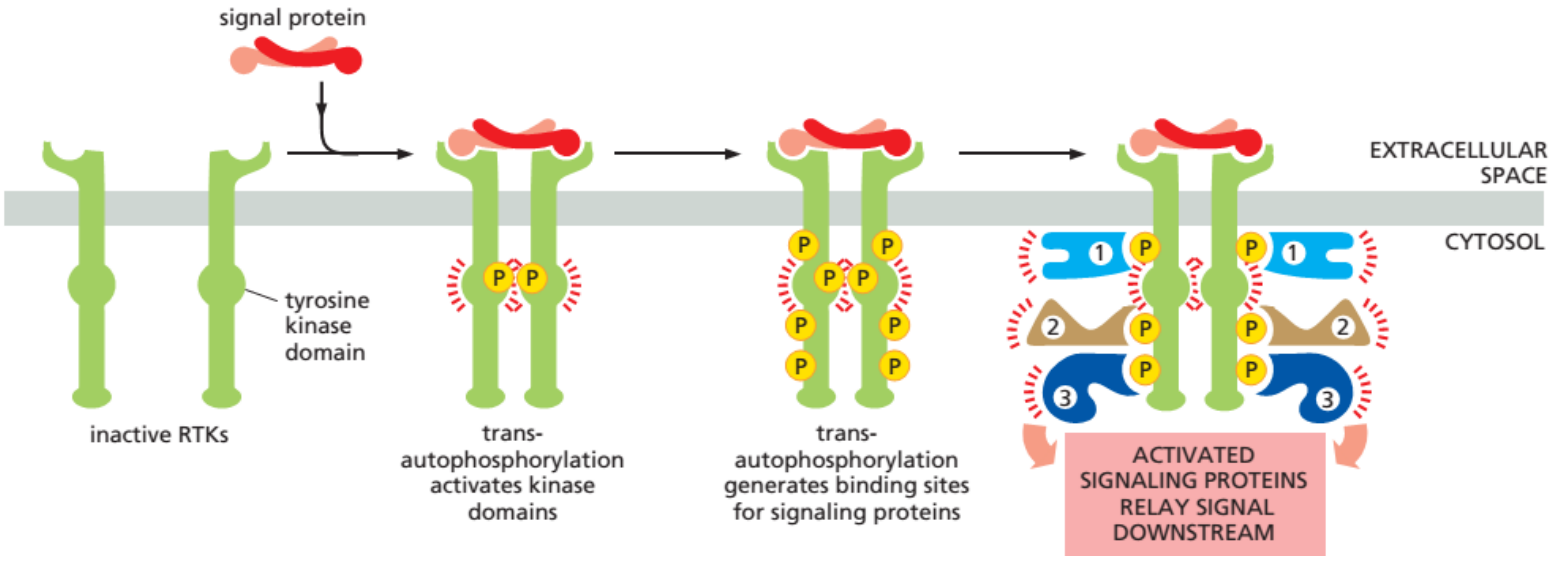
How does the binding of an extracellular ligand activate the kinase domain on the other side of the plasma membrane?

For a GPCR, ligand binding is thought to change the relative orientation of several of the transmembrane α helices, thereby shifting the position of the cytoplasmic loops relative to one another.
It is unlikely, however, that a conformational change could propagate across the lipid bilayer through a **single transmembrane α helix**.

Instead, for most RTKs, ligand binding causes the receptors to dimerize, bringing the two cytoplasmic kinase domains together and thereby promoting their activation.

Dimerization stimulates kinase activity by a variety of mechanisms.

SIGNALING THROUGH ENZYME-COUPLED RECEPTORS



In the absence of extracellular signals, most RTKs exist as **monomers** in which the internal kinase domain is inactive. Binding of ligand brings two monomers together to form a dimer.
In most cases, the **close proximity** in the dimer leads the two kinase domains to phosphorylate each other, which has two effects:
-First, phosphorylation at some tyrosines in the kinase domains promotes the **complete activation** of the domains.

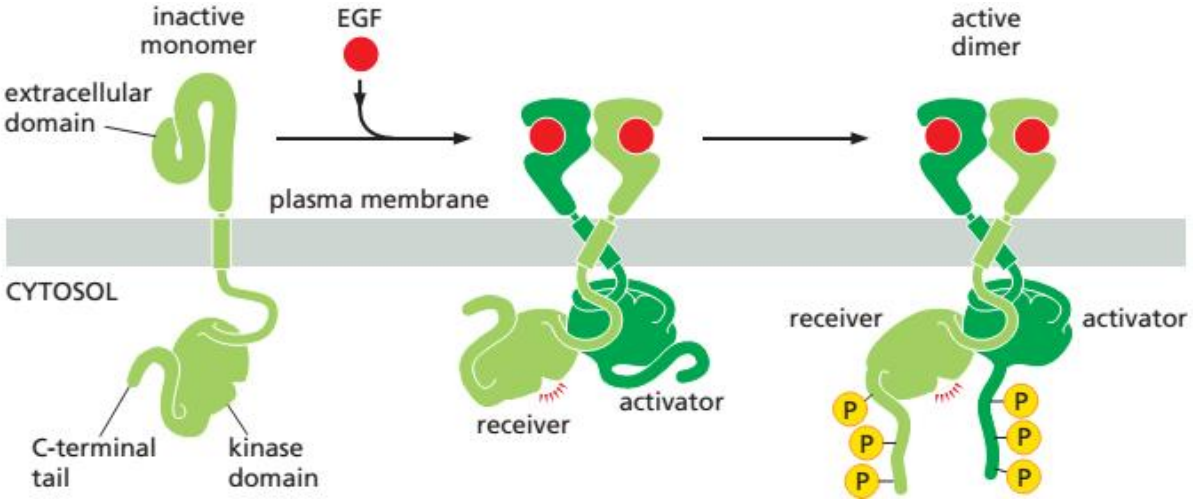
-Second, phosphorylation at tyrosines in other parts of the receptors generates **docking sites for intracellular signaling proteins**, resulting in the formation of **large signaling complexes** that can then broadcast signals along multiple signaling pathways.

- Mechanisms of dimerization vary widely among different RTK family members.
- In some cases, as shown here, the **ligand itself is a dimer** and brings two receptors together by binding them simultaneously.
- In other cases, a monomeric ligand can interact with two receptors simultaneously to bring them together, or two ligands can bind independently on two receptors to promote dimerization.

SIGNALING THROUGH ENZYME-COUPLED RECEPTORS

- In many cases, such as the **insulin receptor**, dimerization simply brings the kinase domains close to each other in an orientation that allows them to phosphorylate each other on **specific tyrosines in the kinase active sites**, thereby promoting conformational changes that fully activate both kinase domains.

- In other cases, such as the receptor for ***epidermal growth factor (EGF),*** the kinase is not activated by phosphorylation but by conformational changes brought about by interactions between the two kinase domains outside their active sites.



In the absence of ligand, the EGF receptor exists primarily as an inactive monomer.
EGF binding results in a conformational change that promotes dimerization of the external domains.
The receptor kinase domain, unlike that of many RTKs, is not activated by transautophosphorylation.
Instead, **dimerization orients the internal kinase domains into an asymmetric dimer**, in which one kinase domain (the "**activator**") pushes against the other kinase domain (the "**receiver**"), thereby causing an activating conformational change in the receiver.
The **active receiver domain** then phosphorylates multiple tyrosines in the C-terminal tails of both receptors, generating docking sites for intracellular signaling proteins

Phosphorylated Tyrosines on RTKs Serve as Docking Sites for Intracellular Signaling Proteins

- Once the kinase domains of an RTK dimer are activated, they phosphorylate multiple additional sites in the cytosolic parts of the receptors, typically in disordered regions outside the kinase.
- This phosphorylation creates **high-affinity docking sites for intracellular signaling proteins**.

- Each signaling protein binds to a particular phosphorylated site on the activated receptors because it contains a **specific phosphotyrosine-binding domain** that recognizes surrounding features of the polypeptide chain in addition to the phosphotyrosine.

-Once bound to the activated RTK, a signaling protein may become phosphorylated on tyrosines and thereby activated.
-In many cases, the binding alone may be sufficient to activate the docked signaling protein, by either inducing a **conformational change** in the protein or simply **bringing it near the protein** that is next in the signaling pathway.

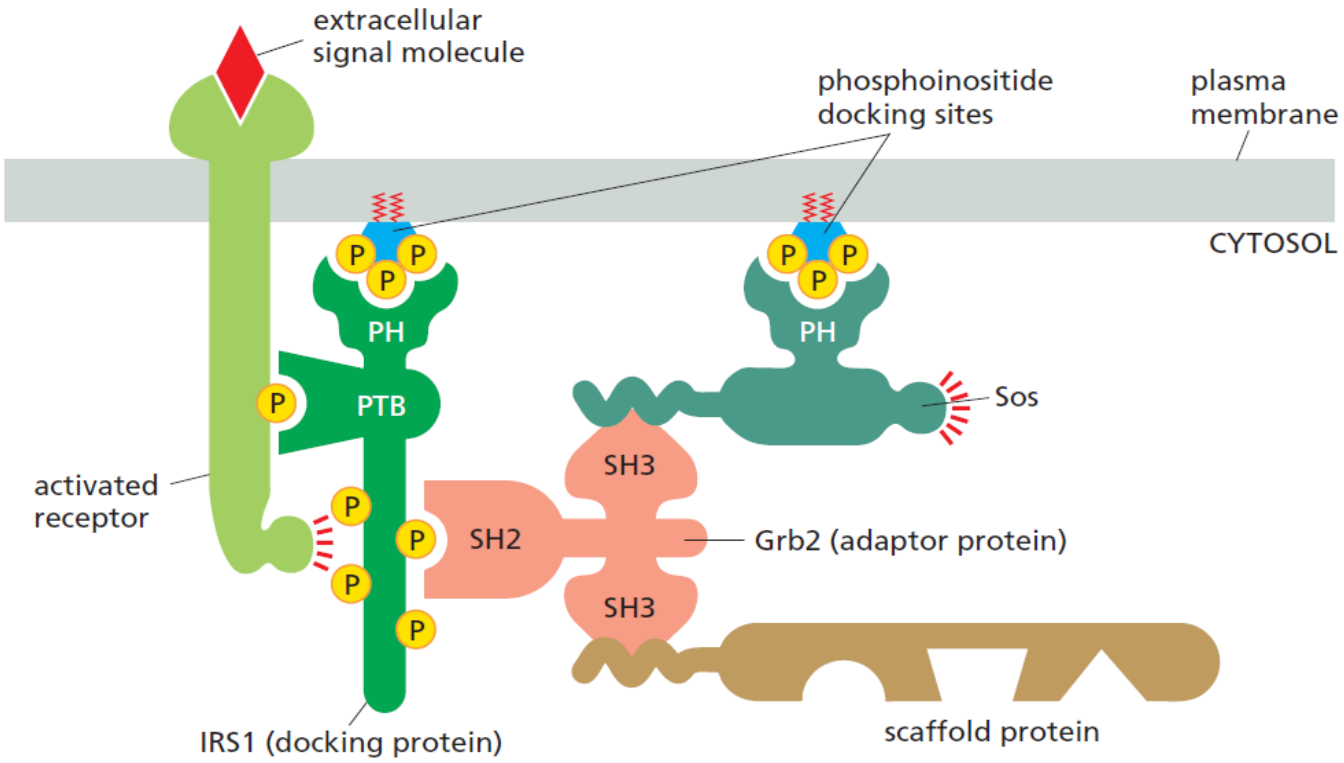
- Thus, receptor phosphorylation serves as a **switch** to trigger the assembly of an intracellular signaling complex, which can then relay the signal onward, often along multiple routes, to various destinations in the cell.

- Because different RTKs bind **different combinations** of these signaling proteins, they activate different responses.

Phosphorylated Tyrosines on RTKs Serve as Docking Sites for Intracellular Signaling Proteins

- Some RTKs use additional docking proteins to **enlarge the signaling complex** at activated receptors:

Insulin and IGF1 receptor signaling, depend on a specialized docking protein called ***insulin receptor substrate 1 (IRS1)***.
IRS1 associates with phosphorylated tyrosines on the activated receptor and is then phosphorylated at multiple sites, thereby creating many more docking sites than could be accommodated on the receptor alone.



Proteins with SH2 Domains Bind to Phosphorylated Tyrosines

- A whole menagerie of intracellular signaling proteins can bind to the phosphotyrosines on activated RTKs (or on docking proteins such as IRS1). They help to relay the signal onward, mainly through chains of protein–protein interactions mediated by modular interaction domains.

-Some of the docked proteins are enzymes, such as **phospholipase C-γ (PLCγ),** which functions in the same way as phospholipase C-β—activating the inositol phospholipid signaling pathway. Through this pathway, RTKs can increase cytosolic Ca^{2+} levels and activate PKC.

-Another enzyme that docks on these receptors is the cytoplasmic tyrosine kinase **Src**, which phosphorylates other signaling proteins on tyrosines.

-Yet another is **phosphoinositide 3-kinase (PI 3-kinase),** which phosphorylates lipids rather than proteins.

- The intracellular signaling proteins that bind to phosphotyrosines have varied structures and functions.

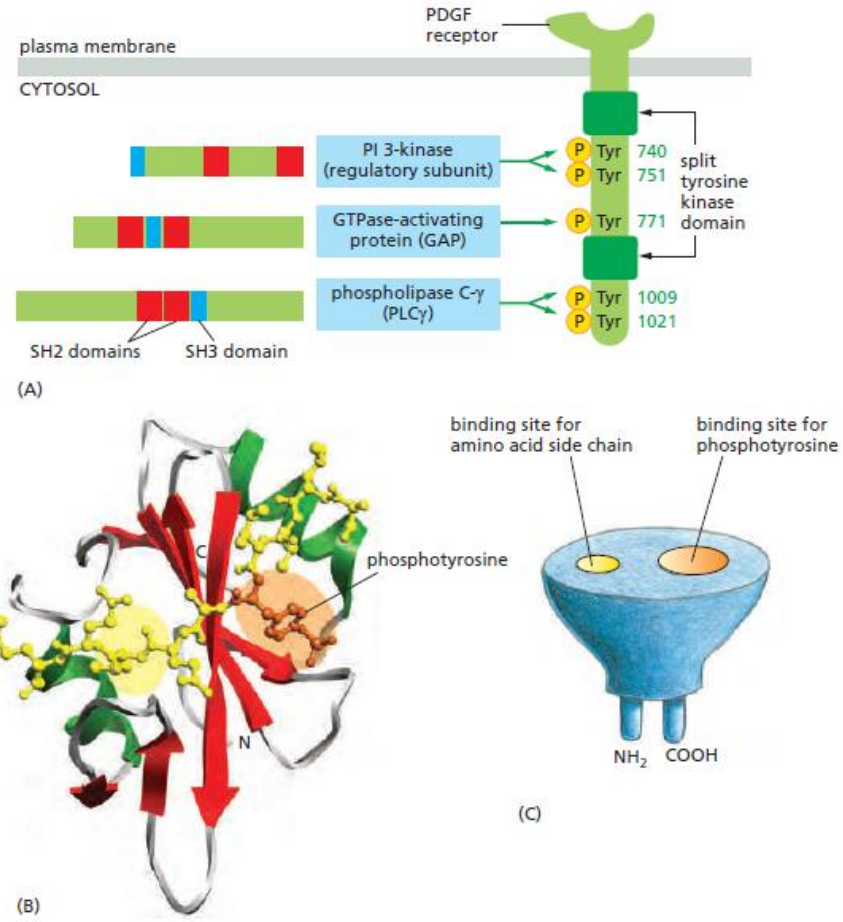
- However, they usually share highly conserved phosphotyrosine-binding domains:

These can be either **SH2 domains** (for Src homology region) or, less commonly, **PTB domains** (for phosphotyrosine-binding).

Proteins with SH2 Domains Bind to Phosphorylated Tyrosines

By recognizing specific phosphorylated tyrosines, these small interaction domains enable the proteins that contain them to bind to activated RTKs, as well as to many other intracellular signaling proteins that have been transiently phosphorylated on tyrosines.

Many signaling proteins also contain other interaction domains that allow them to interact specifically with other proteins as part of the signaling process. These domains include the **SH3 domain**, which binds to proline-rich motifs in intracellular proteins (see Figure 15–11).



This drawing of a receptor for **platelet derived growth factor** (**PDGF**) shows five phosphotyrosine docking sites, three in the kinase insert region and two on the C-terminal tail, to which the three signaling proteins shown bind as indicated.

The numbers on the right indicate the positions of the tyrosines in the polypeptide chain.

These binding sites have been identified by using recombinant DNA technology to mutate specific tyrosines in the receptor.

Mutation of tyrosines 1009 and 1021, for example, prevents the binding and activation of PLCγ, so that receptor activation no longer stimulates the inositol phospholipid signaling pathway.

The locations of the SH2 (red) and SH3 (blue) domains in the three signaling proteins are indicated.

(Additional phosphotyrosine docking sites on this receptor are not shown, including those that bind the cytoplasmic tyrosine kinase Src and two adaptor proteins.)

It is unclear how many signaling proteins can bind simultaneously to a single RTK?

Proteins with SH2 Domains Bind to Phosphorylated Tyrosines

- Not all proteins that bind to activated RTKs via SH2 domains help to **relay** the signal onward.

- Some act to **decrease** the signaling process, providing negative feedback:

-One example is the **c-Cbl protein**, which can dock on some activated receptors and catalyze their ubiquitylation, covalently adding one or more ubiquitin molecules to specific sites on the receptor.
-This promotes the endocytosis and degradation of the receptors in lysosomes—an example of receptor down-regulation.
-Endocytic proteins that contain **ubiquitin interaction motifs (UIMs)** recognize the ubiquitylated RTKs and direct them into **clathrin-coated vesicles** and, ultimately, into lysosomes.
-Mutations that inactivate c-Cbl-dependent RTK down-regulation cause **prolonged RTK signaling** and thereby promote the development of **cancer**.

- As is the case for GPCRs, ligand-induced endocytosis of RTKs does not always decrease signaling.
- In some cases, RTKs are endocytosed with their bound signaling proteins and continue to signal from endosomes or other intracellular compartments:

This mechanism, for example, allows **nerve growth factor (NGF)** to bind to its specific RTK (called **TrkA**) at the end of a long nerve cell axon and signal to the cell body of the same cell a long distance away.
Here, signaling endocytic vesicles containing TrkA, with NGF bound on the lumenal side and signaling proteins docked on the cytosolic side, are transported along the axon to the cell body, where they signal the cell to survive.

- Some signaling proteins are composed almost entirely of **SH2** and **SH3** domains and function as adaptors to couple tyrosine-phosphorylated proteins to other proteins that do not have their own SH2 domains.

Adaptor proteins of this type help to couple activated RTKs to the important signaling protein **Ras**, a monomeric GTPase that, in turn, can activate various downstream signaling pathways.

The GTPase Ras Mediates Signaling by Most RTKs

- The Ras superfamily consists of various families of monomeric GTPases, but only the **Ras** and **Rho** families relay signals from cell-surface receptors.

- By interacting with different intracellular signaling proteins, a single Ras or Rho family member can coordinately spread the signal along several distinct downstream signaling pathways, thereby acting as a **signaling hub**.
- There are three major, closely related Ras proteins in humans: H-, K-, and N-Ras.

- Although they have subtly different functions, they are thought to work in the same way, and we will refer to them simply as Ras.

TABLE 15–5 The Ras Superfamily of Monomeric GTPases

Family	Some family members	Some functions
Ras	H-Ras, K-Ras, N-Ras	Relay signals from RTKs
	Rheb	Activates mTOR to stimulate cell growth
	Rap1	Activated by a cyclic-AMP-dependent GEF; influences cell adhesion by activating integrins
Rho*	Rho, Rac, Cdc42	Relay signals from surface receptors to the cytoskeleton and elsewhere
ARF*	ARF1–ARF6	Regulate assembly of protein coats on intracellular vesicles
Rab*	Rab1–60	Regulate intracellular vesicle traffic
Ran*	Ran	Regulates mitotic spindle assembly and nuclear transport of RNAs and proteins

*The Rho family is discussed in Chapter 16, the ARF and Rab proteins in Chapter 13, and Ran in Chapters 12 and 17. The three-dimensional structure of Ras is shown in Figure 3–67.

The GTPase Ras Mediates Signaling by Most RTKs

- Like many monomeric GTPases, **Ras** contains one or more **covalently attached lipid groups** that help anchor the protein to the cytoplasmic face of the membrane, from where it relays signals to other parts of the cell.

- Ras is often required, for example, when RTKs signal to the nucleus to stimulate cell proliferation or differentiation, both of which require changes in gene expression.

- If Ras function is inhibited by various experimental approaches, the cell proliferation or differentiation responses normally induced by the activated RTKs do not occur.

- Conversely, **30% of human tumors express hyperactive mutant forms** of Ras, which contribute to the uncontrolled proliferation of the **cancer** cells.

- Like other GTP-binding proteins, Ras functions as a molecular switch, cycling between two distinct conformational states—active when GTP is bound and inactive when GDP is bound.

- For monomeric GTPases in general, two classes of signaling proteins regulate Ras activity by influencing its transition between active and inactive states:

-**Ras guanine nucleotide exchange factors (Ras-GEFs)** stimulate the dissociation of GDP and the subsequent uptake of GTP from the cytosol, thereby activating Ras.

-**Ras GTPase-activating proteins (Ras-GAPs)** increase the rate of hydrolysis of bound GTP by Ras, thereby inactivating Ras.

- **Hyperactive mutant forms of Ras** are resistant to GAP-mediated GTPase stimulation and are locked permanently in the GTP-bound active state, which is why they promote the development of cancer.

The GTPase Ras Mediates Signaling by Most RTKs

But how do RTKs normally activate Ras?

- In principle, they could either **activate a Ras-GEF** or **inhibit a Ras-GAP**.
- Even though some **GAPs** bind directly (via their SH2 domains) to activated RTKs (see Figure 15–46A), it is the indirect coupling of the receptor to a **Ras-GEF** that drives Ras into its active state.
- The loss of function of a Ras-GEF has a similar effect to the loss of function of Ras itself.
- Activation of the other Ras superfamily proteins, including those of the Rho family, also occurs through the activation of GEFs.

The particular GEF determines in which membrane the GTPase is activated and, by acting as a scaffold, it can also determine which downstream proteins the GTPase activates.

The GTPase Ras Mediates Signaling by Most RTKs

-The **GEF** that mediates Ras activation by RTKs was discovered by genetic studies of eye development in Drosophila, where an RTK called **Sevenless (Sev)** is required for the formation of a photoreceptor cell called R7.
-Genetic screens for components of this signaling pathway led to the discovery of a Ras-GEF called **Son-of-sevenless (Sos).**
-Further genetic screens uncovered another protein, now called **Grb2**, which is an adaptor protein that links the Sev receptor to the Sos protein; the SH2 domain of the Grb2 adaptor binds to the activated receptor, while its two SH3 domains bind to Sos.
-Sos then promotes Ras activation.

Biochemical and cell biological studies have shown that Grb2 and Sos also link activated RTKs to Ras in mammalian cells, revealing that this is a **highly conserved mechanism in RTK signaling**.

Once activated, Ras activates various other signaling proteins to relay the signal downstream