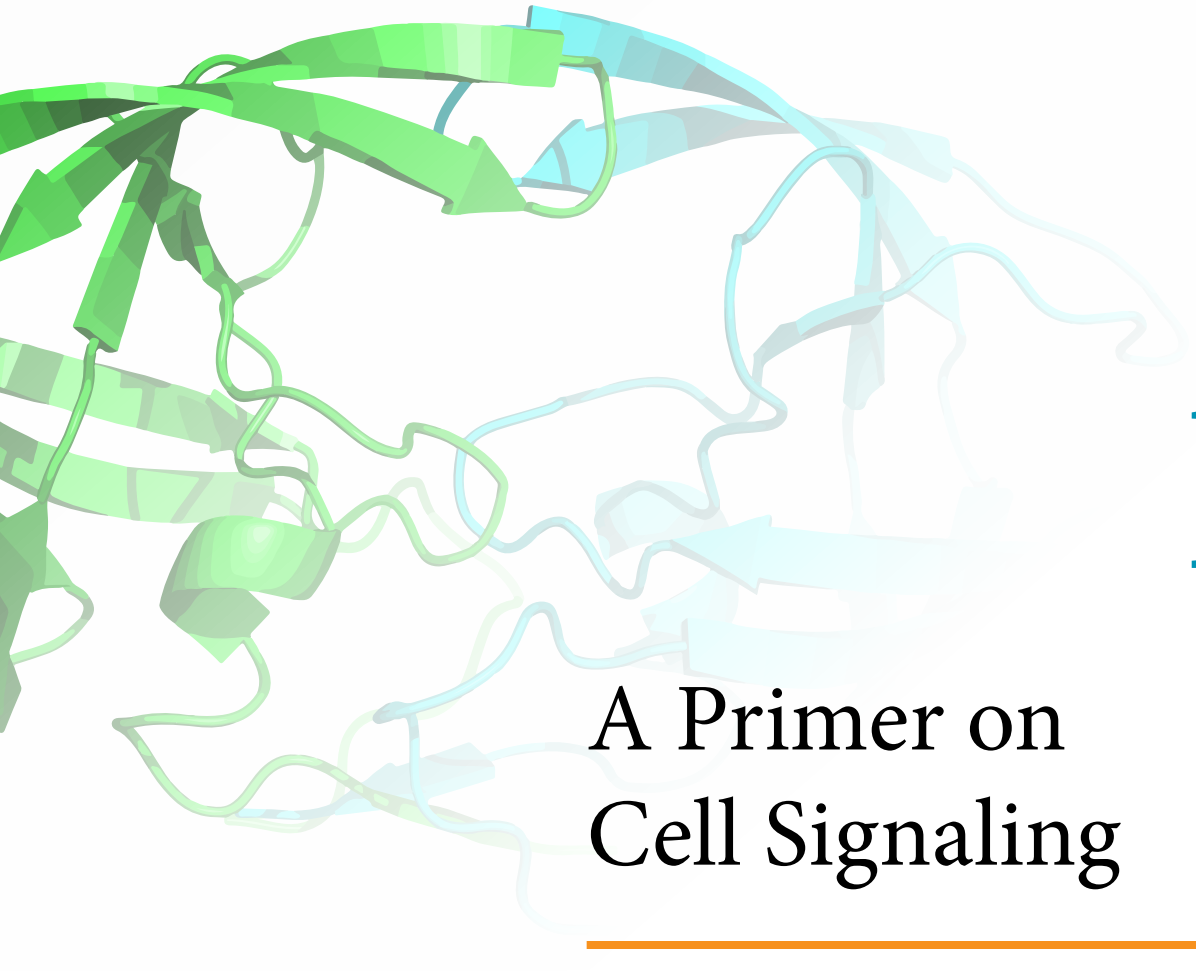

17

A Primer on Cell Signaling

Goal

To understand how cells detect a signal from the environment and how the signal elicits an intracellular response.

Objectives

After this chapter, you should be able to

- explain the role of a membrane receptor in detecting and transducing a signal to the inside of the cell.
- describe how protein kinases catalyze the transfer of phosphates to proteins.
- explain why ATP is both kinetically stable and energetically unstable.
- explain how a GTPase acts as a switch.
- explain how a phosphorylation cascade amplifies a signal.

The fundamental unit of life, the cell, is capable of growing and dividing autonomously. But that doesn't mean that a cell is oblivious to other cells in its environment. On the contrary, cells actively communicate with each other and do so by exchanging chemical signals. Even the simplest life forms, such as bacteria, talk to each other by exchanging small molecules. The cells of higher organisms communicate with each other through a rich repertoire of signaling molecules that range from small molecules, including gases, to proteins, lipids and carbohydrates. These signals communicate information about cell population density, the identity of neighboring cells, whether a cell should stop growing or should divide, whether it should adhere to a surface or should migrate, or whether it should differentiate into a specialized cell type. In short, cells communicate with each other by means of chemical languages in which they release and respond to signaling molecules.

Signaling molecules fall into two categories: those that are capable of diffusing through the cytoplasmic membrane to reach targets inside the cell and those that can't penetrate the membrane and instead act on targets on the cell surface. Examples of signals that can penetrate the outer barrier of the cell are the gas nitric oxide, certain non-gaseous small molecules such as retinoic acid, and steroid hormones, such as corticosteroids and sex hormones. Examples of signaling molecules that act at the cell surface

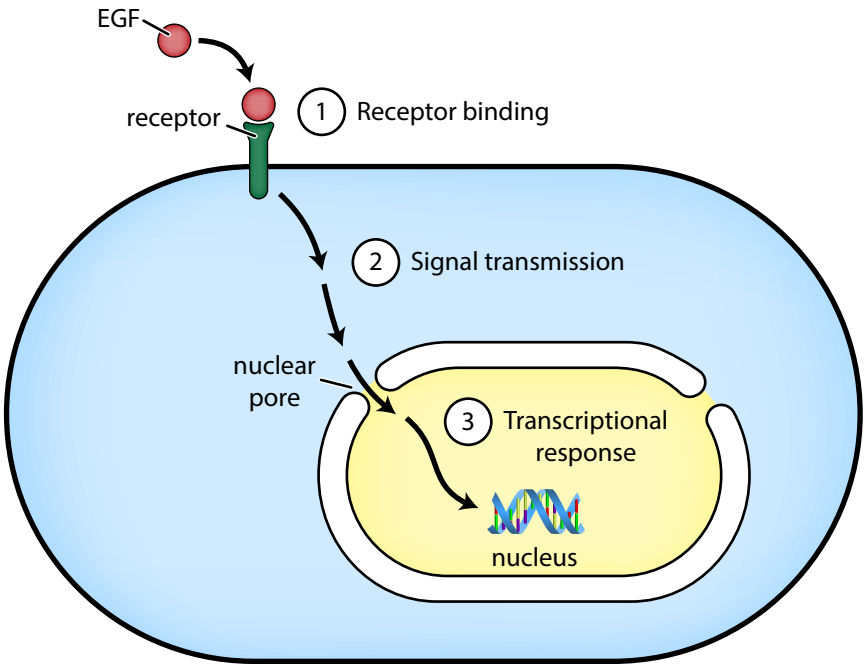

Figure 1 Growth factor signaling results in a transcriptional response

An extracellular growth factor (in this case EGF; see text) binds to a growth factor receptor. This signal is transduced and ultimately transmitted to the nucleus, where it results in a change in transcription.

are neurotransmitters, which as you will recall from Chapter 15 bind to receptors on the surface of neurons to trigger the influx of sodium ions, and growth factors, which are the focus of this chapter.

Cell signaling is a vast subject involving many different kinds of chemical communication. We will not attempt to survey all of the ways that cells converse with each other. Rather, we will restrict ourselves to a single conversation, if you will, one that is mediated by a protein growth factor. The goal is to explain how a signaling molecule can influence the behavior of a cell without entering the cell.

Growth factors stimulate cell growth without crossing the cytoplasmic membrane

Animal cells need to control their growth. They do so in part by relying on growth factors that are produced by other cells and secreted into the extracellular milieu. Because growth factors cannot enter cells, these signaling molecules need to communicate their presence to the inside of the cell. This communication is achieved by **receptor** proteins, which are embedded in the cytoplasmic membrane. The growth factor binds to its receptor, which in turn transduces a signal across the membrane to the inside of the cell (Figure 1). The signal sets in motion a chain of events referred to as a **signaling pathway** that culminates, in the case of the growth factor we will be considering, in a transcriptional response in the nucleus of the cell.

One such growth factor is **epidermal growth factor** (**EGF**). EGF is a protein of only 53 amino acids (Figure 2). It is essential for embryogenesis, and it plays a critical role in wound healing. EGF is also made in large amounts by certain tumors, where it promotes tumor growth.

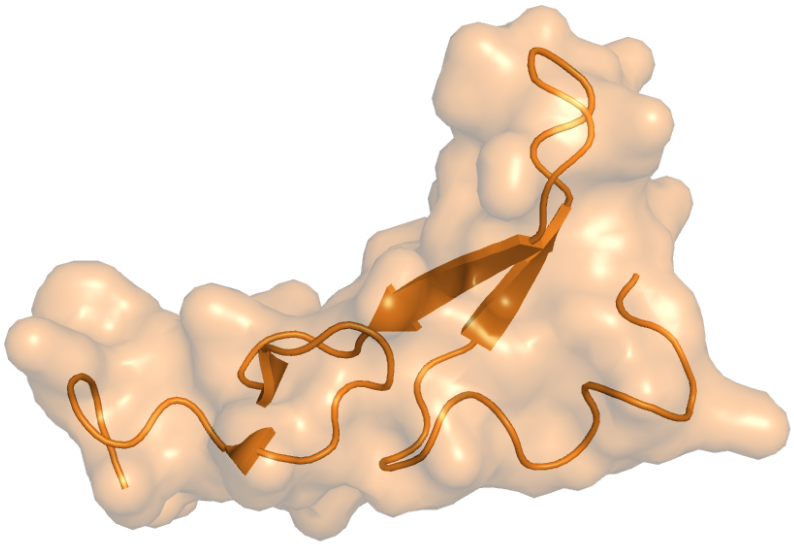

Figure 2 **EGF is a small extracellular protein**

The signaling pathway in overview

The growth factor signaling pathway as exemplified by EGF is a complex cascade involving multiple proteins and enzymes. The proteins in the pathway go by various names, and the pathway has multiple branches and targets. Hence, for the sake of simplicity we will focus on the core features of a single pathway and use generic names as often as possible for its protein components. Our goal is to consider the underlying concepts in signaling, not the details of particular signaling systems.

In broad outline the signaling pathway operates as follows:

- *Growth factor binds to receptor*. The pathway begins with the binding of a protein growth factor such as EGF to its receptor in the cytoplasmic membrane. This binding causes a structural change in the receptor that activates an enzyme intrinsic to the receptor known as a **tyrosine kinase**. The kinase domain is located on the cytoplasmic face of the receptor. Once activated, the kinase attaches phosphate groups to tyrosine residues in the receptor itself in a process known as **autophosphorylation**.

- *Adaptor and GEF are recruited*. In this phosphorylated state, the receptor becomes a binding site for an **adaptor protein**, which in turn recruits a protein known as a **guanine-nucleotide exchange factor** or **GEF**.

- *The GEF activates the small GTPase Ras*. The GEF, in turn, activates an enzyme known as a **small GTPase**, which hydrolyzes GTP to GDP. The prototypical member of the small GTPase family is known as **Ras**. Ras is a switch protein that exists in an ON state in which it is bound to GTP (Ras-GTP) and an OFF state in which it is bound to GDP (Ras-GDP). The GEF activates Ras by promoting the exchange of GTP for GDP.

- *Ras-GTP activates the MAP kinase cascade*. Finally, the GTPase in its active, GTP-bound state triggers a phosphorylation cascade in which it activates a kinase (which phosphorylates serine and threonine residues)

called **MAP kinase kinase kinase**, which in turn phosphorylates a second kinase in the cascade called **MAP kinase kinase**, which in a terminal step phosphorylates a kinase called **MAP kinase**. Finally, the phosphorylated MAP kinase enters the nucleus, where it activates proteins involved in gene transcription.

As you can see, receiving a growth signal from outside the cell and importing that information into the nucleus is no simple matter! To understand how this complex cascade works, let's consider each of the steps in more depth, starting with the receptor.

Growth factor receptors consist of three domains

Growth factors like EGF are extracellular proteins. Since these molecules are present at very low concentrations, cells must have a sensitive mechanism for detecting them. The first step in the detection mechanism involves binding of the growth factor to the extracellular portion of a transmembrane receptor protein that is embedded in the cytoplasmic membrane. This step is known as "recognition," which is a term used to denote a specific interaction between molecules. Interactions between two proteins such as these can involve the hydrophobic effect as well as any of the electrostatic forces (e.g., ionic, polar, or van der Waals) that we learned about in previous chapters. The ability of two molecules to bind to one another selectively in the presence of enormous numbers of other molecules is a fundamental feature of many biological interactions.

The receptors that bind to growth factors, known as growth factor receptors, consist of three domains: (1) an extracellular domain that "recognizes" and

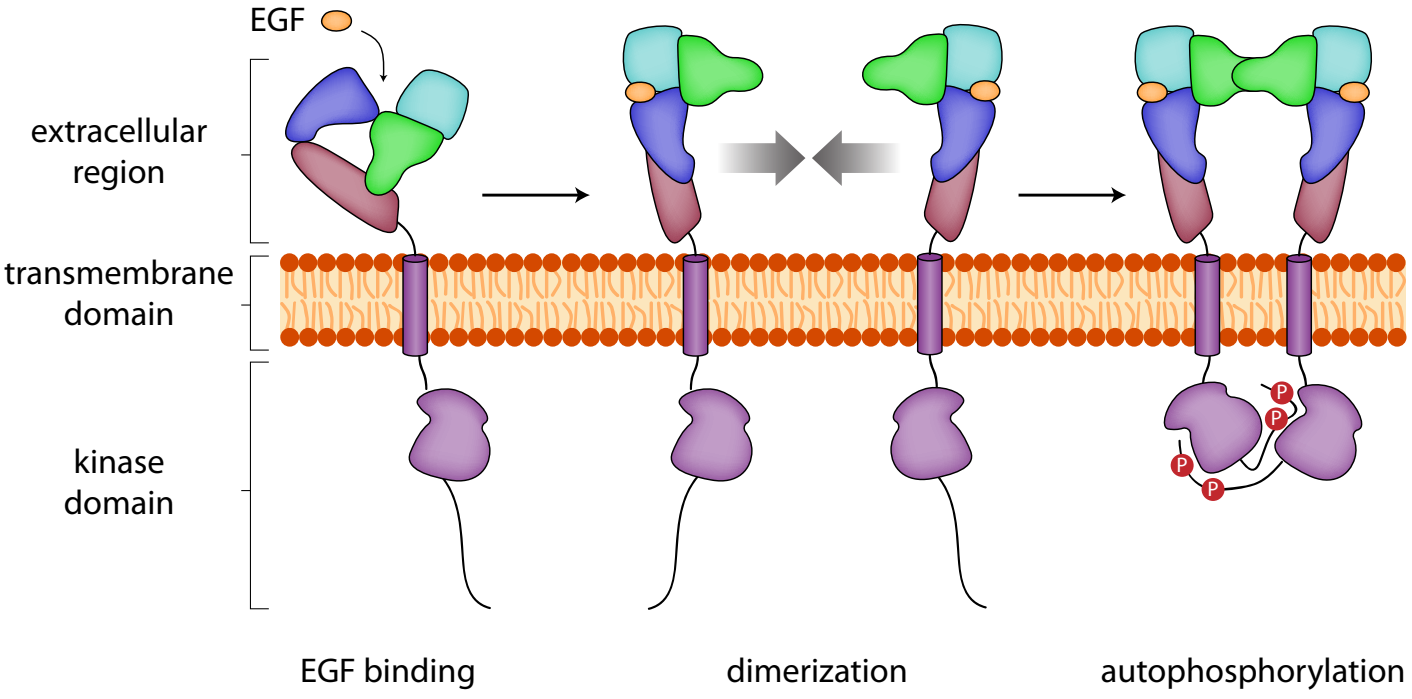

Figure 3 EGF binding causes the receptor to dimerize and undergo *trans* autophosphorylation

Binding of EGF causes a conformational change in the extracellular region of the receptor that promotes dimerization. Dimerization brings the intracellular kinase domains of each receptor close to one another, allowing each kinase domain to phosphorylate the other domain (*trans* autophosphorylation).

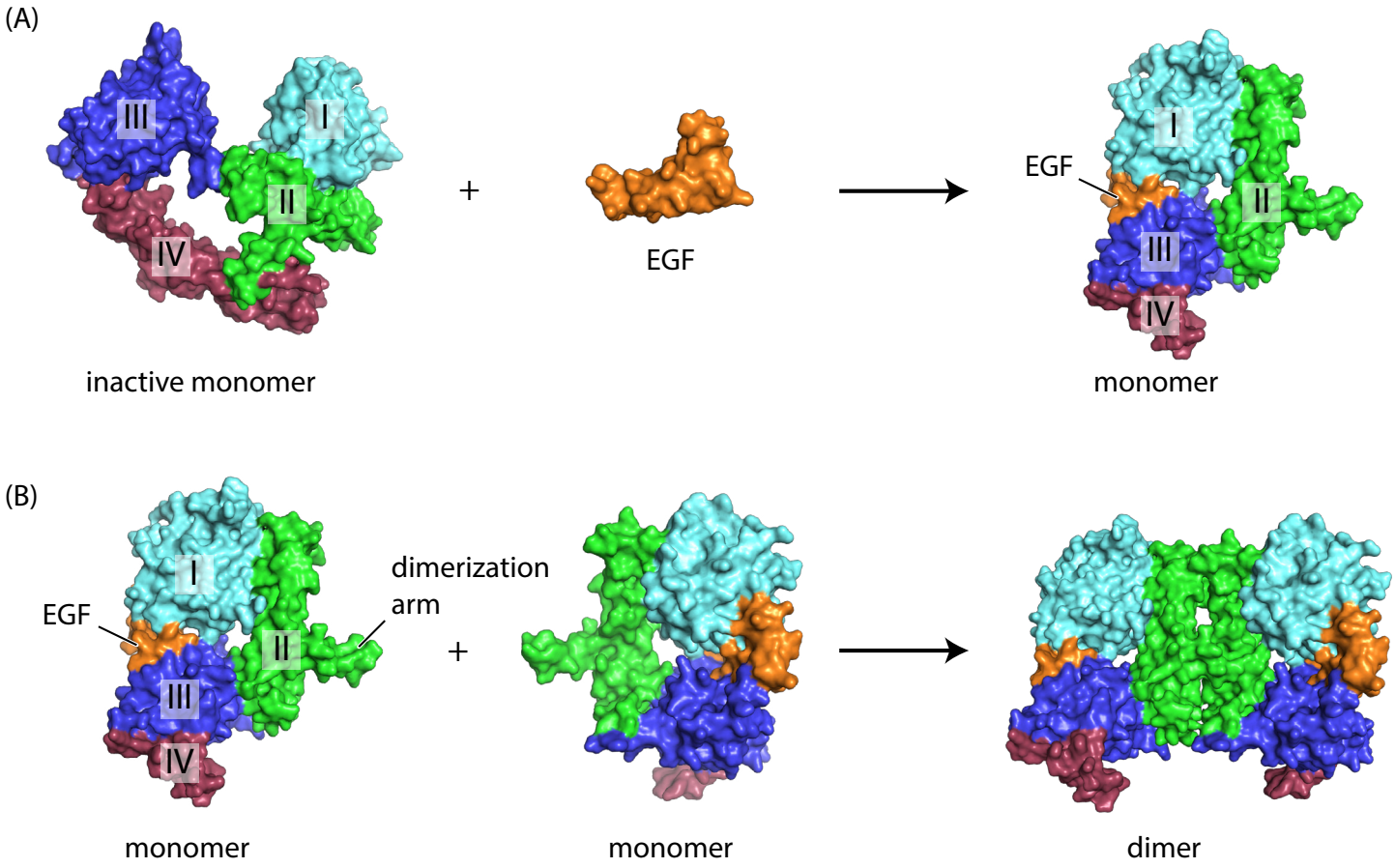

Figure 4 **The receptor is inactive in the absence of EGF**

(A) Shown is the structure of the extracellular portion of the receptor, which consists of four domains termed I-IV. In the absence of EGF, the receptor is in an inactive state in which domains II and IV interact with each other. This interaction prevents dimerization by preventing domain II of one monomer from interacting with domain II of another monomer, which is required for dimer formation. When EGF is present, however, the ligand interacts with domains I and III, causing a conformational change that disrupts the interaction between domains II and IV. (B) Binding of EGF the receptor exposes the dimerization arm of domain II, which binds to a second receptor monomer to form the active dimer.

binds to the growth factor, (2) a transmembrane domain that spans the lipid bilayer of the membrane, and (3) an intracellular domain that has protein kinase activity. The extracellular domain contains a cleft whose shape and charge characteristics are complementary to those of the growth factor. The transmembrane domain typically consists of hydrophobic amino acids arrayed in an α-helical conformation so that all of the backbone hydrogen bond donors/acceptors are internally satisfied in the nonpolar interior of the bilayer.

Dimerization of growth factor receptors activates kinase activity

Growth factor receptors, as well as many other receptors, exist in two states: active and inactive. When no **ligand** (i.e., the molecule to which the receptor binds, such as growth factor) is bound, the receptor exists as a monomer, and the intracellular enzyme activity is turned OFF. Binding of growth factor to the growth factor receptor's extracellular domain changes the shape of the receptor and causes the association, or **dimerization**, of two molecules of growth factor receptor (Figure 3). This dimerization turns ON the kinase activity of the receptor's intracellular domain. Protein

kinases are enzymes that transfer **phosphoryl (-PO_3^{-2}) groups** to reactive amino acid side chains in a process called **phosphorylation** (Box 1). The growth factor receptor is a tyrosine kinase, meaning that it specifically transfers phosphoryl groups to tyrosine side chains, thereby transforming the tyrosine's hydroxyl (-OH) group into a phosphate (-PO_4^{-2}) group. Kinases are specific, acting only on particular side chains. The particular tyrosine side chains that are phosphorylated by the growth factor receptor's intracellular kinase domain are located in the intracellular domain of the receptor itself; thus the receptor undergoes autophosphorylation as we will explain.

How does binding of the growth-factor ligand promote dimerization? When EGF is absent, the receptor adopts a closed conformation. Conversely, when EGF is present the receptor adopts a conformation that can form a dimer. The extracellular region of the receptor consists of four domains, numbered I-IV (Figure 4). The dimer forms when two monomers interact via the dimerization arm of domain II. When the receptor is inactive, however, the dimerization arm of domain II interacts with domain IV, obscuring it and preventing formation of the dimer. The receptor remains in this inactive state until and unless it is specifically activated by binding of the EGF ligand.

Binding of EGF to the receptor stabilizes an extended conformation of the receptor. The reason for this is that EGF can interact with amino acid side chains in domains I and III of the receptor; however, it can only interact simultaneously with both regions when the receptor is in its open, extended conformation. In its open conformation, domains II and IV do not interact, thereby exposing the dimerization arm of domain II and allowing the receptor to dimerize.

How does dimerization promote autophosphorylation? Upon formation of a dimer, the intracellular kinase domains of the two receptor monomers are held in close proximity to one another. Forcing these domains close to one another markedly increases the likelihood that one domain will interact with the other; in other words, formation of a dimer causes a marked increase in the local concentration of the two intracellular kinase domains and their substrates. In a process called ***trans*** autophosphorylation, the kinase domain of one receptor monomer phosphorylates tyrosine residues in the second kinase domain, and vice versa. Phosphorylation drives the

Box 1 Protein phosphorylation

Phosphorylation alters the chemical properties of amino acid side chains

A protein kinase is an enzyme that catalyzes the phosphorylation of amino acids containing reactive side chains, such as tyrosine, serine, and threonine. ATP is the substrate that donates the phosphoryl group that is transferred. In a kinase-catalyzed reaction, the hydroxyl group of the amino acid side chain acts as a nucleophile and forms a new bond to the phosphorus atom of the terminal, gamma (γ) phosphate of ATP, releasing ADP as a leaving group and resulting in the transfer of the phosphoryl group to the hydroxyl group of the amino acid side chain (Figure 5). Receptors for growth factors have tyrosine kinase activity, but other kinds of protein kinases, such as MAP kinases (below), phosphorylate serine and threonine residues. Generally speaking, protein kinases phosphorylate one or a small number of specific target amino acids in a given protein.

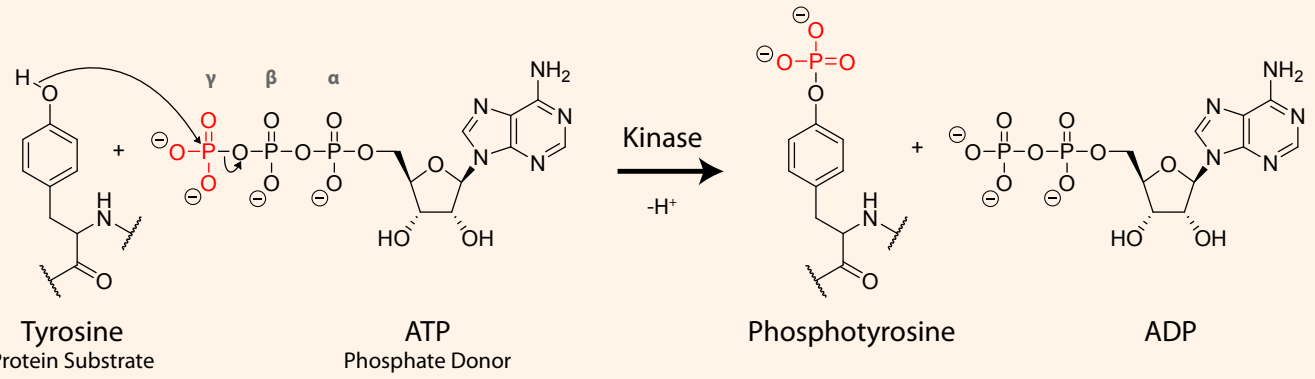

Figure 5 **Phosphotransfer reactions involve ATP and a protein substrate**

Phosphotransfer reactions involve the transfer of a phosphoryl group (*red*) from the γ position of an ATP donor to a hydroxyl group on a protein substrate. In this example, the substrate is the side chain of tyrosine. This reaction is catalyzed by a protein kinase.

Phosphorylation of amino acid side chains significantly changes their chemical properties. The chemical transition that takes place, from a hydroxyl group to a phosphate ester group, changes both the size and the charge of the side chain (Figure 6). Hydroxyl groups are small and polar, whereas phosphate groups are larger and bear a dense negative charge. These changes in the properties of side chains can alter the structures and chemical properties of proteins. This should not be surprising, since we saw in a previous chapter that altering single amino acids can have significant effects on protein structure and folding (e.g., the amino acid substitution in hemoglobin that causes sickle cell anemia).

Phosphorylation regulates protein function in a variety of ways. Examples include controlling enzymatic activity, regulating the localization of proteins within the cell, controlling protein stability (i.e., the rate of protein degradation), and regulating the association of proteins with other molecules. All of these effects on protein function can be traced to changes in protein structure and chemical properties that result from phosphorylation.

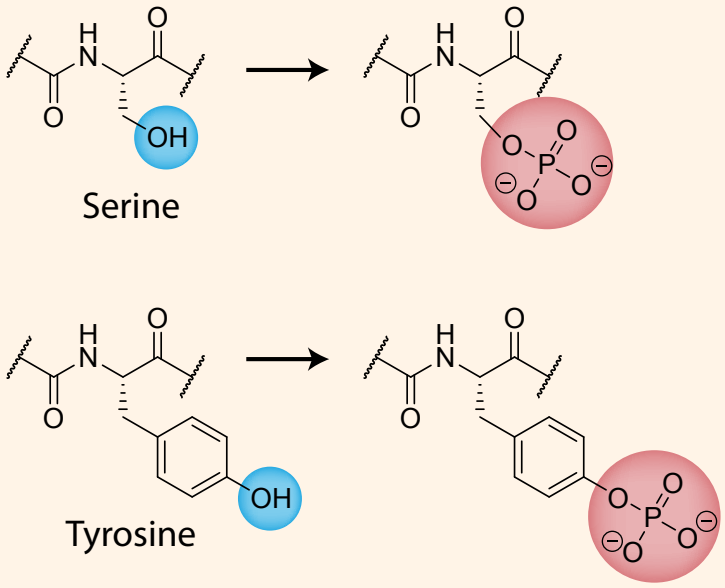

Figure 6 **Phosphorylation of serine and tyrosine**

Shown are the side chains of serine and tyrosine in both their phosphorylated and unphosphorylated states (*right* and *left*, respectively). Phosphorylation alters the charge and physical size of amino acid side chains by transforming a small, polar hydroxyl group (*blue*) into a bulky, densely negatively charged phosphate group (*red*).

Phosphorylation is energetically favorable

ATP is the principal "energy currency" used by the cell to drive reactions. This is because the hydrolysis of ATP is energetically favorable. In fact, the hydrolysis of the γ phosphate of ATP has a $\Delta G°_{rxn}$ value of −7 kcal/mol. This is the case because the phosphoanhydride (P-O) bonds that connect the γ and β phosphates are relatively weak; therefore, breaking one of these P-O bonds requires a small input of energy compared to the relatively large amount of energy that is released by the formation of the new bond that forms between phosphorus and the nucleophilic oxygen atom of the attacking water molecule. As a result, a large amount of energy is released during ATP hydrolysis. From a chemical perspective, the kinase reaction and ATP hydrolysis are similar, but differ in that the attacking group is a hydroxyl in one case and water in the other. As a consequence, the energetics of the kinase reaction are similar to the thermodynamics of ATP hydrolysis.

Why is ATP hydrolysis so favorable? First, the four negative charges in ATP repel one another. Breaking a phosphoanhydride linkage separates these charges, resulting in a lower-energy state in which like charges are moved farther away from one another. In addition, the released phosphate group is stabilized by resonance, as in the case of the peptide bond as you learned earlier. The resonance structures of phosphate (Figure 7) allow the negative charges to be shared between multiple atoms, leading to a lower-energy state in which charges are more distributed and less densely packed.

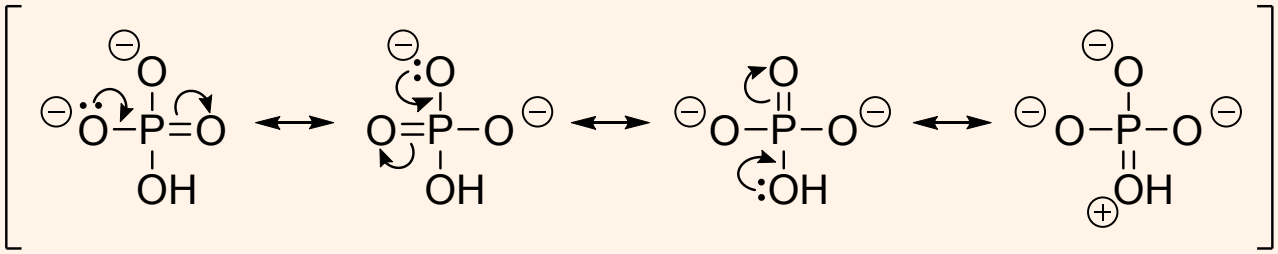

Figure 7 **Phosphate is stabilized by resonance**

Shown are the resonance forms of the free phosphate ion. Its negative charges are distributed in many different ways among its atoms, thereby stabilizing the molecule.

Kinases accelerate the phosphorylation reaction and provide substrate specificity

As we learned earlier, reactions for which the value of $\Delta G°_{rxn}$ is negative have K_{eq} values that are greater than one. Therefore, the equilibrium of a phosphorylation reaction favors the products. Given that phosphorylation is favorable, you might wonder why cells require protein kinases. One reason is that kinases provide specificity so that only the correct amino acids in only the correct proteins are phosphorylated. Another reason is that the kinase is required to accelerate the rate of the reaction. Even though phosphorylation is favorable, it is a slow reaction whose rate is not consistent with a biological timescale. Phosphorylation is slow because the cloud of negative charge that surrounds the phosphate groups repels negatively charged nucleophiles, blocking access to the electrophilic phosphorus atom. The kinase accelerates the rate of the phosphorylation reaction by aligning the reactants, holding them in close proximity to one another, increasing the nucleophilicity of the nucleophilic hydroxyl group by making it more negative, and increasing the electrophilicity of the electrophilic phosphorus atom by making it more positive (i.e., by neutralizing the negative charge that surrounds it).

receptor into a conformation that allows an adaptor protein to bind and thereby initiate downstream signaling events, as we will explain.

Ras carries EGF signals downstream

As we have seen, the binding of EGF to its receptor causes the receptor to dimerize and undergo *trans* autophosphorylation. In its phosphorylated

state, the receptor is able to bind ("recruit") the next protein in the signaling cascade, the adaptor. The adaptor binds specifically to the phosphorylated receptor by interacting with the phosphorylated tyrosines. The adaptor, in turn, recruits yet another protein to the receptor complex called **GEF**, for guanine nucleotide exchange factor. The GEF activates the next protein in the signaling cascade, a membrane-tethered protein (due to a covalently

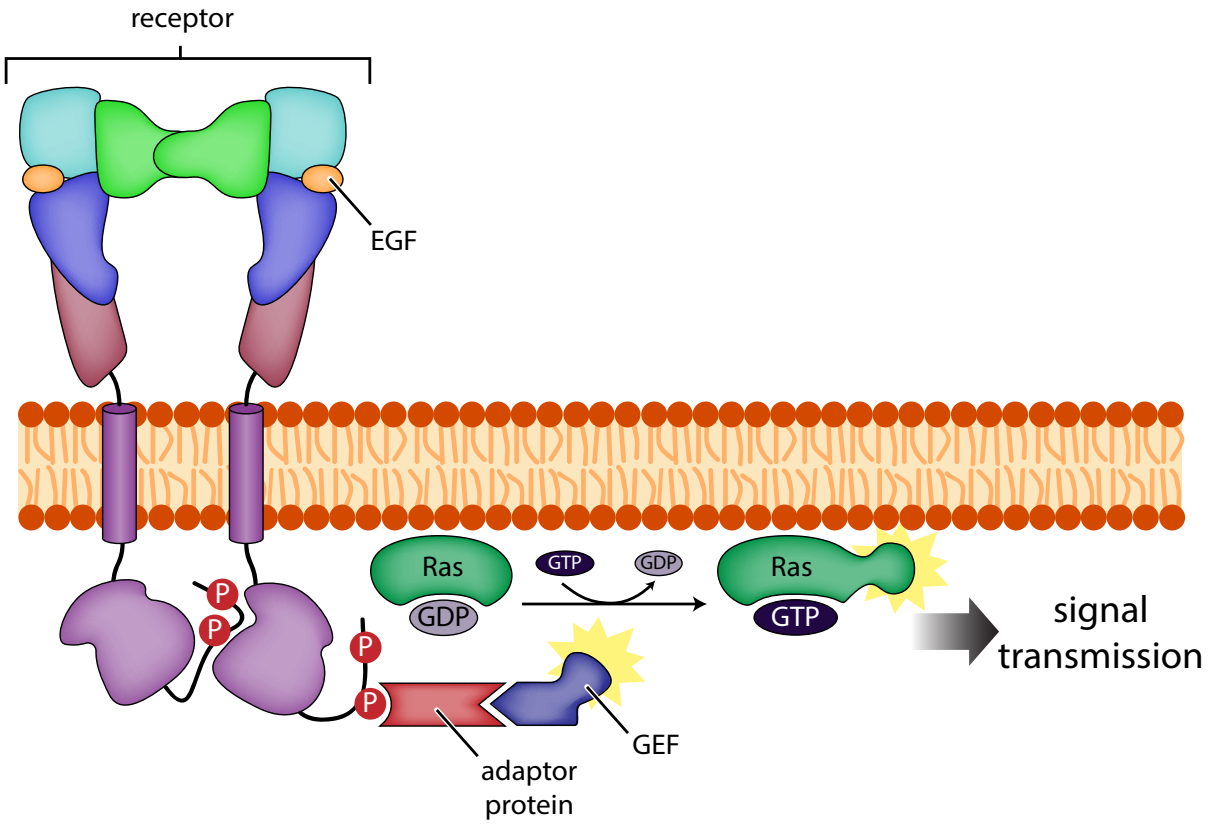

Figure 8 **Autophosphorylation of the receptor triggers Ras activation**

Autophosphorylation creates binding sites for an adaptor protein, which in turn recruits a GEF. The GEF stimulates the dissociation of Ras-GDP, allowing Ras to bind GTP in place of GDP. Upon binding GTP, Ras undergoes a conformational change that triggers downstream signaling events (activation of the MAP kinase cascade as we will come to).

attached lipid) called **Ras** (Figure 8). Ras exists in GDP-bound (Ras-GDP) and GTP-bound (Ras-GTP) states. The GEF activates Ras by promoting the exchange of GDP with GTP, as we now explain.

Ras is a switch protein that exists in GTP- or GDP-bound states

Ras is a member of a family of small GTPases, which hydrolyze GTP to form GDP and phosphate. These proteins can exist in an inactive form that is bound to the nucleotide GDP, Ras-GDP, and an active form that is bound to GTP, Ras-GTP. In its active Ras-GTP form, it binds to downstream protein kinases and activates them.

A GEF activates Ras by binding to Ras-GDP and promoting the release of the bound nucleotide. Ras-GDP is a highly stable complex, and it requires the action of its GEF to undergo dissociation (Box 2). Because the cellular concentration of GTP is higher than that of GDP, free Ras is more likely to bind GTP than GDP. Thus, the GEF, by causing Ras-GDP to dissociate, in effect promotes the exchange of GDP for GTP, thereby activating Ras.

Another important regulator of Ras is its **GAP** (GTPase activating protein).

Ras, like many small GTPases, is a poor enzyme, meaning that it hydrolyzes GTP very slowly. The GAP acts oppositely to the GEF; it binds to Ras-GTP and accelerates the hydrolysis of the bound GTP. In doing so, GAP turns off the activity of Ras-GTP by converting it to inactive Ras-GDP (Figure 9).

Why does the signaling pathway include this extra complication of having a protein that switches between GTP- and GDP-bound states? The answer is that the cycle of Ras activation and inactivation serves an important function in the fidelity of signaling. Because Ras-GTP is constantly converted back to its inactive, GDP-bound state by GAP-promoted hydrolysis, Ras-GTP molecules that arise spuriously are eliminated. This helps ensure that only Ras-GTP molecules that are being actively generated by ligand binding to the receptor reach a high enough concentration to trigger downstream signaling events.

This is the second example we have encountered of the involvement of a GTPase in helping to achieve fidelity in a biological process. You will recall from our discussion of protein synthesis that EF-Tu, which delivers charged tRNAs to the ribosome, is also a GTPase (Chapter 11). In the case of the EF-Tu GTPase, if a proper codon/anticodon match is made, EF-Tu·GTP undergoes hydrolysis (the ribosome acts as a GAP) and releases its cargo of charged tRNA. If not, the EF-Tu·GTP/charged-tRNA complex diffuses away from the ribosome without releasing its cargo.

Underscoring the importance of the GTPase activity of Ras, many human cancers are caused by mutants of Ras that are locked in the GTP-bound, active state. Such tumor cells activate signaling pathways even in the

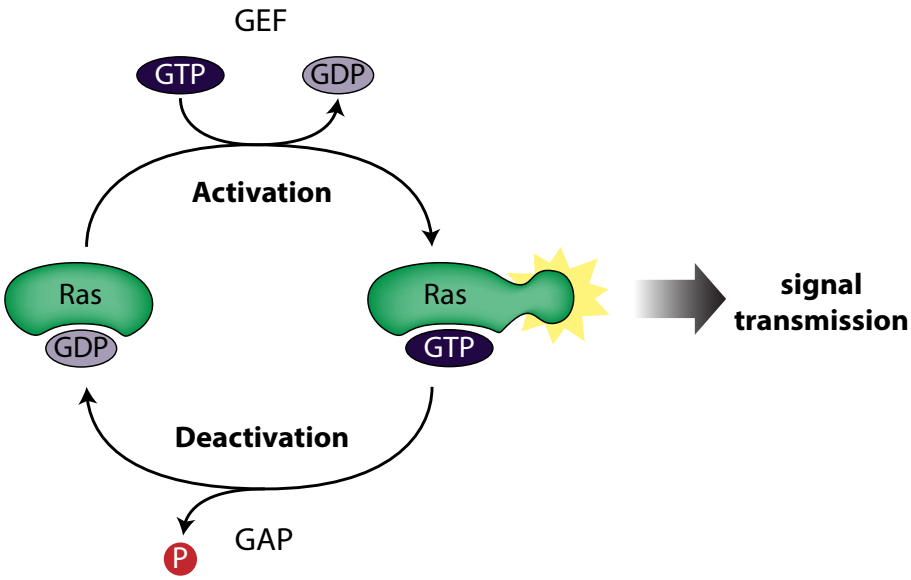

Figure 9 Ras is activated by its GEF and deactivated by its GAP

Ras exists in inactive (Ras-GDP) and active (Ras-GTP) states. When its GEF is recruited to the membrane receptor, it activates Ras by stimulating the release of GDP from Ras-GDP. Since GTP is at a much higher concentration in the cell than GDP, Ras binds to GTP upon releasing GDP. Ras-GTP is deactivated by a GAP that stimulates the GTPase activity of Ras, causing it to hydrolyze its bound GTP to GDP.

Box 2 The lifetime of the Ras-GDP complex can be estimated from its K_d

Ras is inactive when bound to GDP, and in order to become active, Ras must release GDP and bind GTP. Its GEF is required for the exchange of GDP for GTP because Ras binds GDP very tightly; in fact, it binds so tightly that it cannot let go of GDP by itself on a timescale that would be compatible with most biological processes. To understand this slow GDP release, we need to examine the kinetics of Ras-GDP binding and compare them to the timescales over which biological processes occur.

The equilibrium constant for this dissociation reaction (like the equilibrium constant for acid dissociation) is called the **equilibrium dissociation constant** ($\mathbf{K_d}$). As we will see, the K_d for a binding event is inversely correlated with the lifetime of the complex. That is, the more tightly something binds, the lower the K_d and the longer the complex persists. We can estimate the lifetime of a complex if we know the equilibrium dissociation constant for a binding event. Ras-GDP has a K_d of about 10^{-11} M. At equilibrium, the rate of complex dissociation equals the rate of complex formation; therefore, the dissociation rate constant (k_{off}) times the molar concentration of Ras-GDP is equal to the association rate constant (k_{on}) times the molar concentrations of Ras and GDP. Rearranging this equation shows that k_{off}/k_{on} is equal to [Ras][GDP]/[Ras-GDP], which is just the product of the concentration of products over the concentration of reactants. Both of these ratios equal the K_d (Figure 10).

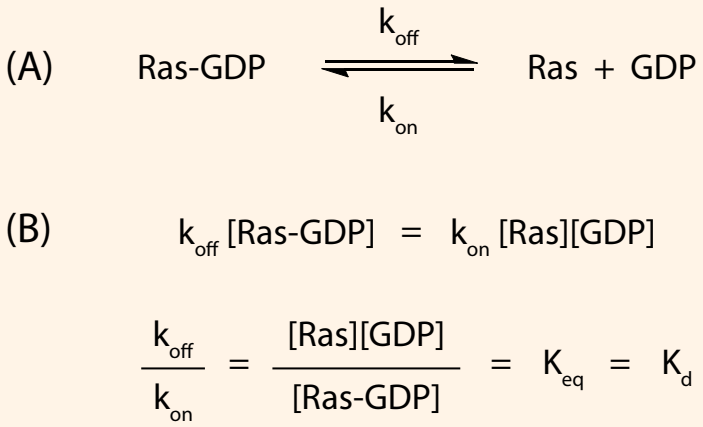

Figure 10 The equilibrium dissociation constant (K_d) equals the ratio of k_{off} to k_{on}

(A) Shown is the dissociation equilibrium for Ras-GDP. The rate constants for the dissociation and association reactions are given as k_{off} and k_{on}, respectively. (B) The rates of dissociation and association are equal at equilibrium; consequently k_{off} [Ras-GDP] equals k_{on} [Ras][GDP] at equilibrium. This equation can be rearranged, as shown, to express K_d, which is the equilibrium constant for the dissociation of a complex.

The rate of association of Ras and GDP to form Ras-GDP (k_{on}) has units of $M^{-1}s^{-1}$. The concentration term reflects the fact that two species must collide in solution for a binding event to occur. The time dependence reflects the fact that the collision frequency for two molecules depends on their size and shape (as well as the viscosity and temperature of the medium). Large molecules move more slowly than small molecules and collide more slowly. Thus, k_{on} values for large molecules tend to be smaller than k_{on} values for smaller molecules. The time dependence of k_{on} also reflects the fact that the molecules not only need to collide but also need to fit together in a productive way. Sometimes the molecules may need to undergo conformational changes to allow a good fit. Values of k_{on} are smaller when such reorganization is required for productive binding. The dissociation reaction is a unimolecular reaction (involving only one reactant); therefore, k_{off} has units of s^{-1}. Dissociation is not concentration-dependent because it involves only a single species. The time dependence of the dissociation reaction reflects how difficult it is to break the favorable interactions the keep the two molecules bound to each other.

The lifetime of Ras-GDP as a function of time can be represented as an exponential decay. A typical plot of complex concentration versus time is shown in Figure 11. Half-life ($t_{1/2}$) is the time it takes for half of the complexes to dissociate, and its value allows us to quantify the lifetime of a complex. The value of $t_{1/2}$ is given by the simple relationship $t_{1/2} = \ln(2)/k_{off}$. We can use this equation to calculate the half-life of any complex for which the value of k_{off} is known.

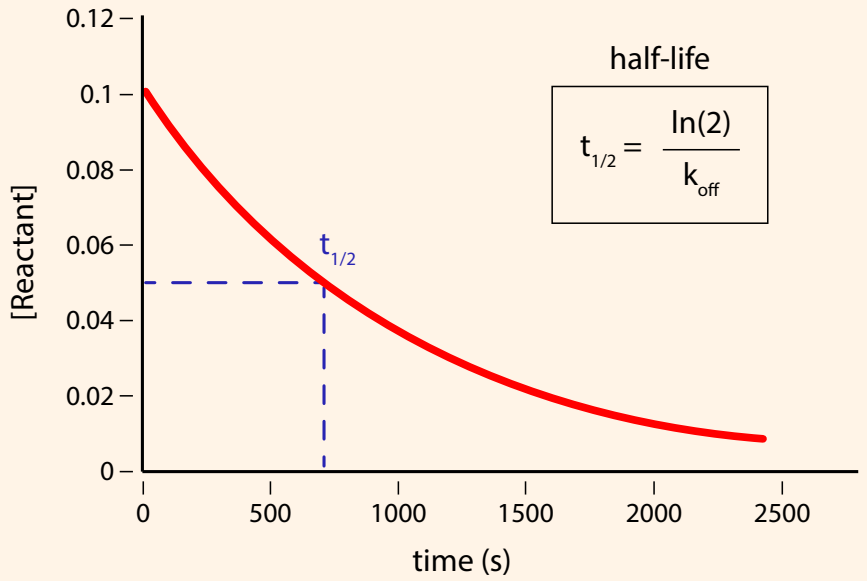

Figure 11 The half-life is the time it takes for the concentration of a reactant to decrease by half

Shown is a graph of a hypothetical dissociation reaction for which the reactant concentration is plotted versus time. The time it takes for [reactant] to reach half of its initial value is called the half-life ($t_{1/2}$), as indicated by the dotted lines. A half-life can be calculated from k_{off} using the equation shown in the inset. As an example, k_{off} equals 0.001 s^{-1} for the hypothetical reaction graphed here. Using the formula, we can calculate the half-life for this reaction to be ln(2)/(0.001 s^{-1}), or about 693 s.

A reasonable estimate of k_{on} for a small molecule binding to a protein is about 10^7-10^8 $M^{-1}s^{-1}$. Using this estimate we can calculate the half-life of the Ras-GDP complex from its K_d, which we noted earlier is about 10^{-11} M. That would mean that the $t_{1/2}$ for the Ras-GDP complex is 6,900 seconds, or more than 100 minutes. Biological reactions must occur on timescales of milliseconds to seconds for biology to work. You can immediately see that a half-life of 100 minutes is not compatible with life. In a cell, however, the lifetime of the Ras-GDP complex can be much shorter if its GEF has been recruited by an adaptor protein so that it is localized near the membrane-associated Ras-GDP complex and is able to facilitate nucleotide exchange. Thus, tight binding interactions in biology allow for complex regulatory mechanisms involving other proteins.

absence of growth factor signals, eliminating the normal controls that keep cell growth and proliferation in check.

Ras activates a cascade of MAP kinases to relay the signal to the nucleus

The activation of Ras triggered by growth factor binding to the growth factor receptor initiates a kinase cascade that relays the growth factor signal to the nucleus. Activated GTP-bound Ras, which is associated with the cytoplasmic face of the membrane, activates a series of kinases called **MAP kinases** that relay the growth factor signal to the nucleus (Figure 12). In their inactive forms, the kinases in the MAP kinase cascade are dephosphorylated. Activated Ras binds to a membrane-associated kinase called **MAPKKK** (MAP kinase kinase kinase), which turns on its kinase activity. MAPKKK then phosphorylates **MAPKK** (MAP kinase kinase), activating it so that it in turn phosphorylates **MAPK** (MAP kinase). The phosphorylation of MAPK causes it to dimerize. Dimerized MAPK is specifically recognized by the protein machinery that imports proteins into the nucleus (see Chapter 16). Once inside the nucleus, MAPK phosphorylates and activates various sequence-specific transcription factors, thus influencing which genes are transcribed. MAPK also phosphorylates RNA polymerase and basal factors, which are transcription factors that are involved in the transcription of almost all genes. Ultimately, the growth factor signal is communicated from

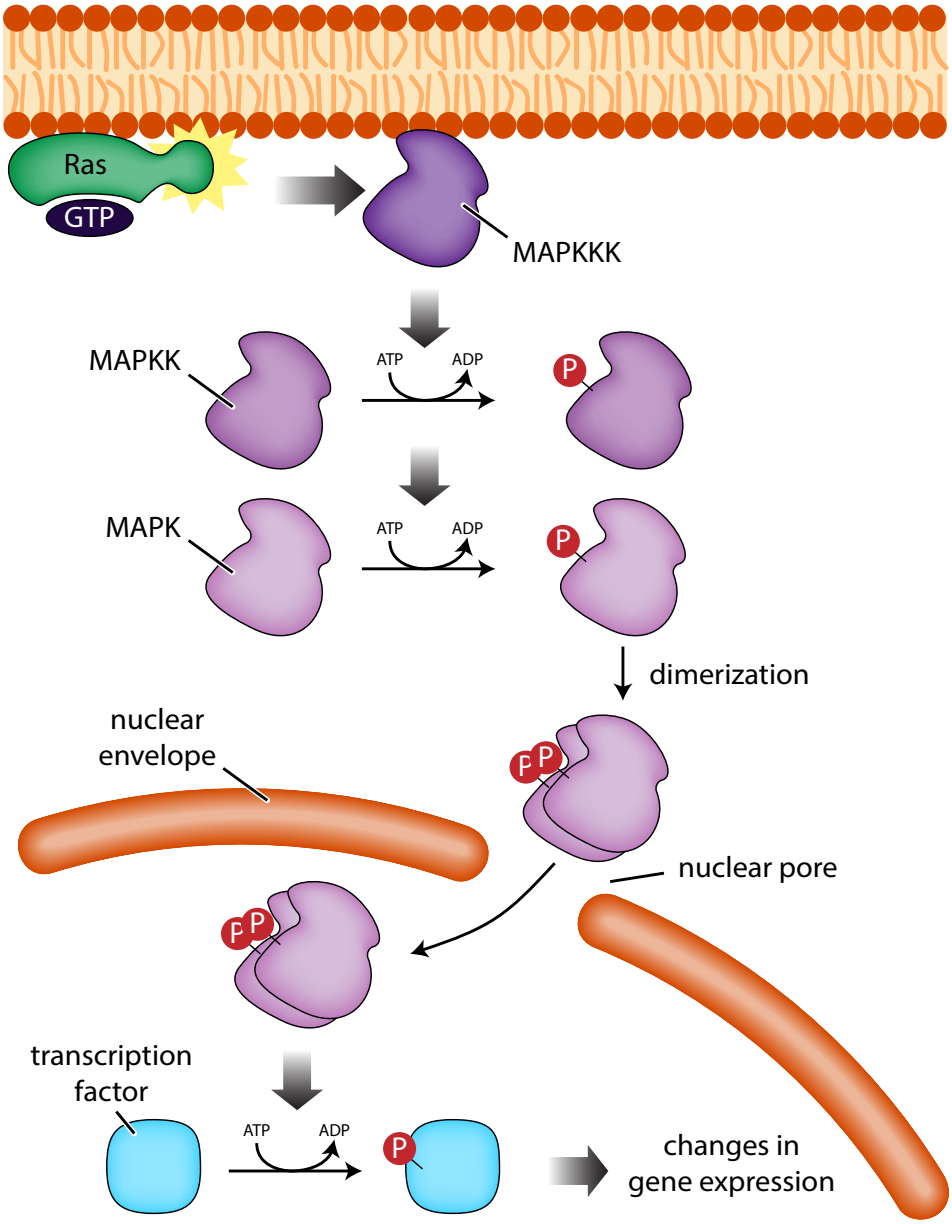

Figure 12 Ras-GTP initiates the MAP kinase cascade, relaying the growth factor signal to the nucleus

Ras-GTP interacts with MAPKKK to activate its enzymatic activity. The active MAPKKK catalyzes the phosphorylation of MAPKK. Phosphorylation activates MAPKK, allowing it to catalyze the phosphorylation of MAPK. The phosphorylation of MAPK causes it to form a dimer that is imported into the nucleus. In the nucleus, MAPK catalyzes the phosphorylation of various transcription factors, thereby causing changes in gene expression.

the outside of the cell to the nucleus, where changes in gene expression occur as a result.

The MAP kinase cascade plays an important role in amplifying the initial signal of growth factor binding to growth factor receptor (Figure 13). Each activated MAPKKK can activate several molecules of MAPKK, each of which in turn can activate several molecules of MAPK. Each MAPK can then phosphorylate multiple copies of the transcription factor substrate. If we consider a simple example of how this might work when each kinase can phosphorylate and activate multiple molecules of its downstream target, activation of a single molecule of MAPKKK will lead to the phosphorylation of many molecules of transcription factor. This signal amplification greatly increases the sensitivity of the signaling pathway, allowing it to sense the

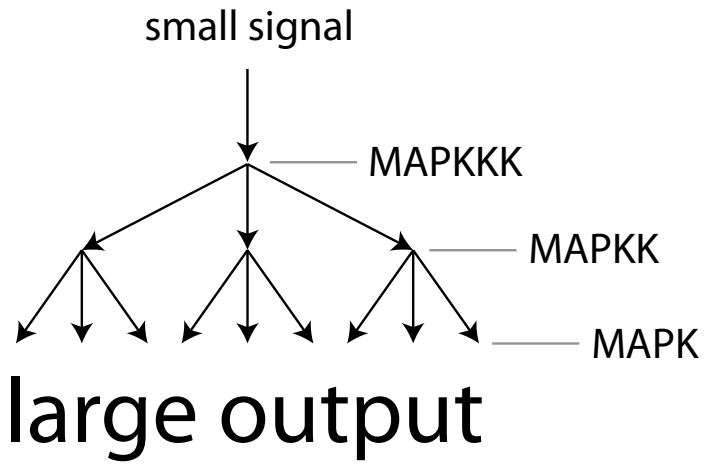

Figure 13 The MAP kinase cascade allows for signal amplification

A single activated MAPKKK protein can phosphorylate and activate multiple MAPKK proteins, each of which can then phosphorylate and activate multiple MAPK proteins. Because each kinase in the cascade can activate more than one downstream target, small input signals can be exponentially amplified to produce large outputs.

presence of very small numbers of bound receptors. This amplification process helps explain how growth factor signaling systems are able to detect the presence of growth factor at very low concentrations.

This ability of the MAP kinase cascade to amplify a signal also explains the importance of the Ras ON/OFF switch as a checkpoint against spurious activation of the signaling cascade. If Ras switched into its GTP-bound ON state spuriously and if Ras-GTP were stable, then the MAP kinase cascade could amplify a signal that did not arise from growth factor binding. Instead, and as we have seen, Ras-GTP is continuously converted back to its inactive GDP-bound state by its GAP.

Scaffold proteins enhance the specificity of MAP kinases

The specificity of MAPKKK for MAPKK and of MAPKK for MAPK is enhanced by **scaffold proteins** (Figure 14), which are large proteins that simultaneously bind to all three MAP kinases. Scaffold proteins bring the kinases close to each other, thus helping each kinase find its substrate. Additionally, each scaffold protein binds specifically to each kinase, thus reducing the chances that a kinase will phosphorylate an incorrect substrate. Binding to the scaffold is dynamic such that each kinase can act on multiple copies of its substrate, as discussed above. If the complex of scaffold protein and kinases was very stable, then signal amplification could not occur.

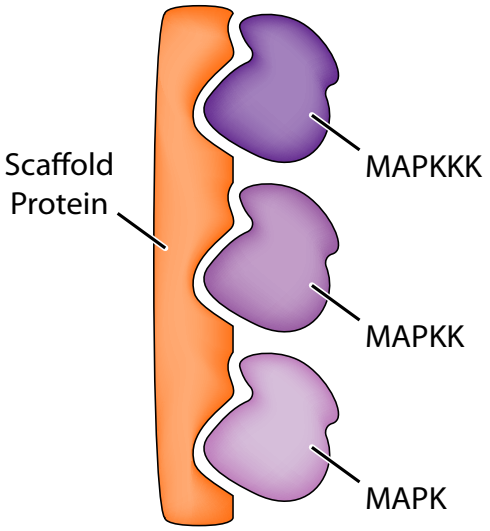

Figure 14 Scaffold proteins enhance the substrate specificity of MAP kinases

Scaffold proteins simultaneously bind to MAPKKK, MAPKK, and MAPK. Each binding site on the scaffold binds specifically to a particular kinase (e.g., the top-most binding site binds specifically to MAPKKK). The scaffold positions each kinase near its substrate, helping it to identify the correct substrate while also decreasing the chance that the kinase will accidentally phosphorylate the wrong substrate. The scaffold/MAP kinase complex is dynamic, allowing each kinase to act on more than one copy of its substrate.

Summary

Cells communicate with each other by exchanging chemical signals. Some of these molecules penetrate the cytoplasmic membrane to reach targets on the inside of the cell. Other chemical signals, such as growth factors and other small proteins, are unable to cross the membrane. Instead, these signaling molecules act by binding to receptors that transduce the signal across the membrane to the inside of the cell.

In the case of the growth factor EGF, signal transduction is mediated by a membrane receptor that dimerizes in response to binding of the ligand. Dimerization activates a tyrosine kinase on the cytoplasmic face of the receptor. In a process known as *trans* autophosphorylation, the intracellular domain of one subunit of the dimer phosphorylates the intracellular domain of the other subunit and vice versa.

Protein kinases are enzymes that transfer a phosphoryl group from a donor molecule of ATP onto the hydroxyl group of a serine, threonine, or tyrosine side chain. This reaction is energetically favorable but occurs extremely slowly in the absence of a kinase. Kinases are substrate-specific, meaning that they only phosphorylate certain hydroxyl groups in particular proteins. Phosphorylation changes the chemical properties of the substrate side chain, replacing a polar hydroxyl group with a large, densely negatively charged phosphate group. This change can have profound effects on the protein's folded structure, and it frequently triggers a conformational change in the substrate protein that alters its activity. Protein kinases are commonly used in signaling pathways due to their ability to modulate protein function via phosphorylation.

The *trans* autophosphorylation of the intracellular domain of the receptor creates a binding site for an adaptor protein. The adaptor protein recruits a GEF to the membrane. The GEF, in turn, binds to a small GTPase known as Ras. Ras exists in an inactive GDP-bound state and an active GTP-bound state. The GEF binds to Ras-GDP, causing it to release its bound nucleotide and bind GTP, generating the active conformation. Ras-GTP, in turn, is deactivated by its GAP, which stimulates Ras's GTPase activity, causing the hydrolysis of the bound GTP to GDP and causing Ras to again assume its inactive, GDP-bound conformation.

When in its active, GTP-bound state, Ras binds to the serine/threonine protein kinase MAPKKK and activates it. MAPKKK then activates MAPKK via phosphorylation. MAPKK, in turn, phosphorylates MAPK. Because each MAP kinase is capable of phosphorylating multiple copies of its substrate, the MAP kinase cascade serves to amplify the original signal generated by the binding of the growth factor to its receptor. The accuracy of the MAP kinase cascade is enhanced by a protein scaffold that holds each kinase near its substrate. Upon phosphorylation, MAPK is imported into the nucleus, where it phosphorylates transcription factors to alter the pattern of gene transcription.