Biochemistry & Molecular Biology of Plants, B. Buchanan, W. Gruissem, R. Jones, Eds.

CHAPTER 18

Signal Perception and Transduction

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CHAPTER OUTLINE

Introduction

Plant cells are constantly bombarded with information to which they must react. **Signal transduction,** the means whereby cells construct responses to a signal, is a recently defined focus of research in plant biology. The application of biochemical and molecular genetic techniques has resulted in major advances in elucidating the mechanisms that regulate gene expression and in identifying components of many signal transduction pathways in diverse physiological systems. Today, signal transduction research contributes to all aspects of plant science, linking many fields of study in much the same way that signal transduction pathways link myriad cellular processes.

18.1 Overview of signal transduction

18.1.1 The stream of signals to which plant cells react is continuous and complex.

Throughout their life cycle, plants and plant cells continually respond to signals that they use to alter their physiology, morphology, and development. Among the stimuli—both external (Fig. 18.1) and internal (Fig. 18.2)—that convey information to plants are light, mineral nutrients, organic metabolites, gravity, water status, turgor, soil quality, mechanical tensions, wind, heat, cold, freezing, growth regulators and hormones, pH, gases (CO_2, O_2, C_2H_4), wounding and disease, and electrical flux. Signals can vary in quality and quantity from minute to minute. Some of the signals are carried by xylem and phloem, the circulatory system, which can accommodate very large and rapid fluxes.

Plant responses to stimulus are modulated by developmental age, previous environmental experience, and internal clocks that specify the time of year and the time of day. For mature plant cells, the response can be physiological and biochemical; for growing cells, it can be morphological and developmental. Integration of various forms of signaling information is usually crucial to determining the final response. In a seed, for example, the decision to germinate can be irreversible and, if timed inappropriately, fatal. The capacity of seeds to react successfully to many physical, chemical, and temporal variables reflects the presence of a complex system for signal recognition and transduction in the living cells of all plants.

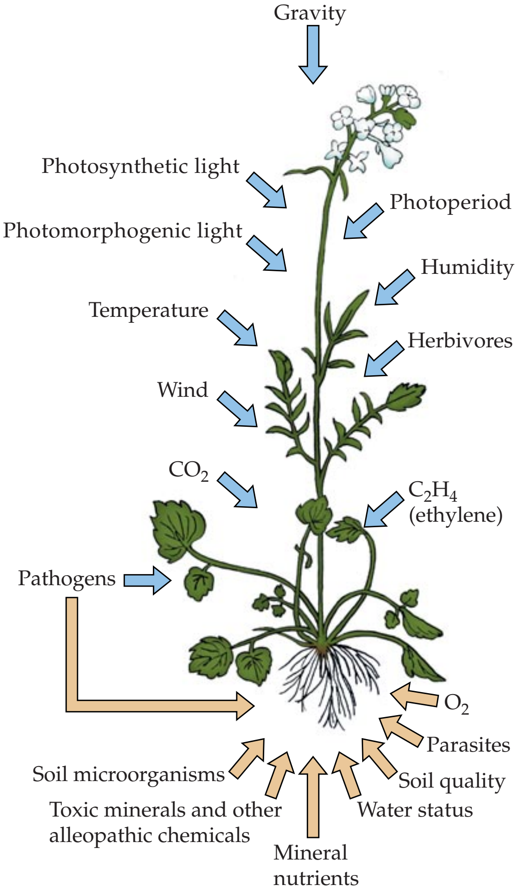


Figure 18.1
External signals that affect plant growth and development include many aspects of the plant's physical, chemical, and biological environments. Some external signals come from other plants. Apart from gravitropic signals, all other signals vary in intensity, often from minute to minute.

18.1.2 Signal transduction uses a network of interactions within cells, among cells, and throughout the plant.

Two of the principal elements in the signal transduction pathways of plant cells are intracellular Ca^{2+} ($[Ca^{2+}]_i$), and protein kinases, enzymes that phosphorylate and thereby alter the activity of target proteins (Fig. 18.3). The term **second messenger** is often used to describe a readily diffusible molecule involved in conveying information from an extracellular source to the principal target enzymes within the cell. In plants, $[Ca^{2+}]_i$ transduces many signals and is a prominent second messenger; it therefore must be maintained in the cytoplasm at concentrations many orders of magnitude lower than the $[Ca^{2+}]$ in the cell wall. During signaling, **Ca^{2+} transients** (brief increases in $[Ca^{2+}]_i$) are often

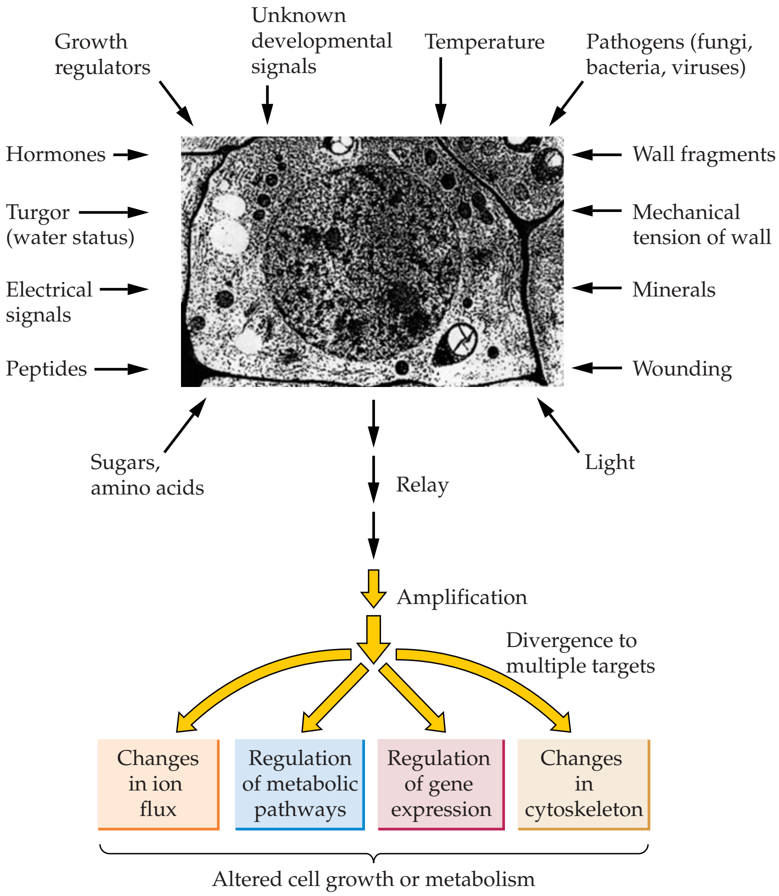


Figure 18.2
A variety of internal signals modify plant cell metabolism, growth, and development. The ability of cells to respond to these signals is not confined to cells that are still growing and developing. Mature cells, too, can initiate metabolic responses and can even reinitiate growth and division in response to signal information.

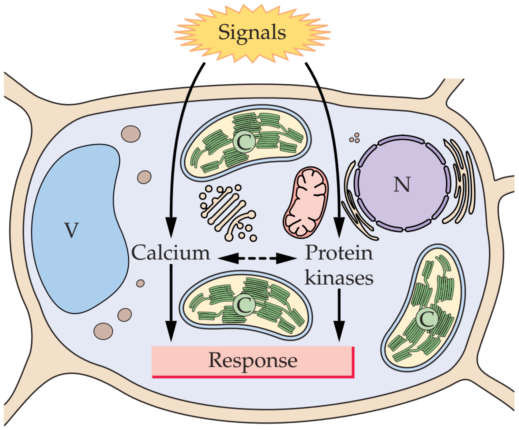


Figure 18.3
Two of the major signal transduction pathways found in plant cells involve cytosolic calcium and protein kinases. Calcium-dependent protein kinases, which are very prevalent in plant cells, connect the two transduction pathways (dashed arrow). V, vacuole; C, chloroplast; N, nucleus.

associated with initiation of responses. The many signal transduction pathways on which $[Ca^{2+}]_i$ acts involve hundreds of different proteins, as well as other second messengers in the cytoplasm and the plasma membrane. Protein kinases are similarly ubiquitous: Genes encoding these enzymes represent an estimated 3% to 4% of the genome; at any one time, cells will be using hundreds of different protein kinases. Together, the signaling pathways that utilize protein kinases and $[Ca^{2+}]_i$ constitute a network of great complexity.

Changes in $[Ca^{2+}]_i$ can initiate diverse responses that vary according to cellular structure and are sensitive to interaction between specific components of signaling systems. For example, increases in $[Ca^{2+}]_i$ can initiate closure of the stomatal aperture in guard cells, reorientation of growth in pollen tubes, or wall thickening in young tobacco seedlings in response to wind. Likewise, a single protein kinase can have many target proteins, but the targets differ among distinct cell types and developmental stages.

Many signals interact cooperatively and synergistically with each other to produce the final response. Signal combinations that induce such complex plant responses include red and blue light (Fig. 18.4), gravity and light, nitrate availability and light (Fig. 18.5), growth regulators (see Chapter 17), and mineral nutrients (see Chapter 23). As illustrated for tobacco in Table 18.1, the chlorosis (yellowing) symptom of iron deficiency is influenced by availability of potassium and phosphorus. Similar synergistic effects have been noted in some aspects of carbon and nitrogen metabolism (see Chapter 16). At some stage in transduction, therefore, the separate signals must affect reactions and proteins that are either strongly interlinked or are identical.

18.1.3 Plant cells contain two information systems: genetic and epigenetic.

The genetic system of information flow in plant cells, DNA → RNA → protein → phenotype, has been a primary focus of research since Mendel's time. This apparent causal sequence implies a certain rigidity and simplicity in result. Some of the genes studied by Mendel, such as those that specify flower

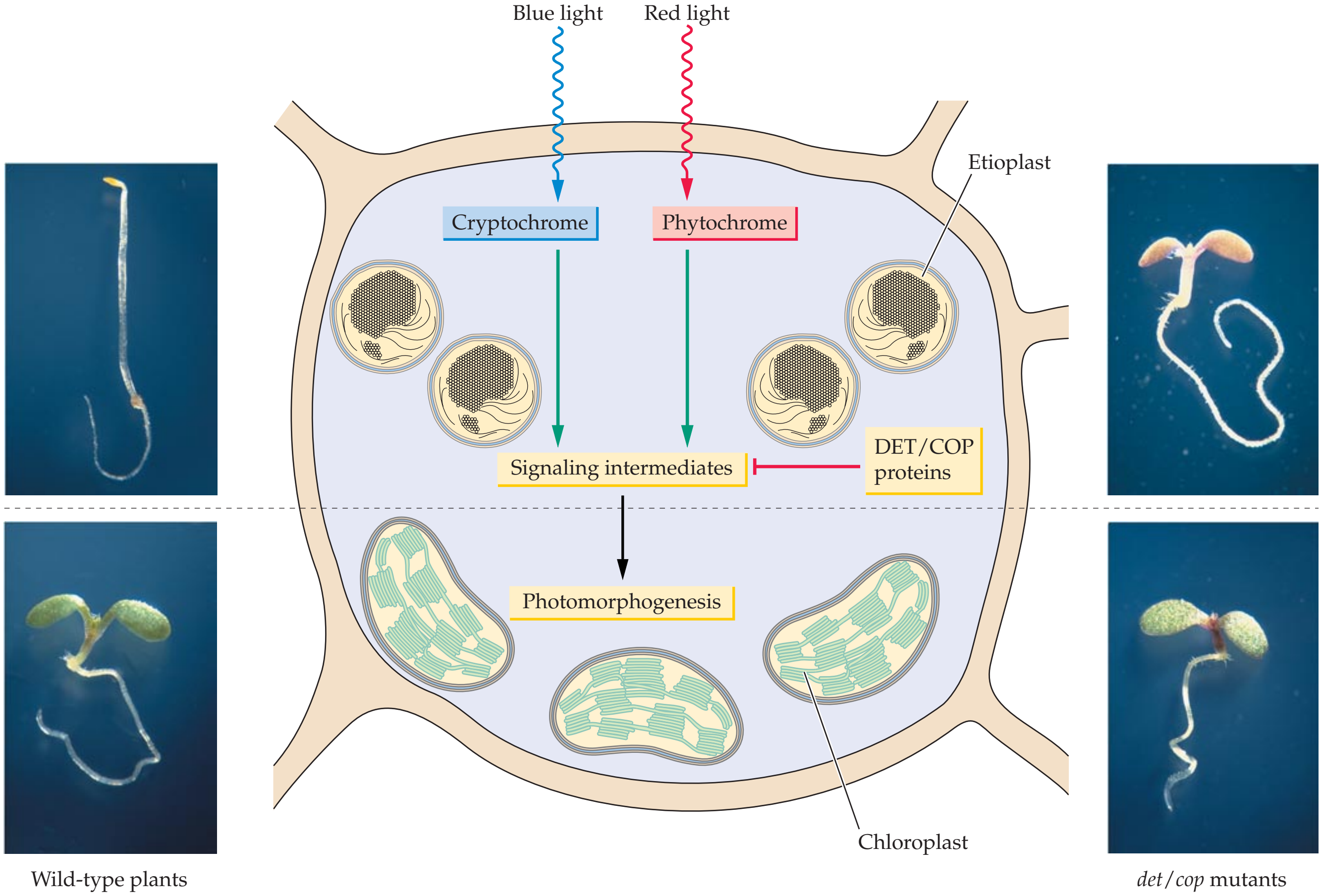


Figure 18.4
Blue light and red light often interact and overlap in their effects on plant development. Two sets of proteins called DET (deetiolated) and COP (constituitively photomorphogenic) ordinarily ensure that the etiolated program is maintained in darkness. The effect of light is to switch off the activity of DET and COP, allowing photomorphogenesis to occur. Repression of DET and COP activity is dependent on the blue light receptor (cryptochrome) and the red light receptor (phytochrome) and the presence of photomorphogenically active light. The effects of red light and blue light on deetiolation indicate that signaling intermediates in the two light-reception pathways form an interactive network.

color or seed morphology (see Chapter 7), are invariant in expression under many different conditions of growth and development (Fig. 18.6A). Geneticists concentrate on the expression of such genes because analysis of their inheritance is relatively simple.

On the other hand, many important phenotypic characteristics are strongly modified by the environment in which the plant grows, including production of biomass, duration of growth, branching, partitioning of photosynthate between reproductive and vegetative structures, and responses to stress. In these cases the phenotypic character is constructed from **epistatic genes,** the products of which alter the expression of other genes that were inherited independently,

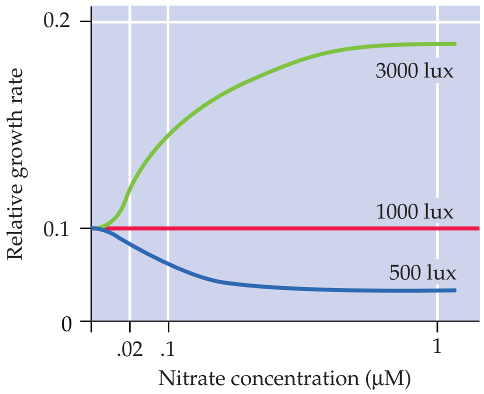


Figure 18.5
Interaction between light and nitrate concentration on relative growth rate of plants. Note that nitrate may inhibit as well as promote growth, depending on the light intensity.

Table 18.1 Effect of phosphate and potassium nutrition on symptoms of mineral deficiency in tobacco

Iron (Fe)	Phosphate (P_i)	Potassium (K)	Apparent symptoms
Low	High	Low	K deficiency in old leaves
High	High	Low	Fe deficiency in young leaves
Low	Low	Low	K deficiency delayed
Low/high	Low	Low	Chlorotic; no K deficiency
High	Low	Low	P_i deficiency

and **pleiotropic genes,** single genes that influence multiple traits. These **epigenetic** characters (see Chapter 7) result from a complex web of interacting gene products enmeshed with signal transduction networks (Fig. 18.6B). Phenotypes associated with such genes can be studied only in rigidly controlled conditions, because the characters vary with the plant's environment (Fig. 18.7).

Signal transduction and epigenetic networks are not easy to conceive; using a topological concept is probably the simplest way to approach them. Picture the network as a landscape with hills and valleys: Information from the signal flows through the valleys like water or a ball rolling downhill (Fig. 18.8). The heights of the hills and the depths of the valleys are initially specified by the genotype; as the environment changes and development progresses, however, the shape of the landscape alters. Changes in the size of the hills modify the shape and position of the valleys. Consequently, the path by which information flows through the valleys varies unpredictably.

Various routes are available for information flow from any one signal, the route actually taken being dictated by the shape of the landscape. Information from the same signal may travel to different regions of the landscape under different environmental conditions. Alternatively, information might arrive at the same point of the landscape after having traveled by very different routes. Thus in *Commelina* plants grown at 10°C to 17°C, abscisic acid (ABA) can initiate

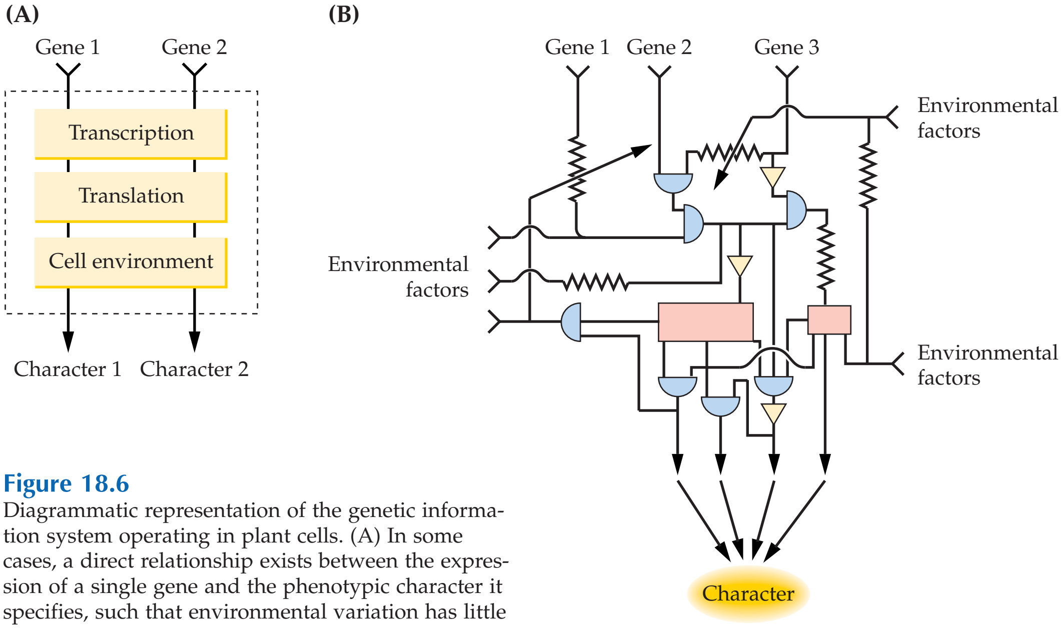


Figure 18.6
Diagrammatic representation of the genetic information system operating in plant cells. (A) In some cases, a direct relationship exists between the expression of a single gene and the phenotypic character it specifies, such that environmental variation has little impact on expression of the gene. Few genes, however, are unaffected by environmental factors (i.e., by signaling events). (B) In the epigenetic information system, a phenotypic character results from complex interactions involving one or more genes and environmental influences that impact signal transduction networks. The computer circuit in the diagram illustrates how difficult it may be to elucidate a precise transduction sequence and predict the influence of any signal. One would expect the actual distribution of information flow through the various branches of the epigenetic network to vary according to environmental cues.

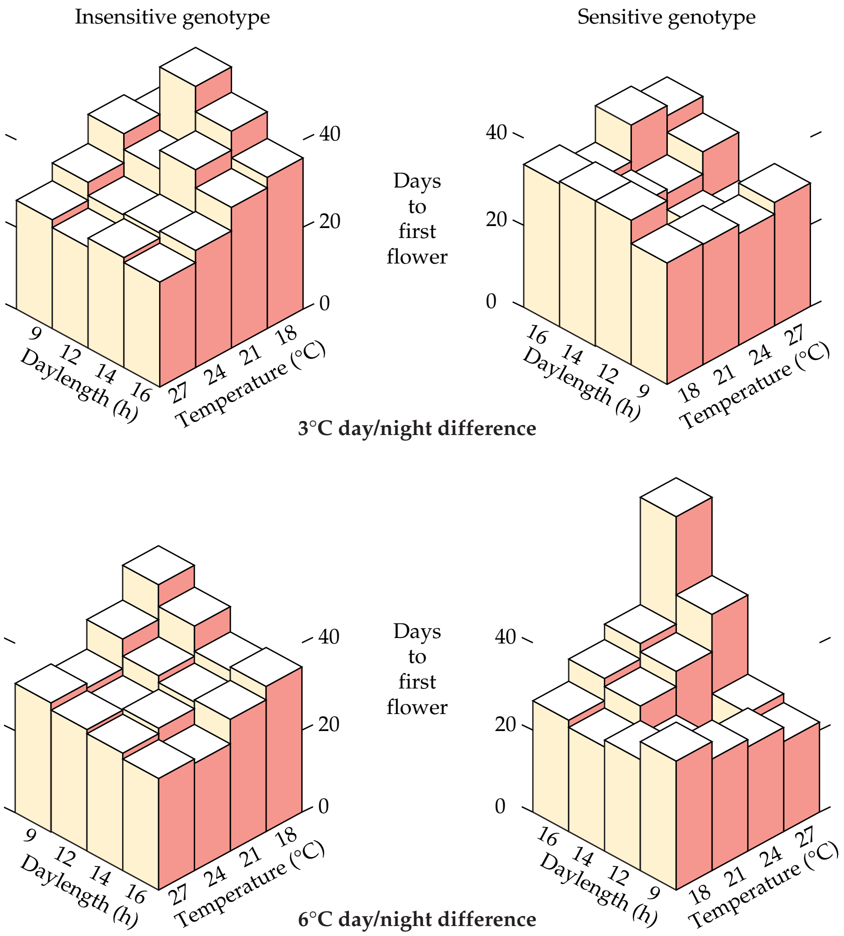


Figure 18.7
Phenotypic characters can result from very complex interactions between genotypes and the environment. Flowering time in two genotypes of beans—one early-maturing and photoperiod-insensitive, the other late-maturing and photoperiod-sensitive—is shown to vary with length of daylight and growth temperature. The tops of the columns can be used to construct an epigenetic surface that best describes the characteristics of development illustrated in Figure 18.8. Inclusion of other variables (e.g., nitrate or light intensity) creates a much more complicated surface.

stomatal closure in the absence of changes in $[Ca^{2+}]_i$. For plants grown at temperatures above 25°C, however, an ABA transduction route involving an increase in $[Ca^{2+}]_i$ seems to be essential.

18.1.4 Different signals affect the transduction network in different ways and at different places, but most modify gene expression.

The signals listed in Figures 18.1 and 18.2 impact the cell at different sites and are perceived by different receptors. However, downstream reactions may meet at what can be termed **nodal points,** that is, at proteins or enzymes that are involved in many

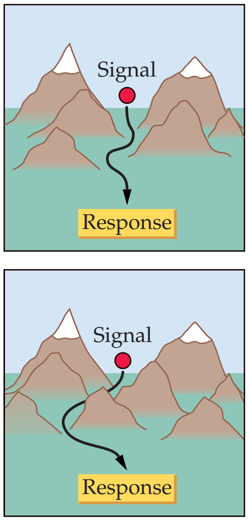


Figure 18.8
The movement of a signal through a transduction network can be thought of as a ball rolling through hills and valleys to reach the final response. Both the environment and development shift the hills and valleys. Achieving similar responses under different environmental conditions involves variations in the activity of different transduction pathways.

transduction sequences. Some responses, e.g., touch-induced leaflet drop of *Mimosa* (Fig. 18.9), occur in seconds. Others, such as shifts in gene expression that accompany touch-induced changes in morphology and development, may take days. Historically, short-term and long-term responses have often been viewed as entirely separate, but we now realize this notion is incorrect. Both fast and slow responses use the same basic transduction network machinery, and both are downstream results of a perceived stimulus. Most signals appear to induce altered gene expression.

18.2 Receptors

18.2.1 Signals can be perceived by protein receptors or through changes in membrane potential.

To initiate transduction, a signal must first be sensed by a **receptor.** Most known receptors are present in the plasma membrane, although some are located in the cytosol or other cellular compartments (Fig. 18.10). At least three different classes of cell surface receptors have been detected in animals (Fig. 18.11), but whether all three exist in plants is still uncertain.

Most identified receptors have turned out to be proteins. For some stimuli, however, protein receptors are not easily identified—for example, the breaking of the dormancy of some buds or imbibed seeds by such chemicals as ethanol, ether, azide, or cyanide. Either these chemicals are able to occupy established cellular receptors or, more likely, many of them modify the membrane potential, the voltage across the plasma membrane (Fig. 18.12).

The membrane potential can act as a receptor. The plasma membrane uses pumps and proteinaceous pores, called **channels,** to control the flux of ions into and out of the cell (see Chapter 3). Selective discrimination against certain ions results in the establishment of a potential difference of –80 to –200 mV. Modifications of membrane

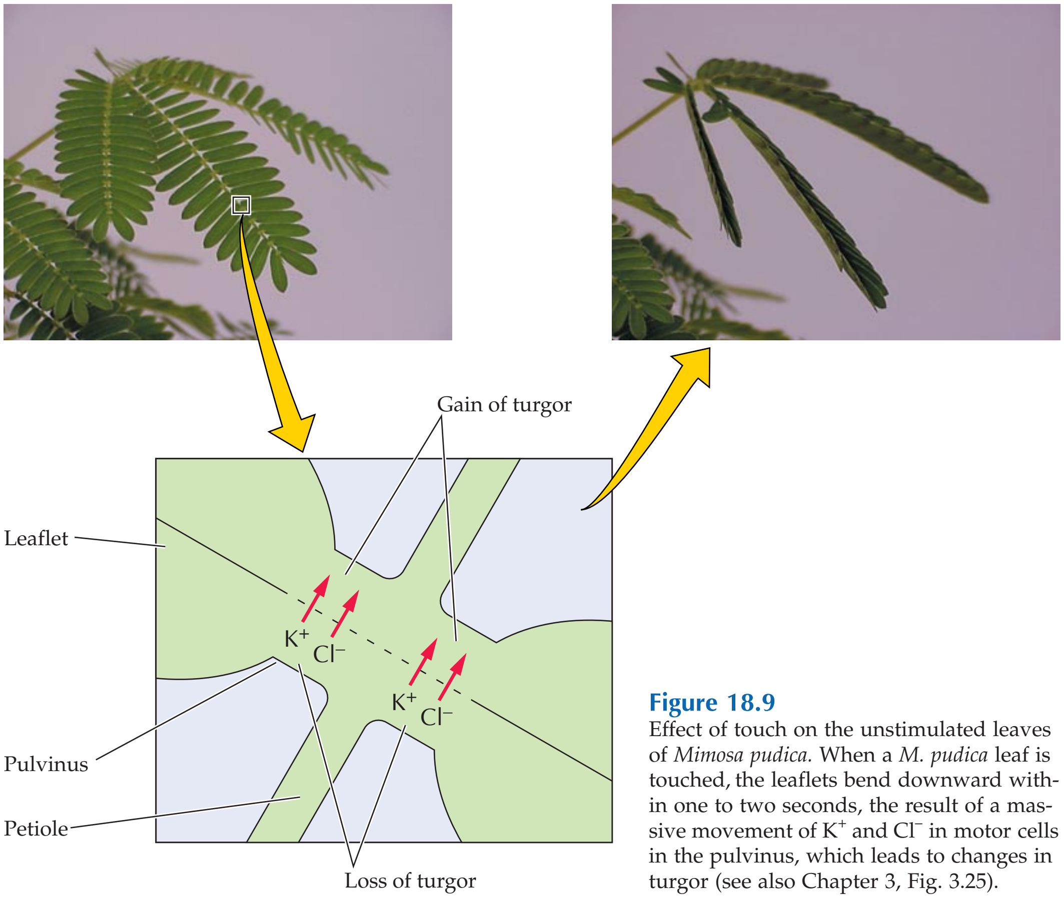


Figure 18.9
Effect of touch on the unstimulated leaves of *Mimosa pudica.* When a *M. pudica* leaf is touched, the leaflets bend downward within one to two seconds, the result of a massive movement of K^+ and Cl^- in motor cells in the pulvinus, which leads to changes in turgor (see also Chapter 3, Fig. 3.25).

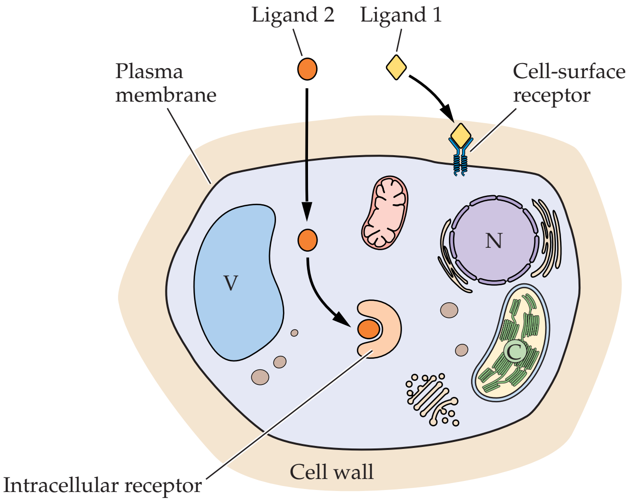


Figure 18.10
Extracellular signals bind either to receptors on the cell surface or to receptors inside the cytoplasm or nucleus. Many hydrophilic molecules, such as peptides and carbohydrates, and osmotic signals cannot easily pass through the plasma membrane and therefore are perceived on the cell surface (e.g., ligand 1). Amphiphilic and hydrophobic molecules, such as growth regulators, can pass through the plasma membrane and may be perceived either by surface receptors or inside the cell (e.g., ligand 2). V, vacuole; N, nucleus; C, chloroplast.

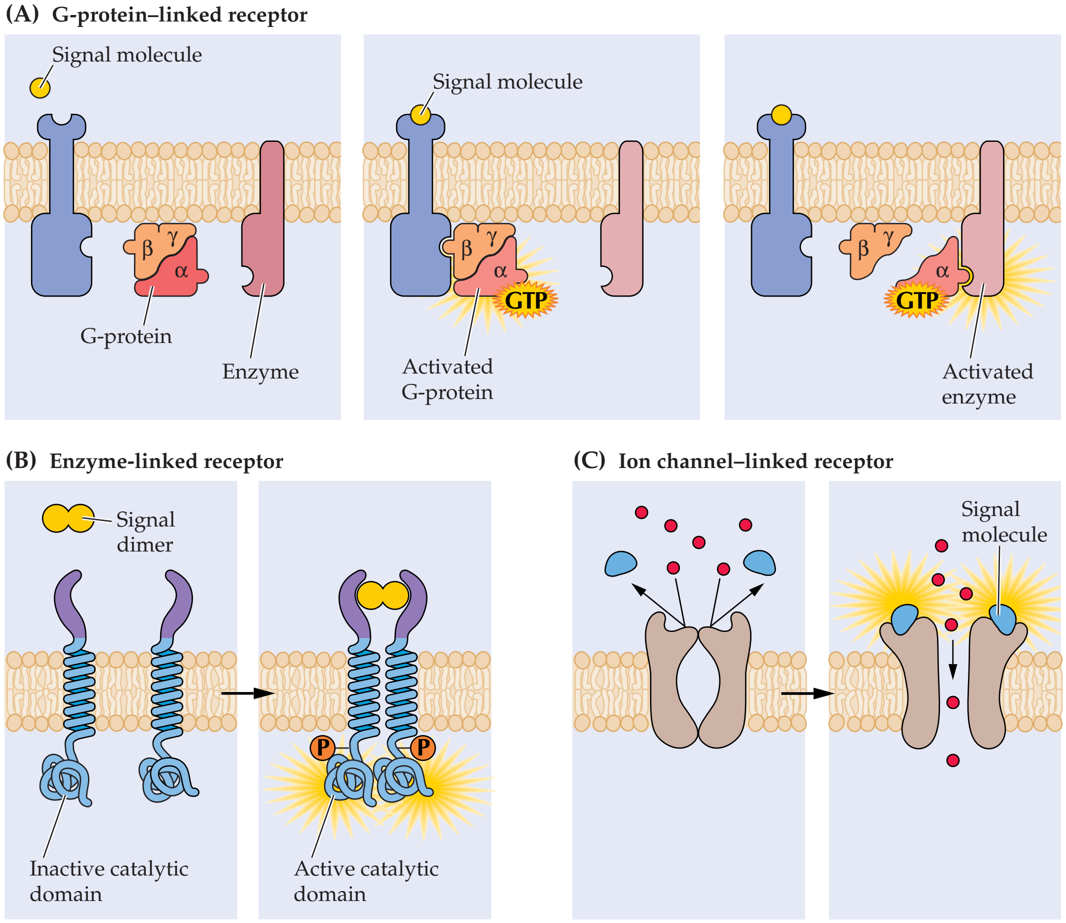


Figure 18.11
Three classes of plasma membrane–located receptors identified in animal cells. (A) When activated, G-protein–linked receptors convey information to a protein that binds GTP as the first stage in transduction. The G-protein α-subunit/GTP complex is usually released from the β/γ-subunits into the cytoplasm, where it can activate other enzymes. (B) Enzyme-linked receptors are commonly protein kinases. Binding of the ligand (signal) causes the receptor to dimerize, leading to intermolecular phosphorylation with activation of the receptor. (C) Ion channel–linked receptors may be coupled directly to important cell surface channels that open when the receptor is occupied. Some ion channel receptors are located on internal membranes as well (see Fig. 18.15 for an example).

potential open a group of voltage-gated channels that allow Ca^{2+} to enter and thus activate a transduction sequence (Figs. 18.12 and 18.13). Ca^{2+} influx, in turn, can cause the subsequent opening of many potassium and chloride channels, resulting in very rapid changes in turgor, such as occur in the pulvini of *Mimosa* (see Fig. 18.9). Many signals are known to modify membrane potential, including red and blue light, fungal elicitors, and many growth regulators.

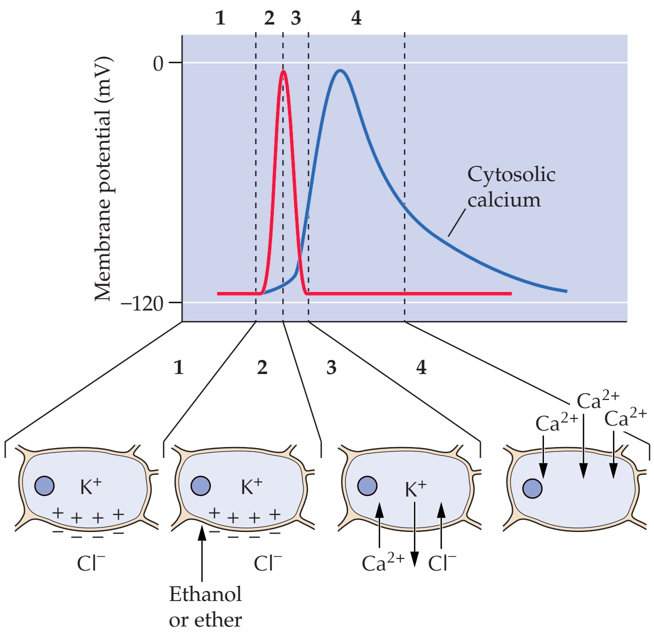


Figure 18.12
Possible sequence of events in transduction of signals such as ethanol, ether, or cyanide. The plant cell membrane potential (in red) is established because the plasma membrane is not equally permeable to K^+ and Cl^- ions (1). Ethanol, ether, or cyanide causes a temporary disruption to the membrane potential (2), which is followed by a brief influx of Ca^{2+} (3). Voltage-sensitive Ca^{2+} channels subsequently open for a longer period, signaling the cell (4). Organic chemicals, minerals, and other depolarizing treatments can also be used to break the dormancy of seeds or buds, increase root formation, and modify flowering.

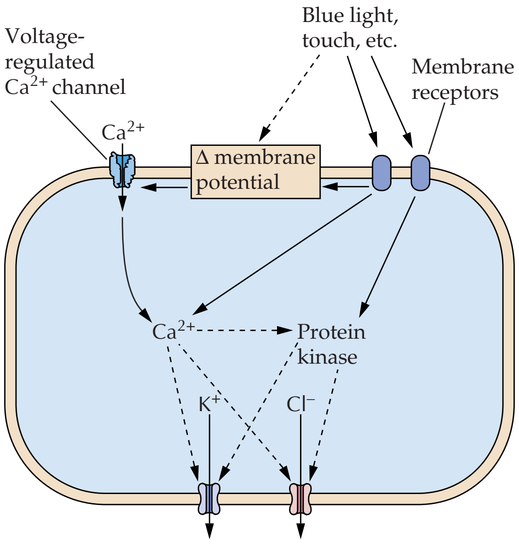


Figure 18.13
Many signals modify the plasma membrane potential and activate voltage-gated channels, permitting entry of Ca^{2+}. When plant cells are exposed to signals such as blue light or touch, detectable changes in membrane potential occur. Voltage-gated channels are opened and concentrations of cytosolic Ca^{2+} are increased. Activation of this transduction pathway can result in opening of the potassium and chloride channels, which leads to loss of turgor (see Fig. 18.9). The separate transduction pathways are linked through Ca^{2+}/calmodulin–regulated protein kinases and other proteins. Blue light and touch may also mobilize intracellular Ca^{2+} by other routes.

18.2.2 Many receptors share similar structural attributes and catalytic activities.

The sequences of many receptors have been determined. Although there is often little sequence conservation among receptors from different organisms that bind the same ligand, a unifying characteristic has emerged. Many receptors have seven hydrophobic domains placed strategically throughout the molecule. These hydrophobic domains are thought to represent regions of the receptor that span the plasma membrane (Fig. 18.14). The ligand-binding site may be located in one of these domains or on the extracellular region. Frequently the N terminus of such receptors is located outside the cell and the C terminus is inside.

In some transmembrane protein receptors, the C-terminal region is phosphorylated by protein kinases. Two possible families of protein kinases are distinguished on the basis of the amino acids they phosphorylate on their substrate proteins: serine/threonine residues or tyrosine residues. A few protein kinases phosphorylate all three amino acids. Protein kinases that phosphorylate tyrosine residues exclusively are rare in plant cells.

Another class of receptors is the so-called **receptor-like protein kinases** (RLKs; see Section 18.7.2). The RLKs of plants typically consist of a large extracytoplasmic domain, a single membrane-spanning segment, and a cytoplasmic domain containing the active site of a protein kinase. Binding of the ligand is thought to cause dimerization of the receptor, bringing the cytoplasmic domains of the protein kinases into close proximity. Intermolecular or intramolecular phosphorylation can then stabilize the activated receptor complex. This phosphorylation can maintain the protein kinase activity of some

RLKs in the absence of ligand. Intermolecular phosphorylation usually modifies serine or threonine residues.

In some cases, the activated complex can phosphorylate and activate other downstream proteins. Alternatively, the active RLK complex interacts with membrane-bound or soluble transduction proteins (such as Ras or other G-proteins in animals) to perpetuate the signal transduction sequence in a different direction. Some RLKs form a membrane complex with a protein phosphatase, which dephosphorylates the activated RLK. The inactive RLK is then free to reassociate with other RLKs in further signaling if its ligand is still available.

Numerous RLKs have been identified in plant cells, including protein kinases with seven membrane-spanning domains. Such RLKs have been detected in the male reproductive tissues of plants, where they are implicated in incompatibility reactions that prevent fertilization (see Section 18.7.2).

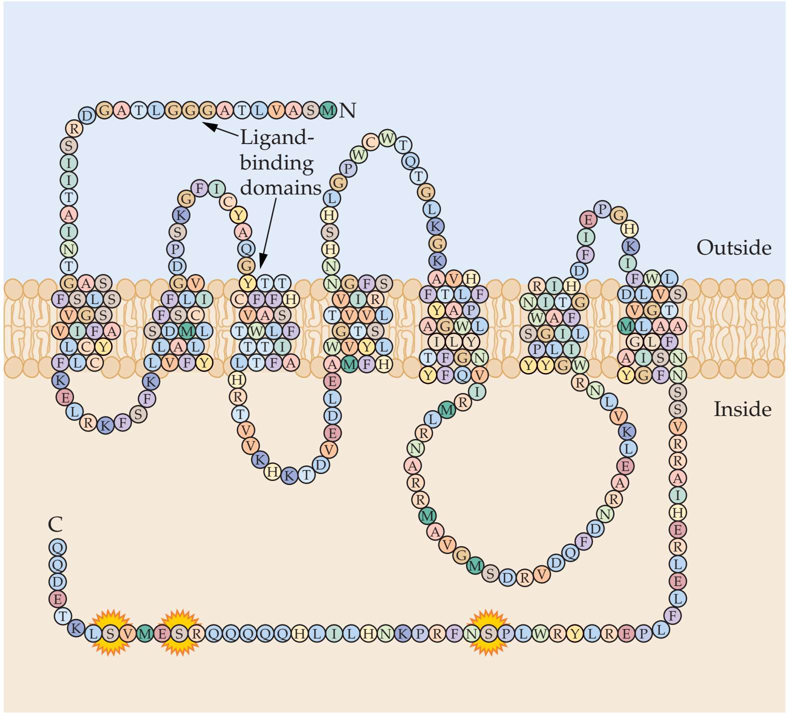


Figure 18.14
Sequence of GCR1, a putative receptor that affects cytokinin sensitivity. The protein structure shown here, with seven membrane-spanning domains and highlighted phosphorylation sites in the C terminus, was deduced from hydropathy plots (see Chapter 3, Box 3.2).

18.2.3 Intracellular receptors can act as ion channels.

Other receptors are located in intracellular membranes and can act as Ca^{2+} channels. The most well-known receptor in this class binds the second messenger inositol 1,4,5-triphosphate (IP_3; Fig. 18.15). Channels for another second messenger, cyclic ADP-ribose (cADPR), have been reported recently. Both these signaling molecules are synthesized by enzymes in the plasma membrane and then translocate to the vacuole and the ER, where their receptors are located. Occupation of the receptor (which may be composed of four subunits) leads to the opening of Ca^{2+} channels and an influx of Ca^{2+} into the cytoplasm from the vacuole and the ER, each of which contains Ca^{2+} many orders of magnitude greater than the cytosolic concentrations (see Section 18.6.1). In contrast to plasma membrane–bound receptors, these protein subunits each have four membrane-spanning domains. Other membrane-spanning proteins also may have important functions in signal transduction. For example, an auxin-resistant mutant, *aux-1,* results from an alteration in a protein with 10 membrane-spanning domains, which is thought to be a permease.

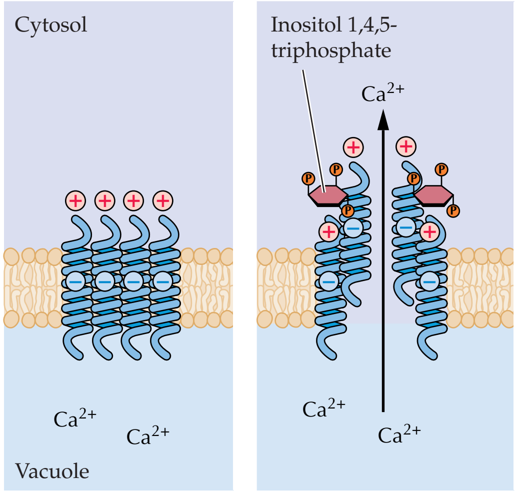


Figure 18.15
The receptor for inositol 1,4,5-triphosphate (IP_3) is located on the tonoplast and ER membranes. Conformational changes in this receptor transduce subsequent signaling. Certain ion channel receptors, including the IP_3 receptor, are composed of four subunits. Each subunit is thought to contain four membrane-spanning domains (not shown). When IP_3 binds to the receptor, conformational changes result in movement of two of the subunits. The distribution of positive and negative charges stabilizes the open conformation of the channel and allows the entry of Ca^{2+} into the cytoplasm.

18.2.4 Affinity labels, sensitivity mutants, and complementation in yeast can be used to identify receptors.

The cellular concentrations of receptors are less than those of other proteins (e.g., enzymes), so their detection requires special approaches. **Photoaffinity labeling** uses isotopically labeled reagents, e.g., reactive carbenes of nitrenes that undergo bond rearrangement on exposure to UV light. When radioactive or fluorescent ligands containing the reactive group are mixed with the receptor preparation and exposed to UV light, the bond rearrangement can covalently cross-link the ligand to the binding site of the receptor; the receptor–ligand complex can then be purified and identified (Fig. 18.16).

An alternative approach is to identify mutant plants that are insensitive to the signal. Molecular mapping techniques (see Chapter 7) and "chromosome walking" (see Chapter 21) can then be used to identify the putative receptor mutation. If the mutation is induced by insertion of characterized fragments of DNA—i.e., a transposon (see Chapter 7) or a T-DNA (see Chapters 6 and 21)—then the gene encoding the protein responsible for the insensitivity can be identified more easily. These methods have identified the receptors for blue light (Fig. 18.17A), for ethylene, and perhaps for ABA but have proven less successful for investigating auxin.

Various other procedures rely on correlations between physiological response and receptor behavior. Identification of **phytochrome** as the red light receptor has relied on the finding that red light effects are frequently counteracted by the immediate exposure of the affected plant to far-red illumination. Purified phytochromes likewise exist in two forms that have different absorption spectra. One form, P_{FR}, primarily absorbs far-red light; the other, P_R, absorbs red light. **Calmodulin** has been functionally identified as a primary Ca^{2+} receptor because the Ca^{2+}–calmodulin complex can activate many other enzymes. Complementation in yeast is potentially an important technology for identifying many plant receptors. For example, responses to osmotic pressure are shared by plants and yeast. Isolation of a yeast mutant that was unresponsive to osmotic pressure led to identification of the yeast receptor involved in osmosensing. This yeast mutant can now be exploited to find plant cDNAs that restore osmotic sensing; such cDNAs might encode plant receptors that signal changes in osmotic pressure (Fig. 18.17B). At present, a plant receptor that senses osmotic status has not yet been cloned.

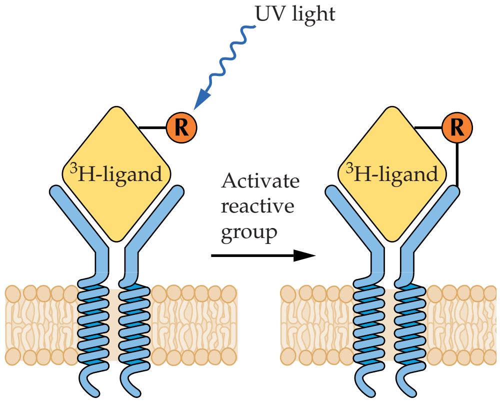


Figure 18.16
A simple way to detect a receptor is to attach a radioactive affinity label (e.g., ^{3}H-nitrene or ^{3}H-carbene) to a ligand. The receptor preparation is mixed with the radioactive ligand to permit binding, frozen to very low temperatures, and then exposed to UV light. On activation of the reactive nitrene or carbene group (R) by UV light, the labeled compound binds irreversibly to the receptor, which can then be purified and identified.

18.2.5 Receptor–ligand binding is reversible and exhibits saturation kinetics.

Identification of a molecule as a receptor is a difficult research objective. The following set of criteria—all of which are fulfilled by the ethylene receptor—can be used to help in the identification when a functional assay is not available. Ligands should bind to specific sites on their receptors. Binding should be of sufficient strength (and thus occupy the receptor for a sufficient length of time) that the associated downstream processes (which usually require direct interactions with other molecules) can be activated.

- Ligand binding should be of relatively high affinity.
- Ligand binding should be reversible, allowing the system to respond to changes in ligand concentration, because receptors are present in limited abundance.
- The binding of the ligand to its receptor should saturate at a certain ligand concentration.
- The receptor should be selective for biologically active molecules, and binding specificity should mimic in vivo physiological activity.

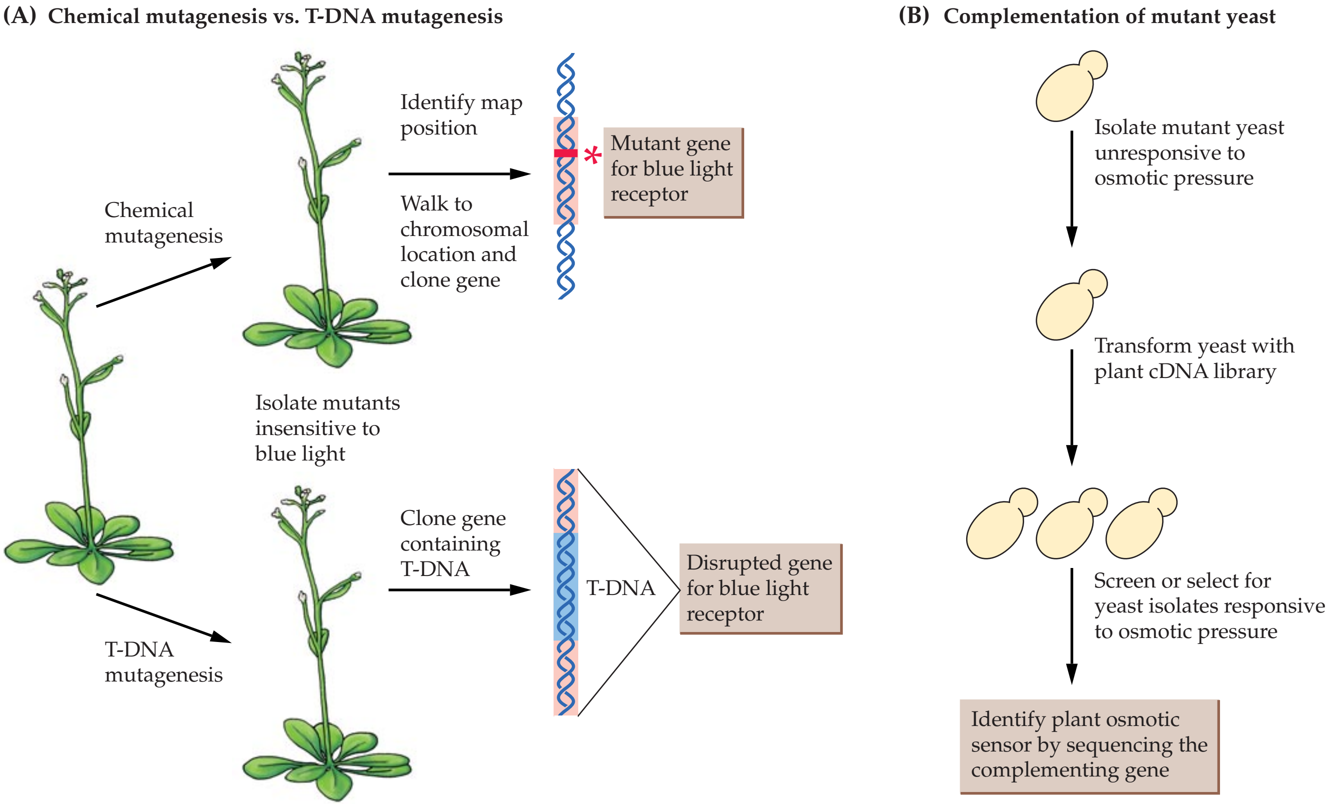


Figure 18.17

(A) Two approaches for identifying plant genes by generating mutant plants with altered phenotypes. Populations of mutants are obtained by chemical mutagenesis and the desired phenotype is identified. Chromosomal regions are identified through selection and mapping. Chromosomal "walks" are conducted to identify the mutant sequence, which is then used to isolate the wild-type sequence and identify the gene of interest. Alternatively, genes can be tagged with T-DNA from the bacterium that causes crown gall disease, *Agrobacterium tumefaciens* (see Chapters 6 and 21). T-DNA is bordered by sequences that facilitate its insertion into the plant genome. Transformation is carried out on seeds or plants, and the required phenotype is identified by screening a population of transformants. The insertion site can be identified by using the known sequence of T-DNA as a tag. Sequencing around the insertion site enables identification of the gene responsible for the phenotypic character. (B) Identifying a plant gene by functional complementation. In this approach, a plant gene restores a wild-type phenotype in a mutant strain of yeast. Complementation requires isolation of a yeast mutant that is deficient in the plant character being investigated. The yeast is transformed with a plant cDNA library, a complemented yeast clone is isolated, and the plant transgene is sequenced. In the hypothetical example shown here, complementation is used to identify a plant osmotic sensor.

- The affinity constant for binding (K_d, Box 18.1) should be similar to the ligand concentration that is active in vivo. (Variations in receptor concentration bring important caveats to this criterion; see Section 18.2.8.)

18.2.6 Specific receptors for many signals have not yet been identified.

The many signals to which plants specifically respond must necessarily interact with specific receptors that couple them to transduction pathways, but most of these receptors have not yet been identified. For example, plant cells can clearly sense their water status and respond in a variety of ways, including osmotic adjustment through accumulation of compatible solutes such as proline or glycine betaine (see Chapter 22), accumulation of ABA (see Chapter 17), and changes in gene expression, development, and morphology. Bacteria sense their osmotic status through a protein receptor, the conformation of which is determined by the amount of bound water; activation of this receptor leads to changes in gene expression. The bacterial system might serve as a model for an equivalent receptor in plants, although

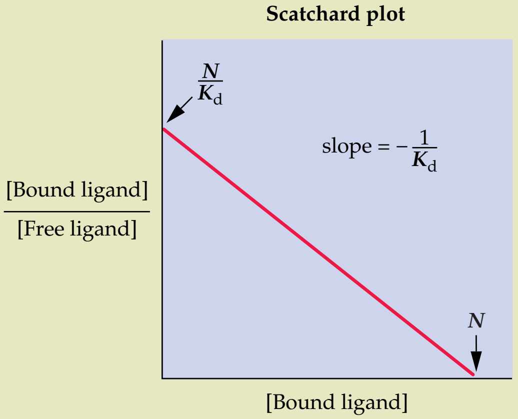
Box 18.1

Measurement of its binding constant is an important characterization of a true receptor.

An important technique for characterizing the interaction of a ligand with its receptor is to measure the affinity constant for binding. Usually such measurements require immobilization of the receptor on a support to expose the receptor to different concentrations of ligand and determine the corresponding amount of binding. According to how the data are treated, important constants characterizing the binding can be determined. The most common way of dealing with binding data is to treat it on the basis of **occupancy theory,** which was first postulated in the 1930s. This theory requires that ligand [L] and receptor [R] bind together in a simple chemical equilibrium. Thus, $L + R \rightleftharpoons LR$. The velocity of the forward reaction is $K_1[L][R]$, and that of the reverse reaction is $K_{-1}[LR]$. At equilibrium, the rates of these two reactions are equal. A binding (or dissociation) constant can be defined as $K_d = K_{-1}/K_1$, where K_d is equivalent to the concentration of ligand at which half of the binding sites of the receptor are occupied. By manipulating this definition of K_d, the Scatchard equation can be derived:

$$B/F = R_T/K_d - B/K_d$$

where B is the concentration of bound ligand (i.e., [LR]), R_T is the total concentration of receptor binding site, and F is the concentration of free, unbound ligand ([L]). For a single class of receptive site, graphing B/F against B yields a linear plot in which the slope = $-1/K_d$ and the intercept on the B-axis equals the maximum density or concentration of receptor sites (see figure).



N = Number of binding sites per unit protein

K_d = Equilibrium constant for binding

$K_d = \frac{\text{[Receptor • ligand complex]}}{\text{[Free receptor] [Free ligand]}}$ at equilibrium

no such putative plant protein has been identifed to date (see Section 18.2.4). However, we have no comparable models for the sensing of carbon dioxide, temperature, and nitrate, even though particular changes in these variables can cause very specific changes in development.

18.2.7 The bacterial two-component system, in which a receptor and an effector interact through phosphorylation of histidine and aspartate residues, may also be present in plants.

The sensory and transduction systems evolved by bacteria enable them to survive and adapt to various different environmental conditions. Bacterial transduction systems facilitate secretion, motility, sporulation, membrane transport, competence, virulence, and metabolic changes in response to a variety of signals. The simplicity of bacterial genetics and transformation technologies has enabled the characterization of many well-defined systems, all of which have some basic elements in common. At least 18 such systems have been identified in *Escherichia coli,* and at least 50 are probably present in prokaryotic cells. These systems, called **two-component systems,** may also be present in plant and fungal cells (Fig. 18.18A).

The first component of a two-component system is usually a receptor protein. The receptor contains a periplasmic domain that binds ligands, includes a variable number of transmembrane domains, and has a C terminal extension. When activated by binding a ligand, a kinase activity located in the C terminus autophosphorylates the receptor, transferring orthophosphate from ATP to a histidine residue. The active receptor is a homodimer in which each monomer phosphorylates the other.

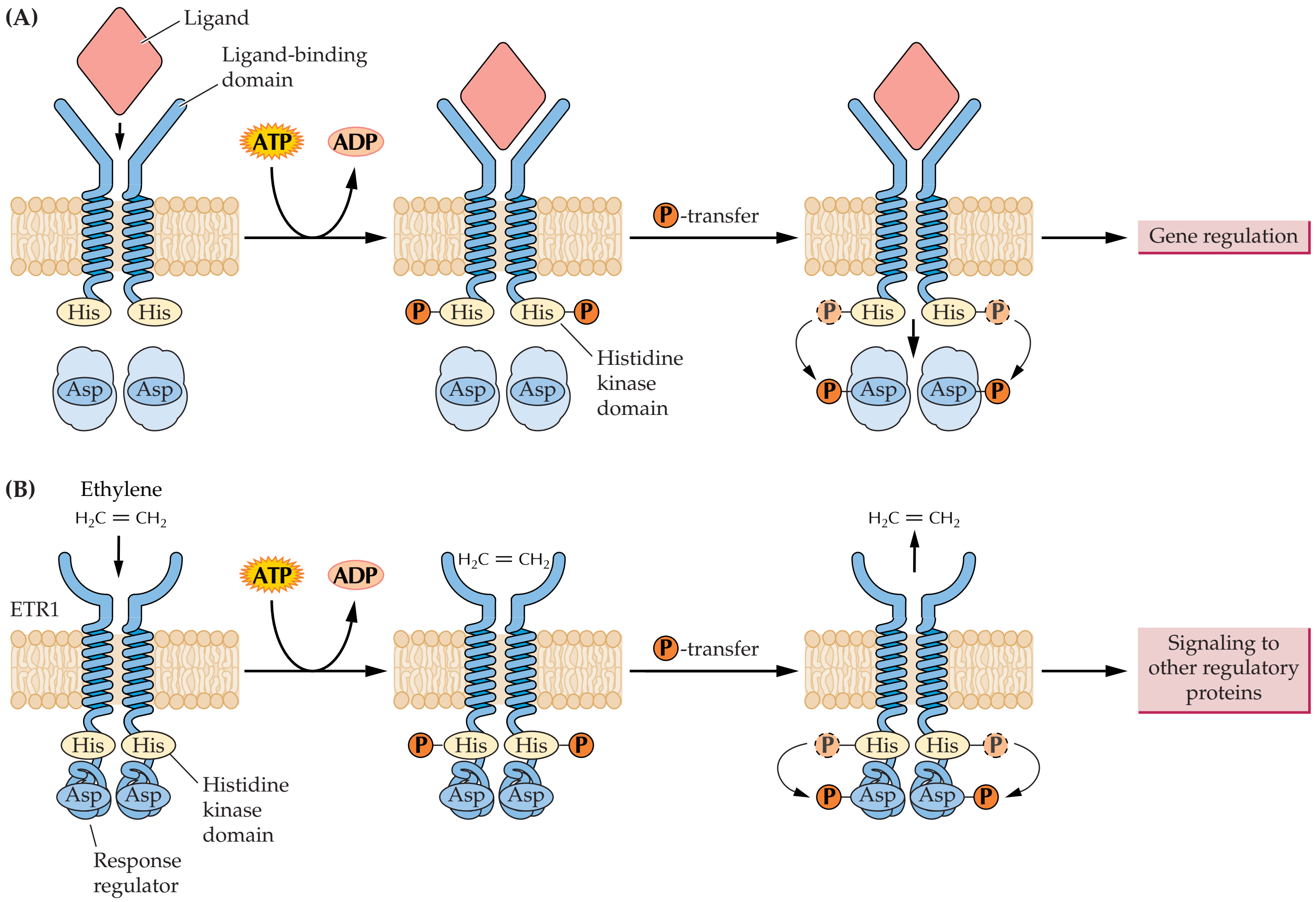


Figure 18.18
The two-component paradigm and the hybrid kinase modification. (A) The two-component system consists of a histidine kinase receptor that, on activation by ligand binding or other processes, autophosphorylates a histidine residue. This phosphate is then transferred to a conserved aspartyl residue on a response regulator, which is often a DNA-binding protein. Gene expression is then altered. (B) In the hybrid kinase system, the histidine kinase is fused with part of a response regulator. An additional response regulator is typically required to control gene expression. The hybrid system may self-regulate the duration of the response by autoinhibition. The *Arabidopsis* ethylene receptor ETR1 is shown here as an example.

Reaction 18.1: Autophosphorylation of receptor

ATP + Histidine kinase →
ADP + Histidine kinase~P

The second component, a **response regulator protein,** is activated when the receptor kinase transfers phosphate from its histidine residue to a conserved aspartate residue on the regulator. Removal of the phosphate group inactivates the response regulator.

Reaction 18.2: Phosphorylation of response regulator

Histidine kinase~P + response regulator →
Histidine kinase + response regulator~P

Reaction 18.3: Dephosphorylation of response regulator

Response regulator~P →
Response regulator + P_i

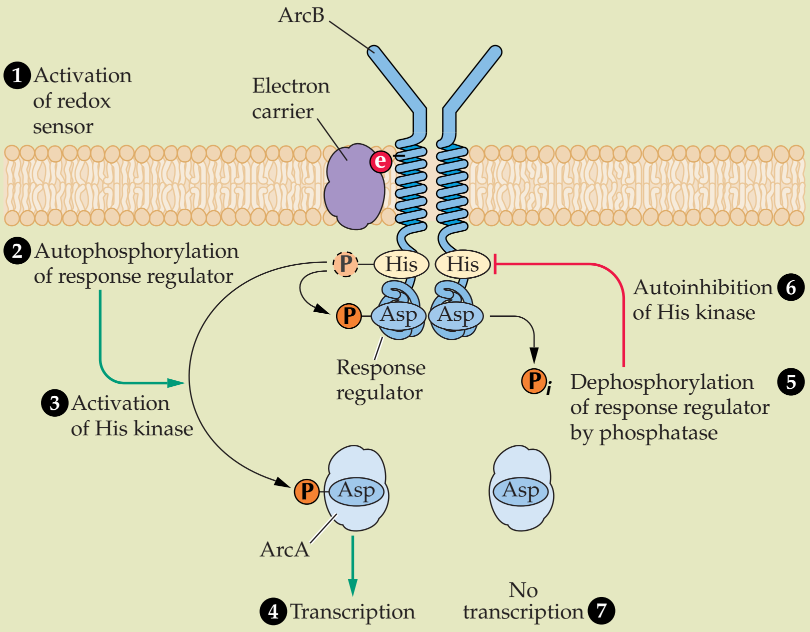
In the phosphorylated form, the response regulator controls a wide range of cellular activities, including motility and gene expression. The extent of phosphorylation of the response regulator is controlled by both the instability of the aspartyl phosphate bond and the protein phosphatase activity. The number of phosphorylated histidyl and aspartyl residues represents a balance between the activating signal and the phosphatase activity.

Box 18.2

Metabolic oxidation/reduction states are sensed in bacteria through a hybrid kinase.

At least 10 hybrid kinases have been isolated from various bacteria. Virulence mechanisms, starvation responses, and adaptation to different redox states all use hybrid kinases as part of the transduction mechanism. ArcB (see figure) is a hybrid kinase created by the fusion of a histidine kinase and a response regulator. ArcB histidine kinase, a redox sensor, is activated by interaction with electron carriers. Subsequently, a conserved histidine residue in the histidine kinase domain of ArcB is autophosphorylated. Transfer of the histidine phosphate to an aspartate residue in the response regulator domain activates the protein. Phosphate is continually transferred from the histidine kinase to ArcA, a transcription factor. This last step results in substantial amplification of the initial signal. Accumulation of many phosphorylated molecules of ArcA initiates specific changes in gene expression. Loss of phosphate from the response regulator domain deactivates the hybrid kinase. Dephosphorylation of the response regulator is controlled by phosphatases, the activities of which are, in turn, modulated by metabolites. A simple means for gating the response is thus present.

Changes in redox state inform plant cells of hazardous situations such as anaerobic conditions, high intensities of light, drought, salinity, and nutrient stress. Changes in redox states may impact cellular responses directly; alternatively, redox states may manipulate concentrations of cytosolic calcium. Bacterial redox-sensing mechanisms may constitute important models for unraveling plant cell pathways for redox sensing and transduction.



In several two-component systems, the histidine kinase and the ligand-binding domains are located on separate proteins, thus enabling many ligands to activate the same kinase. Another prominent variation is a hybrid kinase system, in which the response regulator is fused to the histidine kinase. The sensing of metabolic redox states in bacteria uses a hybrid kinase transduction system (Box 18.2). ETR-1, the ethylene receptor, is a prominent example of a hybrid kinase in plant cells (see Fig. 18.18B and Section 18.3.1).

18.2.8. Regulation of receptor concentrations can change the sensitivity of cells to signals.

Not all tissues or cell types are able to respond to all signals. For example, fruit tissues become sensitive to ethylene at a certain stage of ripening, whereas guard cells are totally insensitive to high concentrations of the gaseous hormone. Different responses by different tissues to the same signal can in part be explained by families of receptors. Auxin, for example, can induce pericycle cells to form adventitous or lateral roots, but in coleoptile cells it promotes elongation. Different receptors are probably involved in each response. However, divergent downstream elements of the signal transduction pathway may also distinguish the developmental responses to auxin exhibited by different cell types. Tissue-specific signal transduction pathways are thus defined not only by the presence or absence of receptors but also by the presence or absence of downstream apparatus required to transduce the responses.

Tissues can adapt or desensitize themselves to continuous signals, and receptor concentrations can change during development. For example, when etiolated seedlings are exposed to red or white light, the cellular concentrations of phytochromes decrease rapidly. The precise cellular mechanism of phytochrome degradation involves ubiquitin-mediated proteolysis (see Chapter 9), phosphorylation, or some other form of sequestration inside the cell. Phytochrome concentrations in green plants may be 100-fold less than those in etiolated seedlings. Consequently, the sensitivity of the green tissues to the greater light fluxes is adjusted so that phytochrome signals can still be sensed and used as a sensitive regulator of development. This behavior is predicted by the **Strickland–Loeb model of receptor action** (see below).

Modification of receptor concentrations can alter the **dose–response** relationship that links specific concentrations of ligand and receptor to physiological responses. Figure 18.19 illustrates the relationship between calcium and calmodulin concentrations and the activation of a calcium/calmodulin–dependent enzyme, cAMP phosphodiesterase. The relationship between calcium, calmodulin, and target enzymes results from unusual binding affinities among the three constituents. The K_d for calcium and calmodulin is approximately 10 μM; those for the calcium/calmodulin complex and the target protein can be as low as 1 nM. Signal transduction pathways take advantage of this relationship. For example, statocytes, the gravity-sensing cells in the root, contain concentrations of calmodulin an order of magnitude greater than those found in other root meristem cells. Statocytes may therefore respond to much smaller increases in cytosolic calcium than other root cells do. Likewise, the mechanical signals that cause transient increases of cytosolic calcium in seedlings, i.e., wind and touch, also substantially increase calmodulin synthesis and accumulation. Thus, stimulation can render seedlings much more sensitive to further mechanical signals.

S. Strickland and J. N. Loeb demonstrated mathematically how variations in hormone receptor concentrations, hormone concentrations, and physiological effects of hormones such as protein kinase activation were interrelated. These workers stipulated certain criteria for the analysis:

- The hormone induces the synthesis or release of a second messenger such as cyclic 3′,5′-AMP (cAMP; discussed further in Section 18.5.1).
- The second messenger interacts with a downstream enzyme or protein such as cAMP-dependent protein kinase A (PKA; see Section 18.5.1).
- The affinity of the hormone for its receptor is weaker than the affinity of the second messenger for its target enzyme. That is, the K_d for the second messenger and its target enzyme is greater than the K_d for the hormone and its receptor.

Two important consequences follow if these criteria are fulfilled. First, hormone responses are activated fully when only a small proportion of the receptors are occupied; most receptors can therefore be described

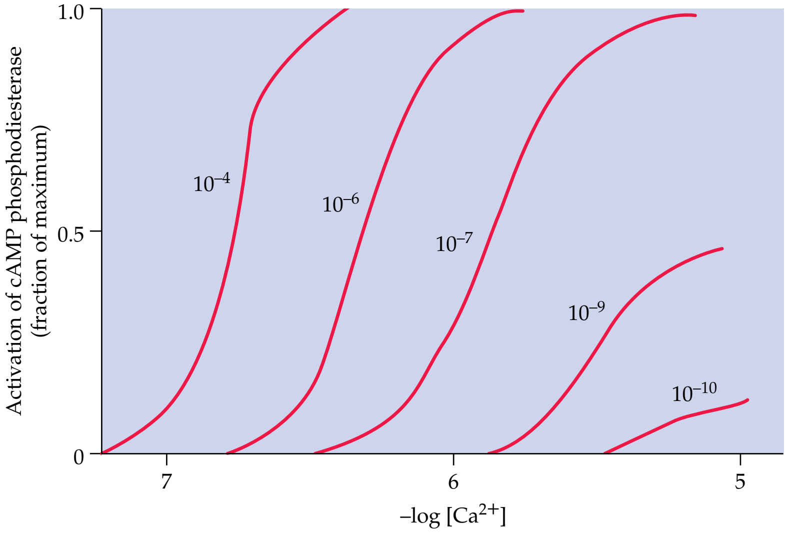


Figure 18.19
Activation of cAMP phosphodiesterase by varying the concentrations of Ca^{2+} and calmodulin. The K_d for Ca^{2+} and calmodulin is about 1 to 10 μM, whereas that for the Ca^{2+}/calmodulin complex and phosphodiesterase is about 1 nM. This binding constant disparity allows full activation of the target enzyme when only a small amount of Ca^{2+}/calmodulin is formed. Activation depends directly on the concentrations of both Ca^{2+} and calmodulin. The x-axis variable is the pCa, the negative logarithm of the Ca^{2+} concentration (M). Illustrated here is cAMP phosphodiesterase activation as a function of calmodulin concentration (the molarity values given next to the individual activation curves).

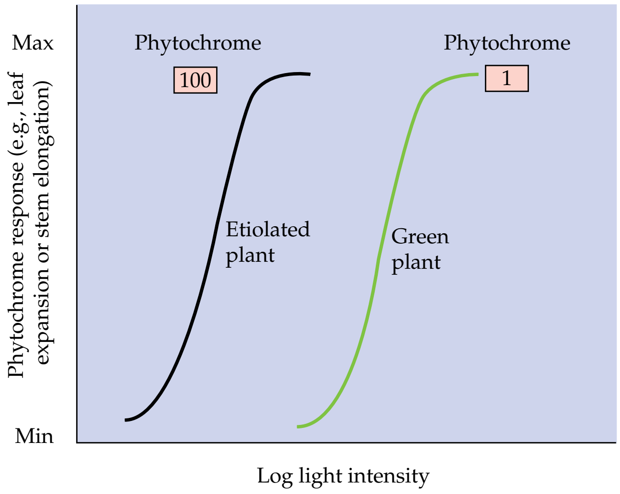


Figure 18.20
Phytochrome concentrations decrease by as much as 100-fold during deetiolation, enabling the plant to retain sensitivity to light over the wide range of light intensities to which dark-grown and green tissues are exposed. Phytochrome concentrations (in boxes) are shown in relative units.

as "spare." Second, changes in the dose–response relationship between hormone concentration and the physiological effects can be produced simply by manipulating receptor concentrations. These proposals suggest a situation analogous to the calcium/calmodulin target enzyme relationship illustrated in Figure 18.19.

If the Strickland–Loeb criteria apply to the deetiolation program, the rapid light-induced decrease in cellular phytochrome adjusts the sensitivity of the tissue to increased light flux. Figure 18.20 indicates a possible relationship between control of hypocotyl or stem length and light fluxes. In this model, the effect of phytochrome degradation is to ensure that green cells can still use phytochrome as a sensitive regulator of growth and metabolism.

18.3 Specific examples of plant receptors

18.3.1 Identification of ethylene receptors provided the first eukaryotic example of a two-component system.

The gas ethylene regulates ripening, germination, elongation, senescence, and pathogen responses. Several ethylene receptors have been cloned through isolation of ethylene-insensitive mutants and subsequent use of molecular technology to identify the mutant gene. ETR1, a 79-kDa protein with a transmembrane domain, was the first receptor cloned from *Arabidopsis.* The C terminus of ETR1 is homologous to a bacterial two-component system hybrid kinase (see Section 18.2.7). ETR1 exists as a dimer in the plasma membrane. Ethylene joins the two monomers together and permits intermolecular phosphorylation (Fig. 18.21).

Mutations in *ETR1* (designated *etr1*) lead to loss of physiological sensitivity to ethylene. Figure 18.22 illustrates some of the physiological effects of expressing an *etr1-1* transgene. ETR1 has also been expressed in yeast to demonstrate that the protein binds ethylene with high affinity. Competitive ethylene antagonists inhibit this binding. Expression of *etr1* in yeast leads to loss of ethylene binding, confirming that ETR1 is thus a true ethylene receptor.

Genes encoding other ethylene receptors have also been identified, including *ERS* (ethylene response sensor), *Nr* (never ripe, a developmentally regulated gene from tomato; see Fig. 18.22A), and *LeTAE1* (a tomato *ETR1* homolog expressed during flower and fruit senescence).

18.3.2 Many auxin-binding proteins have been detected, but whether they represent receptors for different auxin-mediated processes is still uncertain.

Indole 3-acetic acid (IAA, referred to here as auxin) is a growth regulator with a wide variety of functions in cell division and expansion (see Chapter 17). Auxin has been studied

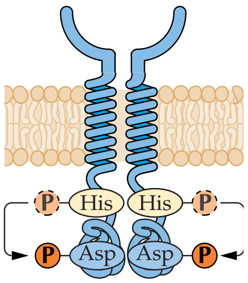


Figure 18.21
The *Arabidopsis* ETR1 protein has been identified as a receptor for ethylene. On binding ethylene, the receptor protein dimerizes and initiates signaling by autophosphorylation and phosphate transfer.

intensively for the past 50 years and, not surprisingly, receptors for the auxin signal have been actively sought. Conventional pharmacological techniques have uncovered one well-characterized **auxin-binding protein** (ABP1). The possible receptor function of this protein was controversial for many years (Box 18.3) but has recently been established.

Use of various affinity-binding techniques and immunoaffinity-labeling approaches has detected several other auxin-binding proteins, including a glutathione *S*-transferase, a (1→3)β-glucanase, and a cytokinin glucosidase. The significance of these proteins to auxin action is unclear at present; perhaps they are expressed by the cell in response to the stressful conditions required for labeling the proteins in vivo. Detecting receptors by isolation of auxin-resistant mutants has yielded some recent success. *AXR1* encodes a protein with similarity to a ubiquitin-conjugating enzyme and may participate in detoxification of auxin (see Section 18.8.3); *AUX1* is a member of a family of closely related genes and encodes an auxin permease with 10 membrane-spanning domains. The roots of auxin-resistant mutants *axr1* and *aux1* have weak or nonexistent

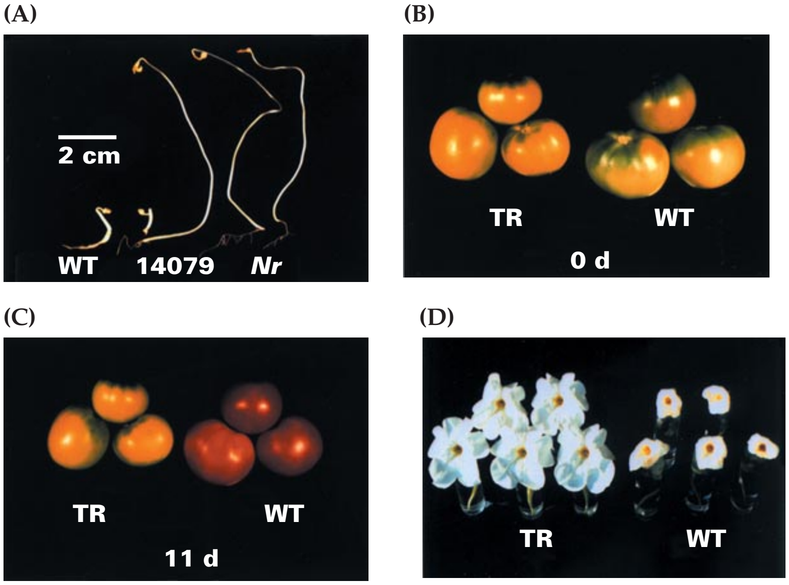


Figure 18.22
Ethylene-insensitive phenotypes displayed by transgenic (TR) plants expressing variants of the *etr1-1* gene. (A) Wild type (WT), TR line containing the *etr1-1* gene (14079), and Never ripe (*Nr*) tomato seedlings germinated in the dark on medium containing the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC). (B, C) Development of three postmature green TR fruits and three mature green WT fruits after storage for 0 (B) and 11 (C) days. (D) Detached TR and WT petunia flowers 16 hours after treatment with ethylene.

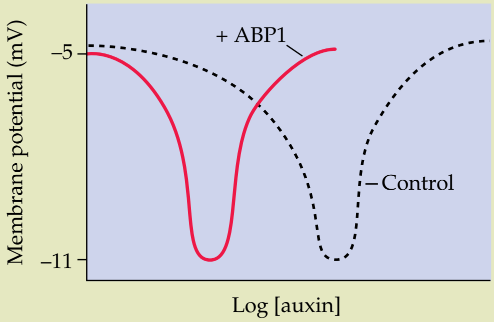
Box 18.3

The role of ABP1 is currently under investigation.

ABP1 is a small family of 23-kDa proteins that bind indole 3-acetic acid (IAA) and naphthalene-1-acetic acid (NAA) as well as other molecules with auxin activity. Despite strong circumstantial evidence for its receptor function, critical evidence of the kind obtained for the ethylene receptor is still lacking; this may soon be resolved, however.

Experiments with tobacco protoplasts provide the best early evidence for the role of ABP1 as an auxin receptor. Adding auxin to tobacco protoplasts causes hyperpolarization of the membrane potential; adding ABP1 to the protoplasts greatly increases their sensitivity to auxin (see figure). Adding antibodies against ABP1 to the protoplasts increases the concentration of auxin required to elicit an equivalent response in an untreated control. Moreover, a decapeptide synthesized from the C terminus of ABP1 has auxin-like activities on *Commelina* guard cells. ABP1 contains a C-terminal HDEL putative ER-retention domain, although small amounts of the protein are found in the plasma membrane as well.

Overexpression of *ABP1* has been achieved in tobacco. Although the phenotype of the transgenic plant remained unaltered and the leaf area unchanged, the size of the average leaf cell increased more than threefold, most probably because of fewer cell divisions. The results indicate a role for ABP1 in controlling aspects of cell expansion but also suggest the presence of other auxin receptors regulating overall leaf growth in coordination with ABP1, which is responsible for just one aspect: cell elongation.



gravitropic responses, but their shoot responses are unaffected, implying that the gravity signal is transduced by different mechanisms in roots and shoots. *AUX1* is a member of a family of closely related genes. Transgenic expression of *AUX1* complements the agravitropic characteristic of *aux1;* expression of *AUX1* is high in root epidermis, where it possibly regulates root growth rates. Some evidence suggests an association of auxin transport with the perception of auxin. More recent investigations have established that AtPIN1, a 67-kDa protein with similarity to bacterial and eukaryotic carrier proteins, is a transmembrane component of the auxin efflux carrier. Mutations in the gene encoding this protein inhibit polar auxin transport and modify production of lateral organs and formation of vascular tissue. In the root, AtPIN2, another member of this auxin efflux family, may control the movement of auxin in the elongation zone. Mutations in the gene for AtPIN2 render roots agravitropic.

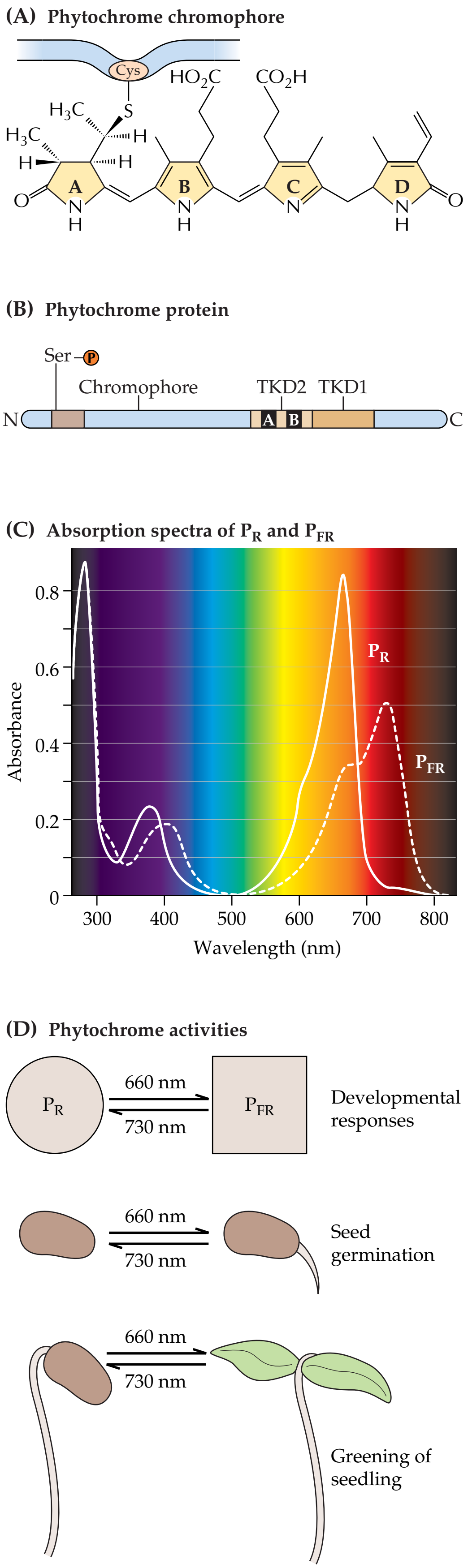


Figure 18.23
Structure and function of phytochromes. (A) The phytochrome chromophore is a tetrapyrrole that binds to the phytochrome protein. (B) The C termini of plant phytochromes may contain two distinct transmitter kinase domains, TKD2 and TKD1. (C) Absorption spectra of P_R and P_{FR} show peaks for red (660 nm) and far-red (730 nm) light, respectively. (D) Phytochromes can exist as either of two interconvertible forms. Isolated phytochromes in a concentrated solution can undergo reversible changes in absorbance induced by illumination with red or far-red light. These light-induced structural changes are coincident with physiological and developmental changes induced by red light or blocked by far-red light.

18.3.3 Phytochrome, a clearly identified receptor for red light, has protein kinase activity in cyanobacteria.

Red light controls leaf expansion, shade-avoidance reactions, germination, and response to photoperiod. A particular characteristic of many red light–induced effects is their reversibility when an inductive flash of red light (660 nm) is followed by an immediate flash of far-red light (730 nm) (Fig. 18.23). This characteristic was used to identify and purify the red-light receptor phytochrome in 1965, one of the primary achievements of 20th-century plant science. The purified receptor protein undergoes reversible changes in structure in vitro when exposed to red or far-red light.

Phytochromes form a family of 120-kDa proteins (Box 18.4). The photoreactive moiety (chromophore) of these proteins is an open-chain tetrapyrrole. Two forms of phytochrome, A and B, can each form dimers in solution, and physiological evidence suggests that both may dimerize in vivo. The far-red–absorbing form, P_{FR}, is considered to be the active form of phytochrome, although most photomorphogenic

Box 18.4

Phytochromes form a family of proteins, each of which has different physiological functions.

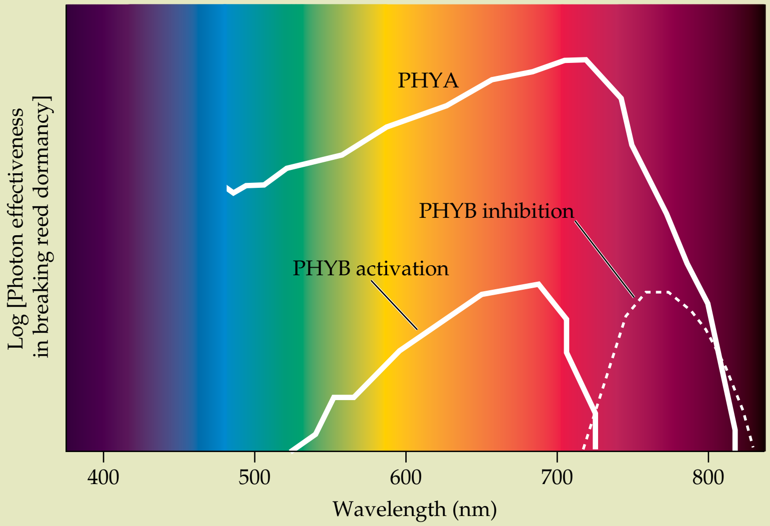
Physiological evidence first revealed that the stability of phytochromes differed in etiolated and deetiolated tissues (see Section 18.2.8). Along with other evidence, this indicated the existence of two pools of P_{FR}, a labile pool and a stable pool—pools that have since been found to represent different phytochromes.

Evidence of distinct types of phytochromes has been provided by the cloning and sequencing of five genes from *Arabidopsis* (designated *PHYA* through *PHYE*) that encode phytochromes A through E. The amino acid sequences of PHYA, PHYB, and PHYC are equally divergent and demonstrate about 50% homology to each other. Mutant analysis has revealed some of the separate functions performed by the various phytochromes. The cucumber *lh* mutant and the *Arabidopsis hy3* mutant, which grow taller than wild-type plants in white light, are deficient in PHYB. The *aurea* mutant of tomato is deficient in PHYA and has been used to dissect phytochrome responses (see Fig. 18.42). A recent mutant screen has identified *PHYC* mutants in *Arabidopsis,* and phenotypic analysis of a *PHYD* mutant in one *Arabidopsis* ecotype indicates that PHYD regulates several of the same responses as PHYB.

Different action spectra have been deduced for PHYA and PHYB. The figure illustrates the capacity of various wavelengths of light to induce seed germination. The action spectrum of PHYA is typically determined on seed imbibed in water for 2 days in darkness; surprisingly, it does not show photoreversibility. The action spectrum for PHYB is determined on seed imbibed in water for very short periods and is reversible by exposure to far-red light.

Several forms of phytochrome response are classified as the high irradiance reaction (HIR) and the induction reaction, based on the fluence and timing of irradiation. The induction reaction is further subdivided into a low fluence red/far-red reversible reaction (LFR) and a very low fluence response (VLFR). LFR and VLFR can be activated by short pulses of light. LFR is the classical low fluence red/far-red reversible control of light-dependent seed germination and is typically detected after only a few minutes of seed imbibition. Mutational studies have implicated PHYB as the prime regulator of LFR. VLFR, a more sensitive response, can be detected in seeds after imbibation for several days in darkness or in many etiolated tissues likewise kept in darkness. Surprisingly, VLFR is not reversible by far-red light, but mutational investigations on seedlings deficient in phytochromes clearly implicate PHYA as the regulator.

HIR requires continuous or prolonged irradiation with high-intensity light. The response in this case is proportional to the irradiance received by the plant; again, photoreversibility is absent. Typical HIR responses are anthocyanin synthesis or inhibition of hypocotyl elongation. Although phytochromes clearly are involved in these responses, evidence indicates that other photoreceptors that absorb UV or blue light contribute to this control.



reactions are thought to result from the cellular ratio of P_{FR} to P_R, the form that absorbs red light.

An early hypothesis suggested that P_{FR} is a protein kinase. Plant phytochromes often copurify with a protein kinase activity, but highly purified or cloned phytochromes, and phytochromes expressed in bacteria, do not appear to have conventional protein kinase activity. A phytochrome cloned from the moss *Ceratodon,* however, contains an additional sequence showing homology to protein kinase catalytic domains. The cyanobacterium *Synechocystis* also contains a phytochrome (Cph1). The N terminus of the prokaryotic protein is similar in sequence to that of plant phytochrome, whereas the C terminus contains consensus sequences that define a two-component histidine kinase (Fig. 18.24). A critical histidine has been shown to be autophosphorylated, and the phosphate can be transferred to a conserved aspartyl residue in Rcp1, a separate protein. Only the P_R form of Cph1 displayed substantial kinase activity. Down-regulation of histidine kinase activity may result from transfer of the phosphate on the histidine to a serine residue in the N-terminal region.

18.3.4 The blue light receptor is a DNA lyase-like protein or a protein kinase.

The most prominent blue light responses are phototropism, inhibition of hypocotyl elongation, stomatal opening, anthocyanin production, and expression of blue light–regulated genes. Blue light initiates changes in plant cell plasma membrane potential (Fig. 18.25), participates in redox reactions and electron transport, and induces at the plasma membrane a change in light absorbance involving a flavin-mediated photoreduction of cytochromes.

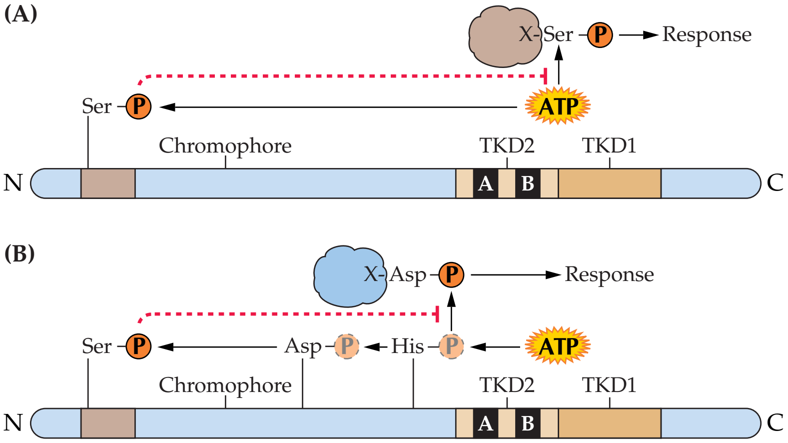


Figure 18.24
Two hypotheses for how kinase activity may mediate phytochrome action in *Synechocystis.* (A) The first model assumes that phytochrome is a serine kinase able to carry out both phosphorylation of a substrate to initiate signaling and autophosphorylation to down-regulate phytochrome activity. (B) The second mechanism assumes phytochrome to be a type of hybrid kinase in which a conserved histidine residue is phosphorylated as a result of light absorption. Transfer of this phosphate to an aspartyl residue and then to a serine residue would down-regulate phytochrome activity.

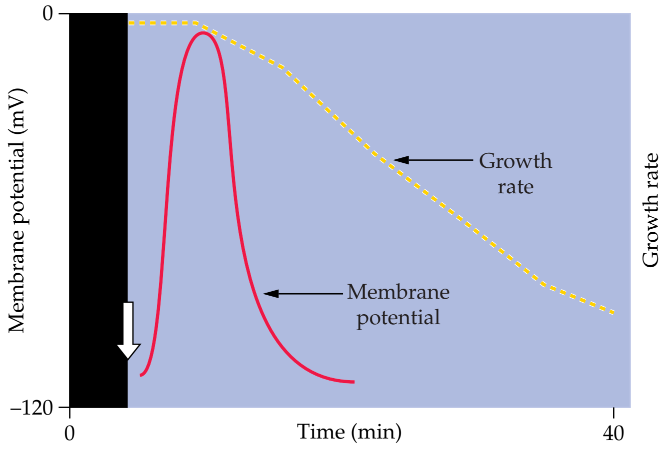


Figure 18.25
Effect of blue light on membrane potential and hypocotyl growth. Etiolated hypocotyls irradiated with blue light (white arrow) undergo a pronounced depolarization of the membrane potential of individual hypocotyl cells, followed by a marked decline in the rate of growth.

Cryptochrome, the blue light receptor encoded by the *Arabidopsis CRY1* gene, has been isolated by screening T-DNA insertion lines for blue light–insensitive mutants (see Fig. 18.17A). Genetic evidence suggests that CRY1 is one member of a family of receptors. Cryptochrome has sequence similarity to DNA photolyases, a rare class of flavoproteins that catalyze blue light–dependent electron transfer reactions in bacteria. However, the cryptochrome encoded by *CRY1* does not have detectable DNA-lyase activity.

The *Arabidopsis* mutant *nph1* (nonphototrophic hypocotyl 1) fails to show a phototropic response in blue light. Sequence analysis and biochemical studies have established that NPH1 has protein kinase activity. Specific amino acid domains in NPH1, termed LOV1 and LOV2, share sequence similarity with proteins from bacteria and eukaryotes. In these organisms, light, oxygen, or voltage regulates the activity of LOV proteins, which participate in redox sensing. It is still unclear whether NPH1 is a primary blue light receptor or a downstream component of a blue light signal transduction pathway. Irradiation of isolated plasma membranes with blue light results in detectable changes in protein phosphorylation. The identification of NPH1 as a protein kinase might explain these early observations. NPH1 is clearly an important transduction component in blue light–controlled processes. Formation of double mutants of *cry1* and *nph1* has shown that a separate blue light sensor regulates stomatal aperture.

18.3.5 Is the ABA receptor a protein phosphatase?

The plant growth regulator ABA controls aspects of plant adaptation to variations in the amounts of soil water and responses to water stress. Probably different ABA receptors are used for processes such as seed germination, stomatal closure, or altered gene expression. Work with caged ABA has provided evidence for the presence of ABA receptors inside cells. (Use of caged molecules is discussed further in Box 18.5.) When caged ABA was loaded into individual guard cells and released, the

Box 18.5

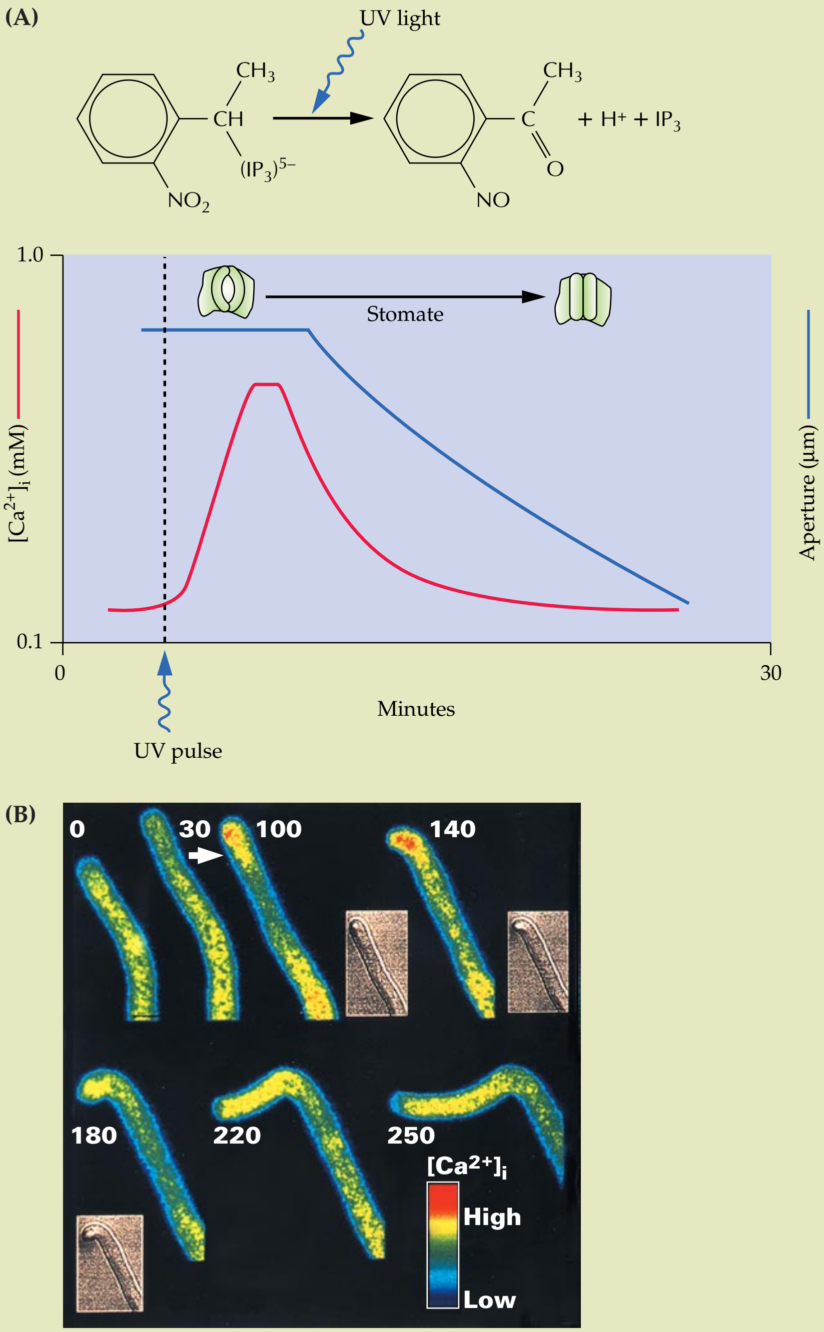
Caged molecules can be used to demonstrate the activity of signaling pathways in plant cells.

One of the primary advances in cell biology technology has been the development and use of photoreactive or "caged" probes. Reaction with a caging group renders the signaling molecule inactive. A flash of UV light can then be used to release the caging moiety. By loading the caged, inactive molecule into cells, the spatial and temporal release of the active molecule inside a cell can be controlled experimentally. A variety of caged molecules are available for study, including hormones (see Fig. 18.26), nucleotides, chelators, and second messengers such as Ca^{2+} and inositol 1,4,5-triphosphate (IP_3). Recent methods have detailed the construction of caged peptides and even caged proteins by modifying critical amino acids. Importantly, loading and photolysis of caged molecules mimic the responses normally induced by ABA, red light, incompatible S-proteins, and treatments that reorient pollen tubes.

Panel A of the figure illustrates an experiment in which caged IP_3 (see structure) is loaded into a guard cell and photolyzed. Brief pulses of UV light release IP_3 into the cell, which is followed by a transient increase of cytosolic Ca^{2+} over several minutes. If a threshold of about 500 nM for $[Ca^{2+}]_i$ is exceeded, the guard cells close (see graph). A similar link between photolysis of caged IP_3 and increased $[Ca^{2+}]_i$ has been determined experimentally in pollen tubes and red light–sensitive protoplasts, indicating the presence of IP_3-sensitive channels in these cell types. Thus, as in animals, IP_3 couples signals to the release of Ca^{2+} from stores within plant cells.

Caged Ca^{2+} (i.e., complexed with diphenyl EGTA) has been released into different regions of the pollen tube to demonstrate that $[Ca^{2+}]_i$ in the pollen tube tip controls orientation of the tube. Pollen tube growth is confined to the apical dome and results from secretion of vesicles containing cell wall material into the wall of the dome. Using focused microbeams of UV light, the cage can be photolyzed in different regions of the pollen tube. A Ca^{2+}-sensitive dye is loaded into the pollen tube and images are generated in which the approximate Ca^{2+} concentrations are color-coded (blue corresponding to low Ca^{2+}, red to high Ca^{2+}), so that the consequences of Ca^{2+} release can be followed. In the fluorescence micrographs shown in panel B of the figure, photolysis was carried out between 30 and 100 seconds after recording started. Images were collected with a confocal microscope (bright-field images of the same pollen tube are included as insets).

When Ca^{2+} was specifically released in one side of the pollen tube tip (indicated by arrow), the pollen tube polarity was altered and the direction of growth was reoriented toward the side having the higher apical concentration of Ca^{2+}.



guard cell closed (Fig. 18.26). Other evidence suggests the existence of external ABA receptors as well.

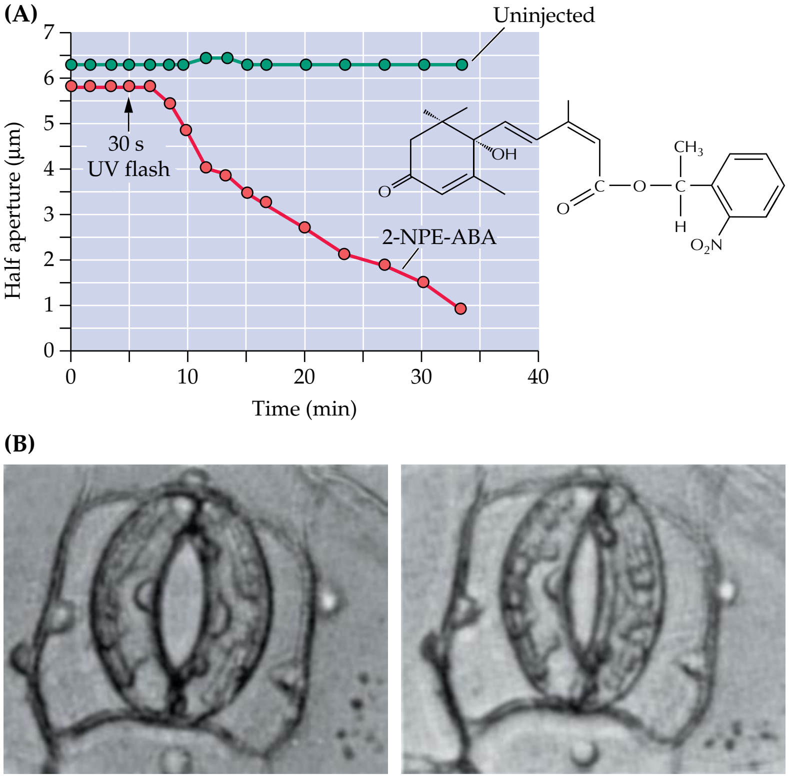


Figure 18.26
Photolysis of caged ABA in guard cells causes closure. (A) A caged species of ABA, 1-(2-nitrophenyl)ethyl-ABA, is labile to UV light. When loaded into guard cells and photolyzed, the caged form is converted to free ABA and causes guard cell closure. (B) Light micrographs showing the impact of ABA on stomatal aperture. The guard cell was loaded with the caged ABA (left panel) and photolyzed, resulting in stomatal closure (right panel).

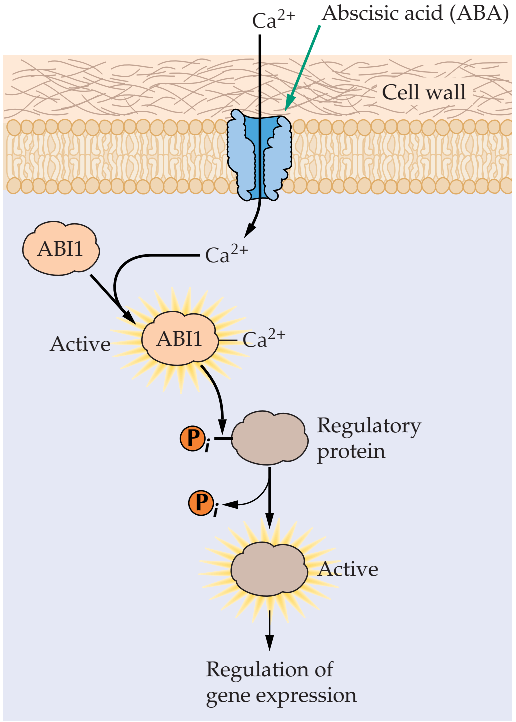


Figure 18.27
Possible transduction route for ABA. ABA is thought to bind to a receptor (not shown) that allows direct entry of Ca^{2+} at the plasma membrane and activates a Ca^{2+}-sensitive protein phosphatase (ABI1).

At least 10 *abi* (ABA–insensitive) mutants have been identified, indicating that a complex signaling network operates in seed dormancy and other processes involving ABA. Many of the mutants exhibit a wilted phenotype, but the *abi1* mutation interferes with the widest range of responses. ABI1 has sequence similarity to a serine/threonine protein phosphatase that binds Ca^{2+} (see Section 18.8.4). A protein kinase cascade is probably activated by ABI1-catalyzed dephosphorylation (Fig. 18.27). ABI1 does not bind ABA, however, and therefore is not activated directly by ABA. Perhaps ABI1 specifically binds to the ABA receptor or to a receptor complex that also involves a protein kinase.

18.3.6 Cytokinin sensing and transduction may use a two-component system and a protein with seven membrane-spanning domains.

Cytokinin-independent mutants such as *cki1* do not require cytokinin for cell division. CKI1, a 125-kDa protein, has weak sequence homology to a hybrid two-component kinase (Fig. 18.28). CKI1 has two membrane-spanning domains, and its N terminus is predicted to reside outside the cell. A second gene, *GCR1,* has been isolated from *Arabidopsis* cDNA libraries. The deduced structure of GCR1 has seven membrane-spanning domains. Expression of *GCR1* in the antisense orientation specifically reduces the sensitivity of the transformants to cytokinins. The precise function of both proteins in cytokinin signaling, however, remains to be clarified.

18.4 G-proteins and phospholipid signaling

18.4.1 G-proteins, a special subset of a GTPase superfamily, may all be concerned with aspects of accuracy of recognition or interaction.

Proteins that bind and hydrolyze GTP are being discovered at a rapidly increasing rate. Each of these many **GTPases** acts as a molecular switch in which the “on” and “off” states are triggered by binding and hydrolysis of GTP. Conserved structure and mechanism in different types of GTP-binding proteins suggest they are all derived from a single primordial protein that has been repeatedly modified during evolution to perform a large variety of functions.

Figure 18.29 illustrates the basic cycle that occurs in all GTPase proteins. The empty state combines with a GTP to form the active state. Hydrolysis of GTP to GDP inactivates the protein, and subsequent dissociation of GDP returns the protein to the empty state. Two protein families control aspects of this cycle. Members of one family, the GTPase-activating proteins (GAPs), accelerate the rate of GTP hydrolysis. The other set, called guanine nucleotide release proteins (GNRPs) or GDP exchange factors (GEFs), controls release of GDP from the inactive GTPase. Two broad classes of GTPases can be distinguished in mammalian cells. **Heterotrimeric G-proteins,** composed of three subunits (α, β, and γ), are specifically involved in recognition of activated receptor states.The other class, **monomeric GTPases,** includes Ras (Fig. 18.30), Ypt, Rab, Rap, Sec, Rho, and EFTu.

Although GTPases act as switches, their primary function may be to improve the accuracy of recognition during signaling, secretion, and protein synthesis. A specific function for a GTPase was first identified in studies of bacterial protein synthesis. In this process the complex of mRNA, ribosomes, and aminoacyl tRNA is joined by a GTPase (see Chapter 9). To ensure accurate codon: anticodon interactions, the stability of this complex is measured against the rate of hydrolysis of GTP. The amino acid is not added to the growing peptide chain until GTP is hydrolyzed. If the recognition is inaccurate, the aminoacyl tRNA/mRNA codon interaction is less stable and dissociates before GTP hydrolysis has occurred. This form of proofreading during translation improves the accuracy of protein synthesis 100-fold.

The requirement for accurate recognition may also explain the involvement of monomeric GTPases in the docking of vesicles with target membranes during secretion as well as G-protein functioning in ligand–receptor interactions. During signal transduction, enhanced accuracy of ligand–receptor recognition ensures amplification of the correct signal.

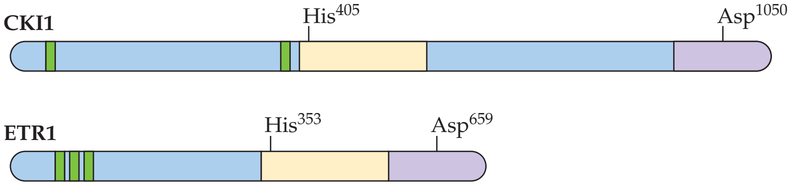


Figure 18.28
Comparison of the structures of CKI1, a putative cytokinin receptor, and ETR1, the ethylene receptor. CKI1 contains two transmembrane domains (shown in green) and conserved histidine and aspartyl residues, which have been recognized by sequence alignment with various two-component hybrid kinases. ETR1 contains three transmembrane domains and conserved histidine and aspartyl residues.

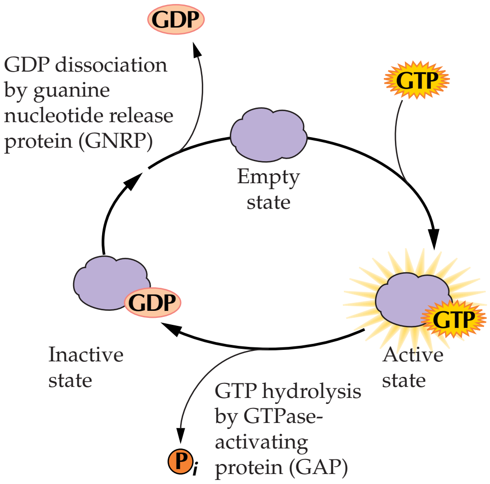


Figure 18.29
The fundamental cycle associated with all signaling GTPases. GTPases are converted from an empty state to an active state by association with GTP. GTP hydrolysis, catalyzed by GTPase-activating protein (GAP), inactivates the GTP-binding proteins. GDP release, catalyzed by guanine nucleotide release proteins (GNRP), returns the GTP-binding protein to the empty state.

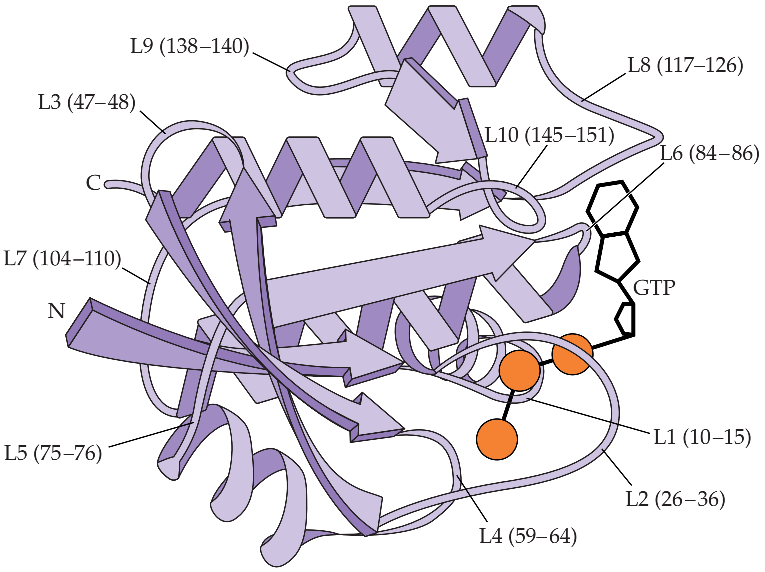


Figure 18.30
Cartoon of the three-dimensional structure of the guanine nucleotide domain of human Ras bound to GTP; the structure was determined by using a nonhydrolyzable GTP analog. The 10 loops, which connect six strands of β sheet and five α helices, are labeled. The structure of Ras serves as a model for other small GTPases found in animals, plants, and fungi.

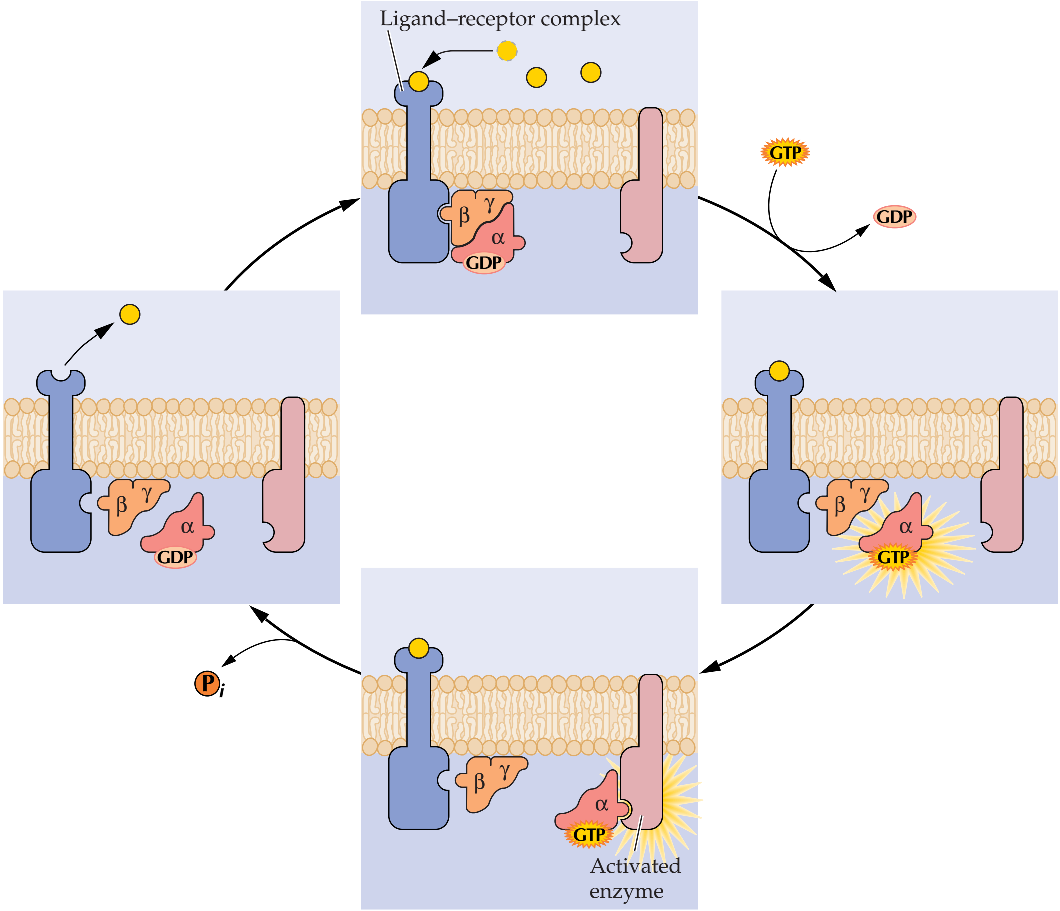
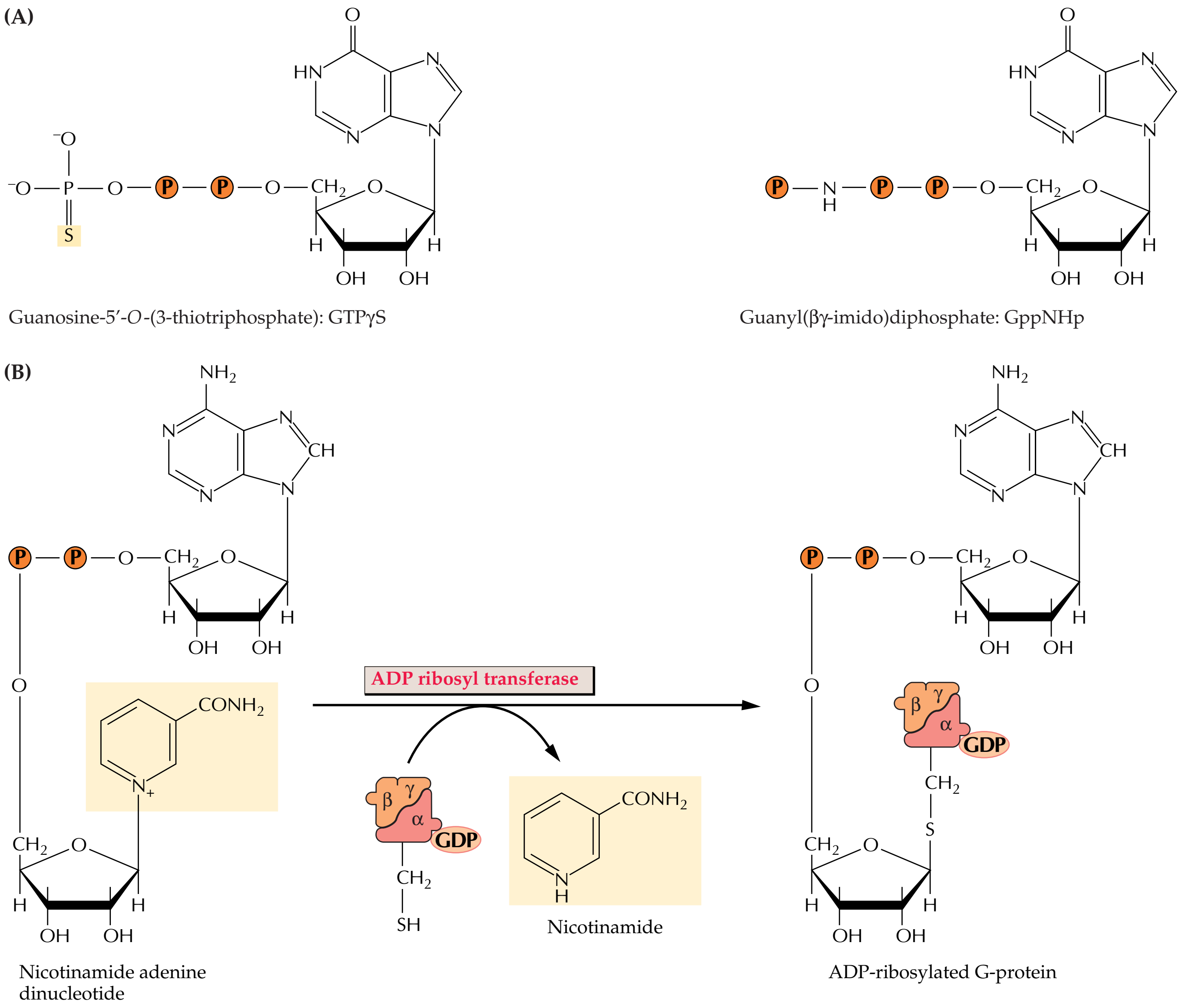


Figure 18.31
The activity cycle of heterotrimeric G-proteins. The ligand–receptor complex acts as the GNRP to catalyze removal of GDP. Binding of GTP to the α-subunit results in its dissociation from the β/γ-subunits. Activation of downstream enzymes such as phospholipase C follows binding by the G-protein α-subunit still bound to GTP. The intrinsic GTPase activity of the α-subunit (stimulated by GAP; not shown) hydrolyzes the GTP to GDP, after which the G-protein returns to the inactive state and the α-subunit reassociates with the β/γ-subunits.

18.4.2 G-proteins found in plant cells may mediate several signals, including blue and red light.

Heterotrimeric G-proteins undergo a modified cycle with GTP (Fig. 18.31). In mammalian cells, GNRP is part of the ligand–receptor complex that, when combined with the inactive G-protein, causes the release of GDP from the α-subunit and its replacement by GTP. Concomitant with GTP loading, the β- and γ-subunits of the G-protein dissociate from the activated α-subunit. Both the β/γ-complex and α-subunit can then interact with and activate other proteins. In plants, the function of heterotrimeric G-proteins and the identities of the downstream target proteins are still uncertain, but phospholipase C (PLC; see Section 18.4.3) and Ca^{2+} channels are prominent downstream candidates. Others may include potassium channels, phospholipase A_2 (PLA_2), and perhaps cGMP phosphodiesterase. The α-subunit has intrinsic GTPase activity and, on hydrolysis of GTP, recombines with the plasma membrane by way of the β- and γ-subunits to complete the cycle. Signal amplification is inherent in the G-protein cycle because one activated G-protein can interact with and activate numerous target proteins.

The involvement of G-proteins in signaling can be investigated by using GTP analogs that are not easily hydrolyzed by GTPase, such as GTPγS or Gpp(NH)p (Fig. 18.32A). When microinjected into cells, these molecules can induce responses in the



Guanosine-5′-*O*-(3-thiotriphosphate): GTPγS

Guanyl(βγ-imido)diphosphate: GppNHp

Nicotinamide adenine dinucleotide

Nicotinamide

ADP-ribosylated G-protein

Figure 18.32
Structure and action of compounds that inhibit the G-protein cycle. (A) Structures of two nonhydrolyzable analogs of GTP: GTPγS and GppNHp. (B) NAD^+ serves as a substrate for cholera and pertussis toxins, which transfer ADP-ribose to proteins. The ADP-ribosyl transferase activity of pertussis toxin can modify and thereby inactivate the trimeric G-protein but does not affect the free α-subunit bound to GTP.

absence of a signal if G-proteins participate in a specific transduction pathway. However, these analogs will not discriminate between heterotrimeric G-proteins and monomeric GTPases. More useful and specific is cholera toxin, which uses NAD^+ to transfer an ADP-ribosyl group to the α-subunit of a heterotrimeric G-protein, inhibiting its GTPase activity so that the activated (GTP-bound) state lasts much longer (Fig. 18.32B). Pertussis toxin also catalyzes transfer of ADP ribose from NAD^+ to the GDP-bound form of the G-protein α-subunit, which results in irreversible inactivation of the G-protein. The involvement of G-proteins in signaling can therefore be assessed by detecting increased or decreased G-protein activity.

The approaches discussed above have revealed the involvement of G-proteins in the transduction of blue light, red light, auxin, and gibberellin signals and in stomatal aperture regulation. For example, GTPγS was found to reduce inward K^+ current in guard cells. Analysis of the expression of the gene for the only G-protein α-subunit known in plants suggests that it is expressed

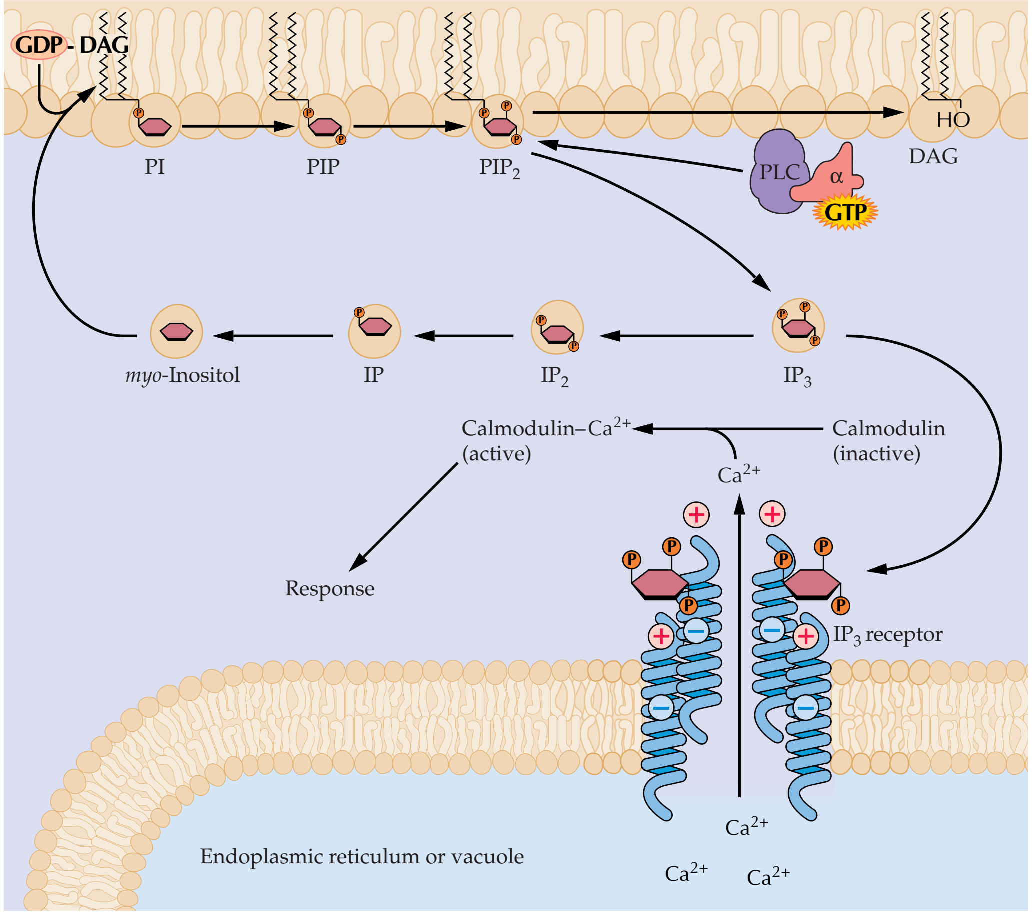


Figure 18.33
Sequence of transduction events leading from activation of phospholipase C (PLC) to increase of cytosolic calcium. Plasma membrane–bound PLC is activated by a G-protein. Phosphatidylinositol 4,5-bisphosphate (PIP_2) is hydrolyzed by PLC to produce the second messengers IP_3 and diacylglycerol (DAG). IP_3 activates the IP_3 receptor attached to the vacuole or the ER, thereby initiating the release of Ca^{2+}. The phospholipid components are recycled phosphatidylinositol (PI), phosphatidylinositol 4-monophosphate (PIP), inositol 1,4-bisphosphate (IP_2), and inositol 1-phosphate (IP).

most strongly in dividing cells. The encoded protein appears to be attached to the plasma membrane and the ER. A gene for a β-subunit protein has also been identified, but no γ-subunit proteins are known in plants.

Rho-like monomeric GTPases have been isolated from several plants. Present indications suggest they have specific functions in pollen tube growth and in cytoplasmic streaming. Rho-like proteins may also be involved in the regulation of actin/myosin–dependent reorganizations of the cytoskeleton during cell division (see Chapter 5).

18.4.3 Phospholipases in the plasma membrane can be activated by G-protein–coupled receptors.

PLC participates with several kinases and phosphatases in an important cycle of inositol phospholipid synthesis and breakdown. PLC, which has been purified from several plants, appears to be a family of proteins that respond to different signals, including one PLC that is Ca^{2+}-dependent. The substrate for PLC—phosphatidylinositol 4,5-bisphosphate (PIP_2)—is cleaved to yield two products: **inositol 1,4,5-triphosphate** (IP_3), a soluble second messenger, and **diacylglycerol** (DAG), which remains membrane-bound (Fig. 18.33). PIP_2 formation is catalyzed by two kinases that successively convert phosphatidylinositol (PI) to phosphatidylinositol 4-phosphate (PIP) and then to PIP_2. A current model suggests that occupation of appropriate receptors by ligands in animals or activation of receptors by red or blue light in plants results in exchange of bound GDP for GTP on the G-protein. The α-subunit bound to GTP is released and activates PLC (Fig. 18.33). IP_3 diffuses freely in the cytoplasm and binds to specific Ca^{2+} channels in the vacuole and the rough ER. Opening these channels releases Ca^{2+} into the cytoplasm.

Inositol phospholipids can also interact with cytoskeletal proteins and thus signal important changes in cellular structure. PIP_2 has been shown to bind to two important proteins involved in microfilament organization. Profilin (see Chapter 5, Box 5.2) is able to bind actin monomers. When phosphorylated, profilin releases actin, which can then participate in the formation of microfilaments. A regulatory domain that binds PIP_2 has been detected in profilin. Gelsolin, a Ca^{2+}-activated F-actin–fragmenting protein, also possesses a PIP_2-binding domain, and PIP_2 is known to modulate gelsolin activity.

18.4.4 The IP_3 signal is constrained by active phosphatases that are sensitive to lithium.

IP_3 is able to mobilize the stores of Ca^{2+} inside the vacuole and rough ER by binding to specific receptors that also act as calcium channels (Fig. 18.33). Both these organelles can accumulate substantial amounts of Ca^{2+}. Associated with the ER are proteins such as calreticulin and calsequestrin, which have many low-affinity binding sites for Ca^{2+}. The IP_3-binding protein has been detected in plant cells and, as in animal cells, its binding is inhibited by heparin. The IP_3 signal is truncated by the activities of phosphatases that successively remove the phosphates to yield sequentially IP_2, IP, and inositol, this last then being reused for synthesis of PI (Fig. 18.33). The phosphatase that degrades IP to inositol has been cloned and, like the animal enzyme, is inhibited by Li^+ ions. Li^+ can thus be used as a general inhibitor of the IP_3 signaling pathway.

18.4.5 Do diacyl lipids play any function in plant cell signaling?

The hydrolysis of phosphatidylinositols generates IP_3 and DAG. In animal cells both products are used to activate downstream signaling components. Some signals activate phospholipases that hydrolyze PIP and PI as well as PIP_2, giving rise to a massive increase in DAG. DAG then activates protein kinase C (PKC) by altering its sensitivity to Ca^{2+} ions. PKC is involved in many signaling events because its target proteins function in the signaling pathways involving cell growth and division (Fig. 18.34). Although typically PKC is activated by a combination of DAG and Ca^{2+}, enzyme variants have emerged that are DAG-activated but Ca^{2+}-insensitive. All PKCs require phosphatidylserine for activity, and some use unsaturated fatty acids or lysophospholipid as cofactors. PKC is permanently activated by phorbol esters, plant products suspected as carcinogens in mammalian cells (see Chapter 24).

A true plant PKC—i.e., a protein with PKC activity, cofactor requirements, and sequence homology to PKCs in other systems—has not yet been detected, but plant protein kinases activated by phosphatidylserine and Ca^{2+} and activated by phosphatidylserine, Ca^{2+}, and phorbol esters have been reported. Other plant protein kinases activated by free fatty acids or lysophospholipids have been identified, some of which require Ca^{2+}. These proteins may represent functional equivalents to PKC. The absence of a signaling role for DAG in plants seems unlikely, and some evidence points to its direct involvement in the growth of pollen tubes and regulation of stomatal aperture.

18.4.6 Phospholipases A and D can generate other signaling molecules and may be regulated through G-proteins.

In animals, phospholipase A (PLA) catalyzes the hydrolysis of phospholipids to yield lysophospholipids and free fatty acids (Fig. 18.35). The most common free fatty acid

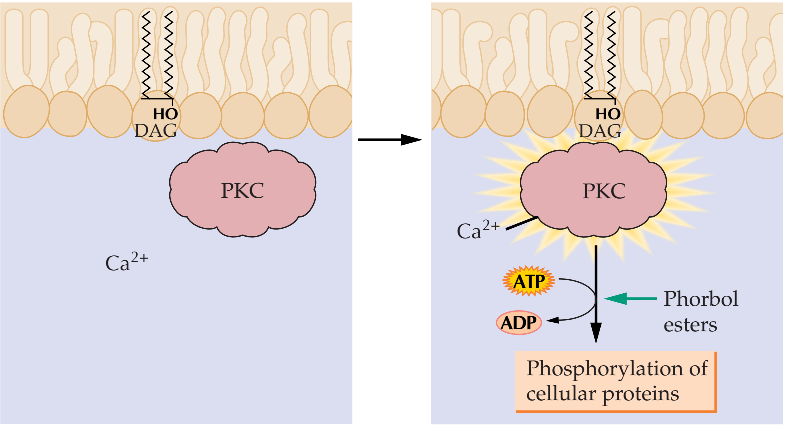
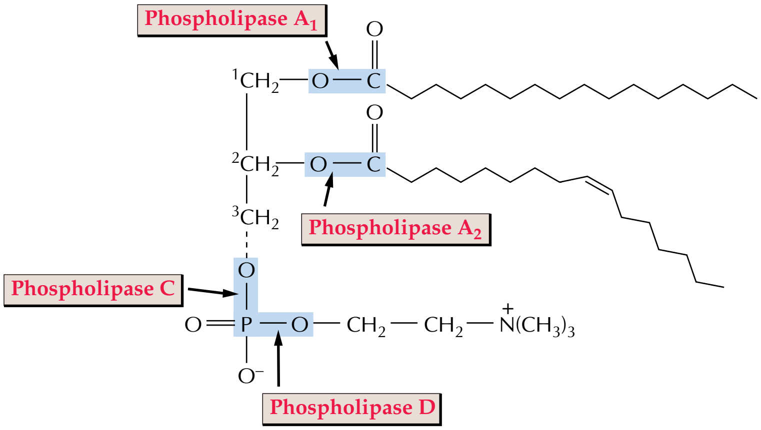


Figure 18.34
Production of DAG by PLC is required for other downstream transduction processes. DAG acting with Ca^{2+} activates protein kinase C (PKC), to which many important functions have been ascribed, including control of cell division and cell growth. A true PKC has yet to be isolated from plant cells but functional protein kinase equivalents have been described. Because PKC is the phorbol ester receptor, its activation can be mimicked by treatment with phorbol esters.



Enzyme	Products of phosphatidylcholine cleavage
PLA	Free fatty acid and lysophospholipid
PLC	DAG and phosphocholine
PLD	Phosphatidic acid and choline

Figure 18.35
Specific phospholipases degrade membrane phospholipids at defined cleavage sites.

released in mammalian cells is arachidonic acid. Lysophospholipids can also serve as a precursor to platelet aggregating factor (PAF), which functions in blood clotting. Two forms of PLA, PLA$_1$ and PLA$_2$, can be distinguished, but only PLA$_2$ can be regulated by G-proteins, proteins kinases, and Ca^{2+} in animal cells.

In plants, free fatty acids and lysophospholipids increase the activity of the plasma membrane H$^+$-ATPase. Auxin treatment may also increase the rate of formation of lysophospholipids. Unsaturated fatty acids trigger stomatal opening, which might reflect direct effects on potassium channels. A plant type of PLA$_2$ may also mediate the action of fungal elicitors and the associated oxidative burst responses. Several enzymes such as (1→3)β-glucanase are up-regulated as the result of PLA$_2$ activation. One product of PLA$_2$ action, linolenic acid, is a precursor in the octadecanoid pathway that synthesizes jasmonic acid (JA), an important signal generated in response to wounding (Fig. 18.36). PAF, a lysophospholipid-like derivative of phosphatidylcholine, may also influence proton transport through the plasma membrane.

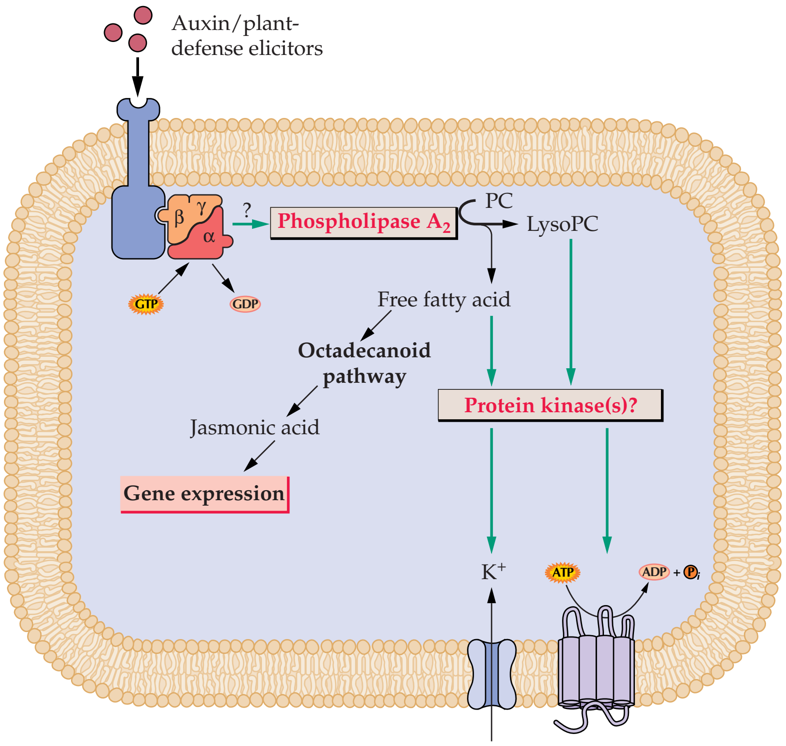


Figure 18.36
Putative role of phospholipase A$_2$ (PLA$_2$) in plant cell signaling. Activation of PLA$_2$ hydrolyzes phosphatidylcholine (PC) to lysophosphatidylcholine (LysoPC). The free fatty acid released can be used for synthesis of jasmonic acid, which mediates induction of gene expression. Although the exact relationships that link all the constituents have not been fully defined, this pathway is undoubtedly important in the transduction of stress signals.

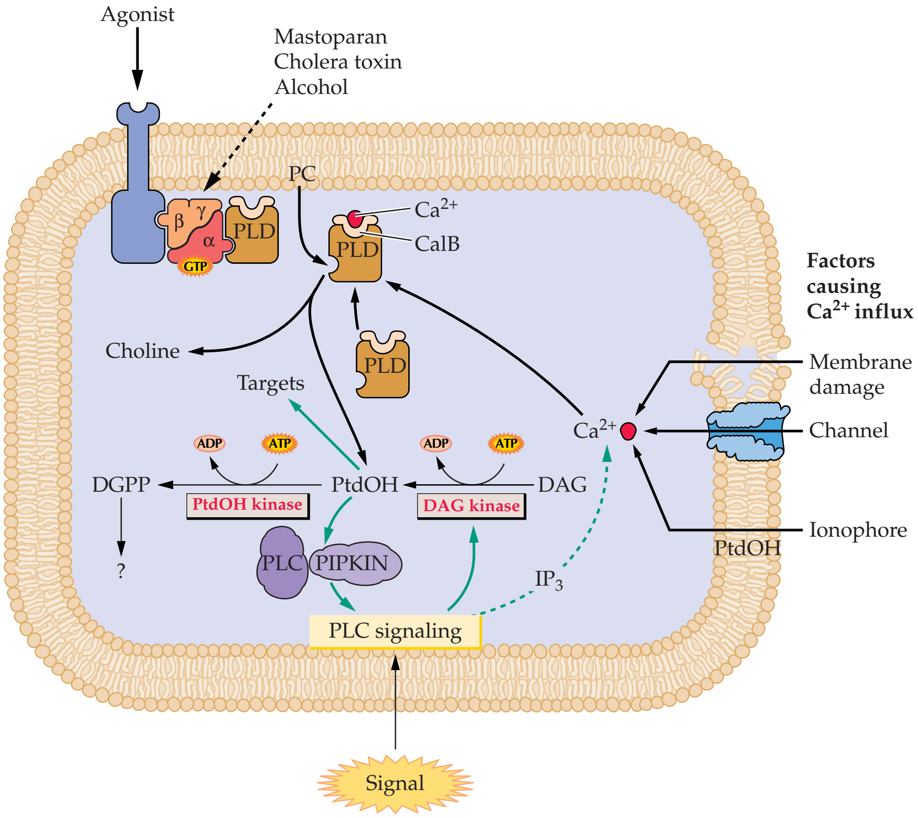
Phospholipase D (PLD) hydrolyzes phosphatidylcholine to release phosphatidic acid (PtdOH; see Fig. 18.35). In plants, PLD has been implicated in senescence (see Chapter 20), fruit ripening, stress (see Chapter 22), and wounding. PtdOH is thought to act as a Ca^{2+} ionophore, permitting the free movement of Ca^{2+} across the plasma membrane (Fig. 18.37). The cellular activities and events regulated by PtdOH include protein kinases, GTP-binding proteins, actin assembly, vesicle trafficking, secretion, and the oxidative burst response to pathogens. When plant cells are challenged by pathogens, PLD is translocated to the plasma membrane and activated in a G-protein–dependent process and by Ca^{2+}. Several PLD isoforms have been identified in plants and are expressed at different times and in different cellular locations. The proposed complex interrelations of PLD and PLC activation are diagrammed in Figure 18.37.

18.4.7 A signal transduction pathway involving phosphatidylinositide 3-kinases has recently emerged.

Phosphatidylinositide 3-kinase (PI3K) phosphorylates PI, PIP, and PIP_2 at the 3′ position (Fig. 18.38). The 3′-phospholipid products are thought to act as second messengers. To date, an enzyme that releases free inositol 3′-phosphates from phospholipids has not been discovered in plants. However, PKC in animal cells can be activated by inositol 3,4,5-P_3.

PI3K appears capable of interacting directly with several proteins to initiate cell division, cell death, and vesicle movement. *Arabidopsis* PI3K, which can complement yeast *PI3K* mutants, contains a Ca^{2+}-dependent lipid-binding domain. Expression of *Arabidopsis PI3K* in the antisense orientation produces severely deformed and stunted plants, probably the result of impaired vesicle transport to the vacuole.

Figure 18.37
Speculative view of possible interactions between phospholipase C (PLC) and phospholipase D (PLD) in plant cell signaling. The release of phosphatidic acid (PtdOH) from membrane phospholipids by PLD may induce several signaling changes involving Ca^{2+} and other targets (e.g., protein kinases, actin assembly and secretion). Various factors, including channel opening, agonist-stimulated activation of heterotrimeric G-proteins, and membrane damage by heat, cold, or parasites, can activate PLD through a calmodulin-binding domain (CalB) and free Ca^{2+}. Mastoparan (a peptide from bee venom), cholera toxin, and alcohol might directly mimic G-protein activation. Activated PLD is recruited to the plasma membrane, where it hydrolyzes phosphatidylcholine (PC). PtdOH can act as an ionophore, enabling more Ca^{2+} to enter the cytoplasm. PtdOH can also be synthesised from diacylglycerol (DAG) in a reaction catalyzed by DAG kinase. Activation of PLC with associated synthesis of DAG will further increase DAG kinase activity. PtdOH can be inactivated through conversion to diacylglycerol pyrophosphate (DGPP) by PtdOH kinase. Any increase in PtdOH can amplify the PLC signaling cascade by activating phosphatidylinositol-4-phosphate-5-kinase (PIPKIN), which converts PIP to the PLC substrate PIP_2.



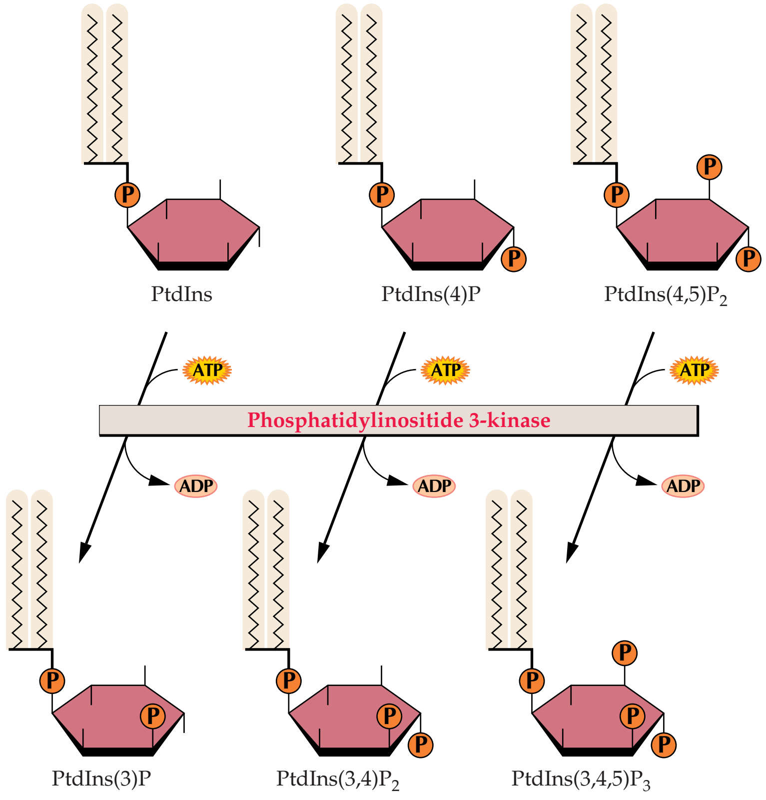


Figure 18.38
The enzymatic action of phosphatidylinositide 3-kinase on phosphatidylinositol phosphates. PtdIns, phosphatidylinositol; PtdIns(4)P, phosphatidylinositol 4-phosphate; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PtdIns(3)P, phosphatidylinositol 3-phosphate; PtdIns(3,4)P_2, phosphatidylinositol 3,4-bisphosphate; PtdIns(3,4,5)P_3, phosphatidylinositol 3,4,5-trisphosphate.

18.5 Cyclic nucleotides

18.5.1 Adenyl cyclase, an important signaling enzyme in bacteria and motile algae, has recently been detected in plants.

cAMP, synthesized from ATP by adenyl cyclase and degraded to 5′-AMP by cyclic-AMP phosphodiesterase (Fig. 18.39), is an important second messenger in animals, fungi, and many prokaryotes but its role in plants is still controversial. Several different types of adenyl cyclase enzymes are responsible for cAMP synthesis in animal cells. Adenyl cyclases are large proteins (about 120 kDa); some isoforms are soluble in the cytoplasm, whereas others are located in the plasma membrane (Fig. 18.40). Some forms of adenyl cyclase are Ca^{2+}-dependent. Signals that activate or inhibit adenyl cyclase directly are usually mediated by specific types of G-proteins.

In mammalian cells, cAMP concentrations are sensed by PKA, which contains both a catalytic subunit and an inhibitory subunit that binds cAMP (Fig. 18.41). In the presence of cAMP, the catalytic subunit is released and can phosphorylate enzymes that control glycogen metabolism and many other metabolic reactions. PKA also regulates transcription factors such as the cAMP response element–binding proteins (CREBs). The activated transcription factors can then bind to cAMP response elements (CREs) in the specific promoters, thereby modifying the transcription of cAMP-regulated genes.

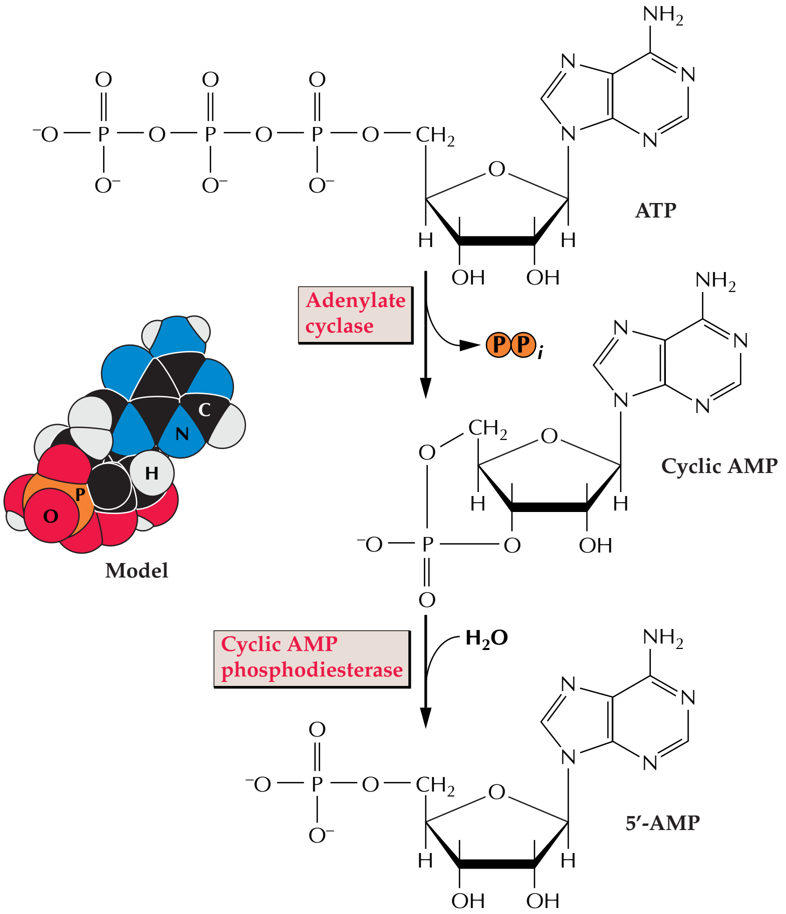
The function of cAMP in higher plant cells was disputed for many years. Detection of plant proteins that synthesize cAMP and have sequence similarity to adenyl cyclase has recently been reported. DNA sequence analysis has also detected putative CREBs and CREs in plants, and several plant and viral promoters contain CRE sequences. Expression of these promoters in transgenic yeast appears to be regulated by cAMP-binding proteins. The nucleotide sequence of the *ETR1* promoter also has a DNA sequence motif that suggests regulation of the gene by cAMP.

Involvement of cAMP has been indicated in many plant-specific processes. For example, signals that initiate stomatal closure may be transduced through adenyl cyclase. The activity of guard cell channels is modifed by cAMP-dependent phosphorylation, and cloned plant potassium channel proteins also have a cAMP-binding region. Pollen tube growth is reportedly regulated by cAMP, and adenyl cyclase has been proposed to mediate incompatibility between stigma and pollen tube. Both the plant cell cycle and the rhizobial interactions with root hairs are accompanied by changes in cAMP concentrations. Much more information on the function of this multifunctional second messenger in plants can be expected in the near future.

18.5.2 Guanyl cyclase in plant cells may be more important than adenyl cyclase and may mediate aspects of light signal transduction.

Cyclic 3′,5′-GMP (cGMP) is synthesized by guanyl cyclase from GTP. In certain

Figure 18.39
Synthesis and degradation of cyclic 3′,5′-AMP by adenyl cyclase and cyclic AMP phosphodiesterase. cAMP is shown as a structural formula and a space-filling model.



specialized animal cells, most notably rod cells, cGMP helps transduce the visual image. In plant cells, cGMP may participate in transducing signals of fungal invasion, red light signals mediated by phytochrome, and gibberellin signals that regulate synthesis of aleurone amylase.

The tomato *aurea* mutant, which lacks PHYA (see Box 18.4), has been used to demonstrate the involvement of cGMP in phytochrome transduction. Individual etiolated cells injected with cloned and reconstituted phytochrome become sensitive to red light (Fig. 18.42). The transformed cells can then form chloroplasts when irradiated with red light. To elucidate the transduction events that control these processes, researchers microinjected signal transduction components into *aurea* cells and, to monitor the effects of the microinjected transduction component, coinjected DNA constructs containing phytochrome-regulated promoters linked to a reporter gene. Cellular responses observed by microscopy indicated that several transduction pathways were involved in transducing the PHYA signals leading to chloroplast formation. Transduction of red light–activated PHYA signals by G-proteins was observed by using cholera toxin and GTPγS (see Section 18.4.2). Injection of Ca^{2+} alone induced partial chloroplast formation. The expression of genes such as those encoding the chlorophyll *a/b*–binding protein were also induced by microinjection of Ca^{2+}. However, complete differentiation of chloroplasts required concomitant injections of cGMP and Ca^{2+} (Fig. 18.42). Injection of cGMP on its own induced anthocyanin formation. Advanced imaging techniques have demonstrated that brief red light irradiation of etiolated wheat leaf protoplasts induces transient increases in cytosolic Ca^{2+}. Whether these second messengers are active under all cellular circumstances in which phytochrome regulates plant growth and development is currently unknown. However, the molecular events mediating deetiolation seem to involve cGMP and Ca^{2+}.

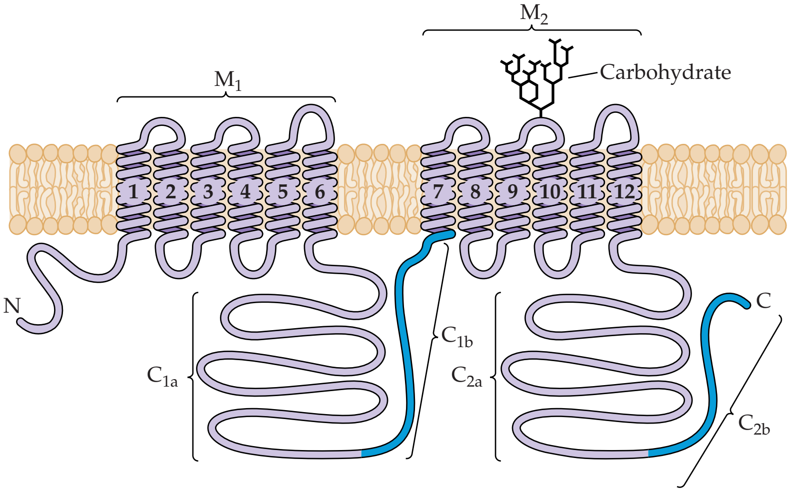


Figure 18.40
Structure of animal adenyl cyclase. This complex enzyme may contain 12 membrane-spanning domains in two groups (M_1 and M_2) and two large intracellular domains (C_{1a} + C_{1b} and C_{2a} + C_{2b}). The extracellular domains may be glycosylated. The amino acid sequences in C_{1a} and C_{2a} are highly similar in all known membrane-bound adenyl cyclases.

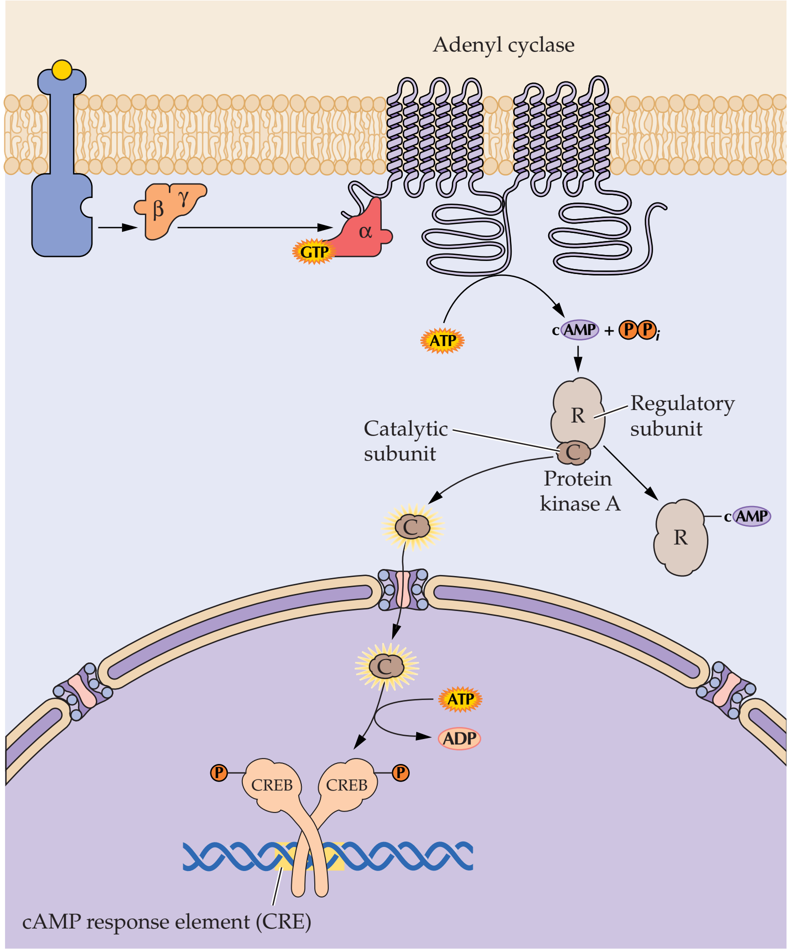


Figure 18.41
The interrelations of heterotrimeric G-proteins, cAMP, and the regulation of gene expression in animal cells. After synthesis by a plasma membrane–bound adenyl cyclase, cAMP can regulate gene expression through phosphorylation of a cAMP response element–binding protein (CREB) by an activated protein kinase A. The catalytic and regulatory subunits of protein kinase A are thought to form a heterotetramer (not shown).

18.6 Calcium

Cytosolic calcium, $[Ca^{2+}]_i$, occupies a pivotal position in plant cell signal transduction. The plant signals thought to be transduced through $[Ca^{2+}]_i$ include touch, wind, temperature shock, fungal elicitors, wounding, oxidative stress, red light, blue light, anaerobiosis, ABA, applied electrical fields, osmotic stresses, and mineral nutrition.

Two practical criteria define the dependence of signaling processes on $[Ca^{2+}]_i$. First, the signal must stimulate observable changes either in $[Ca^{2+}]_i$ or in Ca^{2+} flux across membranes, which in turn must precede physiological responses. (Increased fluxes across membranes or in their vicinity can be crucial transducing events but are often difficult to detect.) Second, the physiological responses associated with changes in $[Ca^{2+}]_i$ must be duplicated artificially by mimicking the observed $[Ca^{2+}]_i$ transient (e.g., with caged Ca^{2+}; see Box 18.5).

18.6.1 Calcium signaling involves a separation of different concentrations of Ca^{2+} by membranes, and the signals must be regulated.

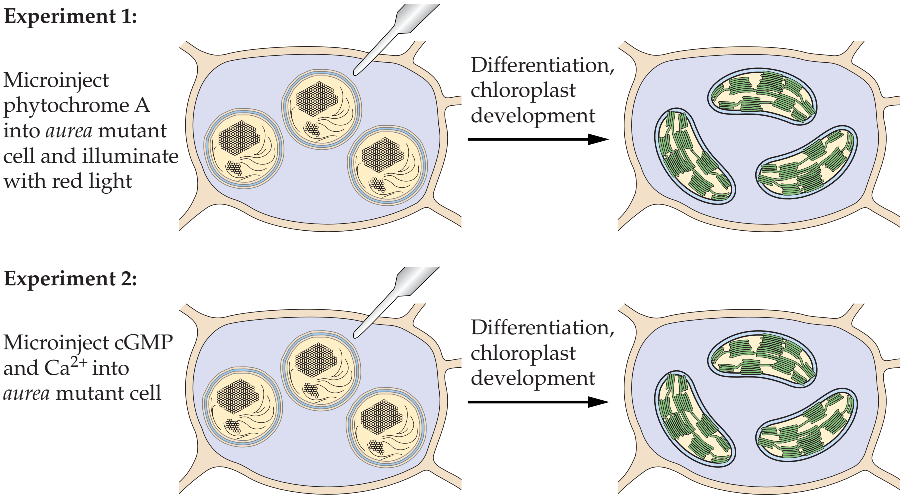


Figure 18.42
Cells of the tomato *aurea* mutant lack PHYA and are impaired in chloroplast development. The mutant cells can be induced to perform the normal red light–controlled development of chloroplasts by microinjection of purified phytochrome followed by exposure to red light (Experiment 1). The effects of this phytochrome/red light treatment can be mimicked by injecting mixtures of cyclic 3′,5′-GMP (cGMP) and Ca^{2+} (Experiment 2). Injection of either second messenger by itself may induce partial chloroplast development. Injection of only cGMP may also induce anthocyanin formation.

Ca^{2+} signaling depends on the transmembrane electrochemical gradients of Ca^{2+} across the plasma membrane and intracellular membranes. Cells maintain very low resting concentrations of cytosolic Ca^{2+} (100 to 200 nM) to facilitate signaling processes (Fig. 18.43). The vacuole and rough ER constitute large intracellular stores of Ca^{2+} (typically about 1 mM), which can be mobilized by IP_3 and other signals synthesized by the plasma membrane. In the cell wall, where Ca^{2+} is used as a structural molecule, the concentration of Ca^{2+} is estimated to be about 0.5 to 1 mM. Mitochondria, chloroplasts, and even the nucleus may act as Ca^{2+} stores as well. These organelles can also contain other elements of Ca^{2+} signal transduction such as calmodulin, a ubiquitous Ca^{2+} receptor. The nuclear membrane also contains the essential elements of an IP_3-generating system.

When cells receive signals, Ca^{2+} channels are transiently opened and $[Ca^{2+}]_i$ increases rapidly. Numerous Ca^{2+}-binding proteins are thus activated, including calmodulin and Ca^{2+}-dependent calmodulin-like domain protein kinases (CDPKs). Once formed, the Ca^{2+}/calmodulin complex transduces the Ca^{2+} signal by binding to and activating target proteins. Plant cells probably contain several hundred Ca^{2+}- or Ca^{2+}/calmodulin-binding proteins.

$[Ca^{2+}]_i$ signals are truncated by the activity of ATPases located in the plasma membrane, the tonoplast, and ER membranes. These pumps restore and maintain low concentrations of cytoplasmic Ca^{2+} (Fig. 18.43). Just as the H^+-ATPases of the plasma

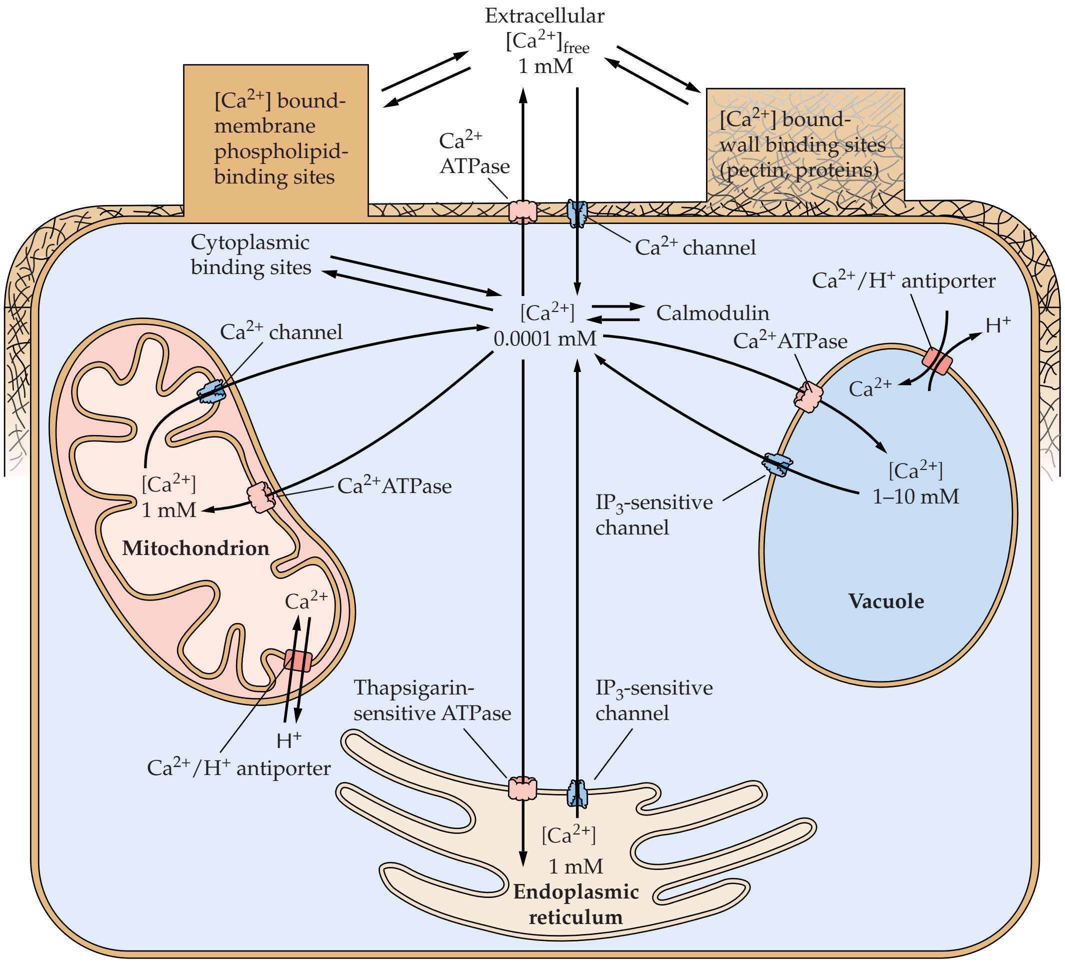


Figure 18.43
Interactions of intracellular and extracellular Ca^{2+} in cell signaling. The relationship of Ca^{2+} stores in plant cells are known to be complex, concentrations of Ca^{2+} being high in organelles and in the cell wall and low in the cytoplasm. When the cell is signaled, channels are opened in various organelles or in the plasma membrane, allowing Ca^{2+} to enter the cytoplasm by diffusing down its electrochemical gradient. Ca^{2+} ATPases and perhaps Ca^{2+}/H^+ antiporters return the cytoplasmic concentration to its resting value. Where known, subcellular concentrations of Ca^{2+} are indicated (quoted values for the ER vary from 0.1 to 1 mM). The concentration of cytoplasmic binding sites has been measured at about 0.5 to 1 mM. Free cytoplasmic Ca^{2+} is in equilibrium with these binding sites. Increases of cytosolic calcium, $[Ca^{2+}]_i$, activate calmodulin, thereby initiating subsequent downstream events. IP_3, inositol 1,4,5-trisphosphate.

membrane and tonoplast remove protons from the cytosol, Ca^{2+}-ATPases use the free energy released by ATP hydrolysis to translocate Ca^{2+} into extracytosolic compartments against its electrochemical gradient. Some of the Ca^{2+}-ATPases are Ca^{2+}/calmodulin-dependent. Inhibitors of calmodulin binding therefore increase $[Ca^{2+}]_i$. A plasma membrane–localized Ca^{2+}-ATPase from plants has membrane-spanning domain sequences similar to those of the well-characterized Ca^{2+}-ATPase from red blood cells. The active sites of both enzymes require a phosphorylated aspartyl residue as an intermediate in the use of ATP and appear to be controlled by phosphorylation/dephosphorylation reactions as well. Ca^{2+}-ATPases in the ER and nuclear membrane are inhibited by thapsigargin and cyclopiazonic acid. Application of these two inhibitors to plants can substantially increase $[Ca^{2+}]_i$. Ca^{2+}/H^+ antiporters in the tonoplast and inner mitochondrial membrane may also help maintain low $[Ca^{2+}]_i$ (Fig. 18.43).

During signaling, $[Ca^{2+}]_i$ can transiently reach very high concentrations, particularly near the mouths of open channels (Fig. 18.44). Special luminescence technology using microinjected aequorin (Box 18.6) has detected 10 to 100 µM $[Ca^{2+}]_i$ in local regions of the cytoplasm during the passage of a single action potential in animal cells. These high concentrations cannot be tolerated for long, because Ca^{2+} can interfere with cellular metabolism by competing with Mg^{2+} for ATP. Some carrier proteins, including a Ca^{2+}/H^+ antiporter in the tonoplast, a Ca^{2+} uniporter in the inner mitochondrial membrane, and the mitochondrial phosphate/OH^- antiporter (see Chapter 14, Fig. 14.38), are activated by high Ca^{2+} concentrations (Fig. 18.45), providing transport mechanisms to control Ca^{2+} toxicity. The $[Ca^{2+}]_i$ system is poised for immediate and rapid response.

18.6.2 Mechanisms exist for sensing the state of intracellular stores of Ca^{2+} and replenishing them as needed.

A $[Ca^{2+}]_i$ transient induced by red light irradiation of an etiolated leaf protoplast is illustrated in Figures 18.46 and 18.47. When plant cells are signaled and channels are opened, there is an immediate influx of Ca^{2+}

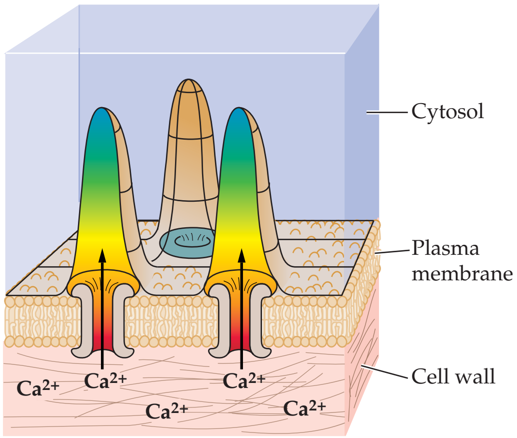


Figure 18.44
Distribution of Ca^{2+} in the cytoplasm within the vicinity of open Ca^{2+} channels. The channels are assumed to be voltage-regulated and open only for about two milliseconds. The color range indicates the concentration of Ca^{2+}, with red being the highest and blue the lowest.

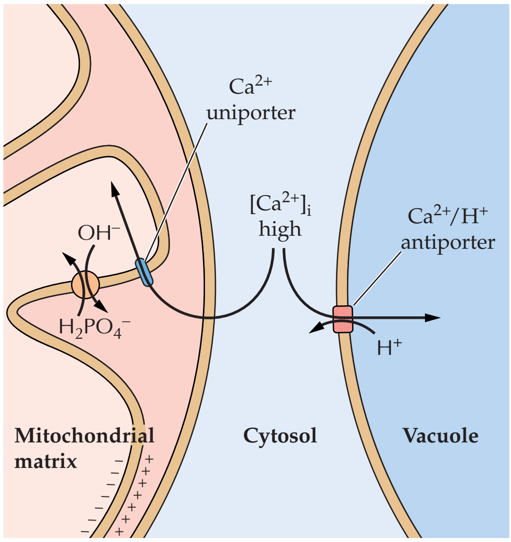


Figure 18.45
Several transport mechanisms activated by high $[Ca^{2+}]_i$. The function of uniporters, such as that shown here on the inner mitochondrial membrane, and antiporters, such as the Ca^{2+}/H^+ transporter on the vacuolar membrane and the phosphate/hydroxide antiporter on the inner mitochondrial membrane, is described in detail in Chapters 3 and 14. The transport of Ca^{2+} into the matrix via the mitochondrial uniporter is electrogenic (i.e., not directly compensated by coupled import of an anion or coupled export of a cation). Ca^{2+} uniporter activity is energized by membrane potential and pH gradients across the inner mitochondrial membrane. Under experimental conditions in which isolated mitochondria take up large amounts of Ca^{2+}, the inflow is accompanied by movement of an anion (usually $H_2PO_4^-$) from the intermembrane space to the matrix. Movement of the $H_2PO_4^-$ is catalyzed by the phosphate/hydroxide exchanger.

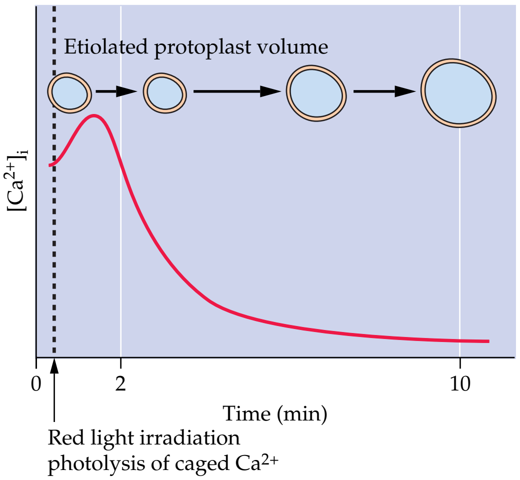


Figure 18.46
Red light induces changes in the concentration of cytosolic Ca^{2+} and the volume of etiolated wheat leaf protoplasts. Red light irradiation of etiolated wheat leaf protoplasts leads to transient increases in cytosolic Ca^{2+} lasting less than two minutes and a subsequent increase of protoplast volume of much longer duration. Photolysis of loaded caged Ca^{2+} or caged IP_3 results in similar changes in volume with a similar 2-minute lag.

down its electrochemical gradient. A single open Ca^{2+} channel can conduct an estimated 10^6 ions per second. In some cases, a counterbalancing activation of Ca^{2+}-ATPases immediately follows the increase of $[Ca^{2+}]_i$. Because calcium released from organelles can be expelled to the wall by plasma membrane–localized Ca^{2+}-ATPases, continuous signaling can rapidly exhaust intracellular stores, which must then be refilled before signal transduction or sensing can continue.

Intracellular stores can communicate their state of emptiness to the plasma membrane. When the stores are empty, special channels are opened in the plasma membrane. These channels have flux rates that are orders of magnitude less than those of the plasma membrane Ca^{2+} channels involved in signaling and remain open until the stores are filled. The intracellular store thus acts rather like an electrical capacitor, and this type of Ca^{2+}-uptake behavior is referred to as **capacitative Ca^{2+} signaling.** The channels involved are called **I_{CRAC}**, for the current *(I)* carried by a release-activated Ca^{2+} channel. The precise means whereby the store communicates its state of emptiness to the plasma membrane is unknown, but both chemical and structural signals controlling interactions between ER and plasma membrane have been implicated.

18.6.3 Ca^{2+} diffuses slowly in the cytoplasm.

The rate at which free Ca^{2+} diffuses in the cytoplasm is much slower than that in free solution. When isotopes ^{45}Ca and ^{42}K first became available in the late 1950s, they were injected into squid axons and their distributions were measured after several hours. Most surprisingly, ^{45}Ca showed very limited diffusion and remained at the site of injection, whereas ^{42}K diffused readily. Recent measurements have confirmed that the diffusion constant of Ca^{2+} in the cytosol is at least two orders of magnitude less than that

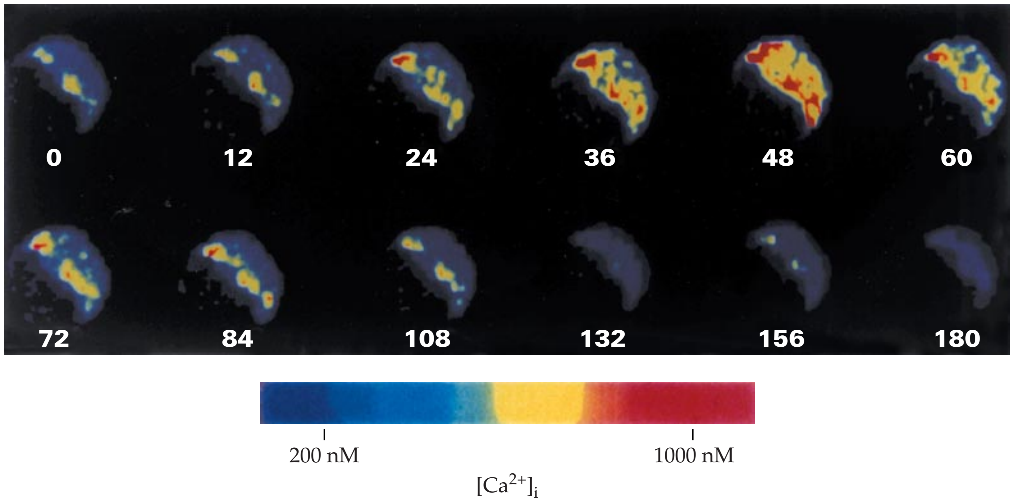


Figure 18.47
Red light–induced changes in cytosolic Ca^{2+} in single wheat leaf protoplasts loaded with a Ca^{2+}-sensitive fluorescent dye, fluo-3, as measured by confocal microscopy imaging. The scale used represents high Ca^{2+} concentration as red color and low Ca^{2+} as blue color. The times (in seconds after dye loading) at which the images were taken are indicated above each protoplast. Only half of the protoplast is visible because the fluorescent dye does not enter the vacuole.

in free solution. Impediments to Ca^{2+} diffusion include uptake into organelles (e.g., chloroplasts, mitochondria, ER, and vacuole) and binding to proteins that are free in the cytosol or are attached to the cytoskeleton.

The low rate of diffusion is an important part of $[Ca^{2+}]_i$ signaling. Because $[Ca^{2+}]_i$ does not disperse quickly in the cytoplasm, standing gradients of Ca^{2+} can form, as in growing pollen tubes (Fig. 18.48). Maintenance of the standing $[Ca^{2+}]_i$ gradient is essential for vesicle fusion and continued growth. In general, the spatial segregation of $[Ca^{2+}]_i$ at defined sites in the cytoplasm can promote signaling specificity (see Section 18.6.7).

18.6.4 Ca^{2+} channel activity can be detected by patch clamp technologies.

Three families of Ca^{2+} channels have been detected in plants by using patch clamp technology (see Chapter 3, Box 3.7). Ca^{2+} channels have been found in the plasma membrane, rough ER, and tonoplast; they may also be present in mitochondria.

Voltage-gated channels have their opening probability determined by a particular value of the membrane potential. The vacuole and the plasma membrane contain a considerable number of different members of the Ca^{2+} channel families that form subfamilies, each of which can be distinguished by the membrane potential required for activation and by the kinetics of opening. In animal cells, the voltage-gated Ca^{2+} channel contains at least four or more separate subunits; if the structures in plants are similarly complex, their isolation will present a difficult problem (Fig. 18.49).

Receptor- and second messenger–regulated Ca^{2+} channels form a second group. In animal cells, the plasma membrane contains Ca^{2+} channels that are opened by interaction with G-proteins. In plants, both the vacuole and most probably the ER and nuclear membrane contain Ca^{2+} channels that bind IP_3. The vacuole membrane also contains channels that are opened by the cyclic nucleotide second messenger cyclic ADP-ribose (cADP-R). In plants, the synthesis of cADP-R is regulated by ABA (see Section 18.8.4).

A third group of Ca^{2+} channels is found in both the vacuole and plasma membrane. These "stretch" channels sense tension in the membrane and are opened when the tension is altered. Mechanical signals and turgor status may be mediated through stretch channel activity. Mechanical signals (e.g., touch, wind) modify the interrelation of the plasma membrane and the wall and thus promote a change in tension. Water stress or altered activity of water channels modifies the turgor pressure and hence the pressure exerted between the wall and the plasma membrane. Stretch channels can be prominent in regions of active growth (e.g., the tip of a pollen tube).

Channel activity is not a binary function. Channels may exist in a closed, open, or inactivated condition (see Chapter 3 and Fig. 18.50). Although channels are often described as opening or closing in response to a stimulus, this behavior is not uniform for a population of channels. More accurately stated, the probability of a given channel being open or closed is influenced by the stimulus involved. Phosphorylation of the channel protein can also regulate the probability of opening.

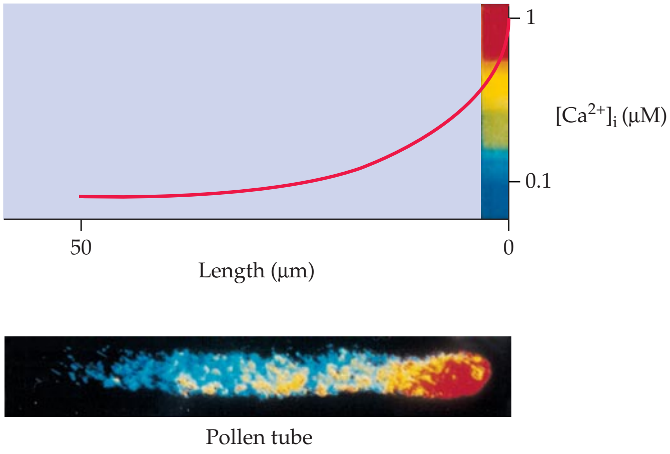
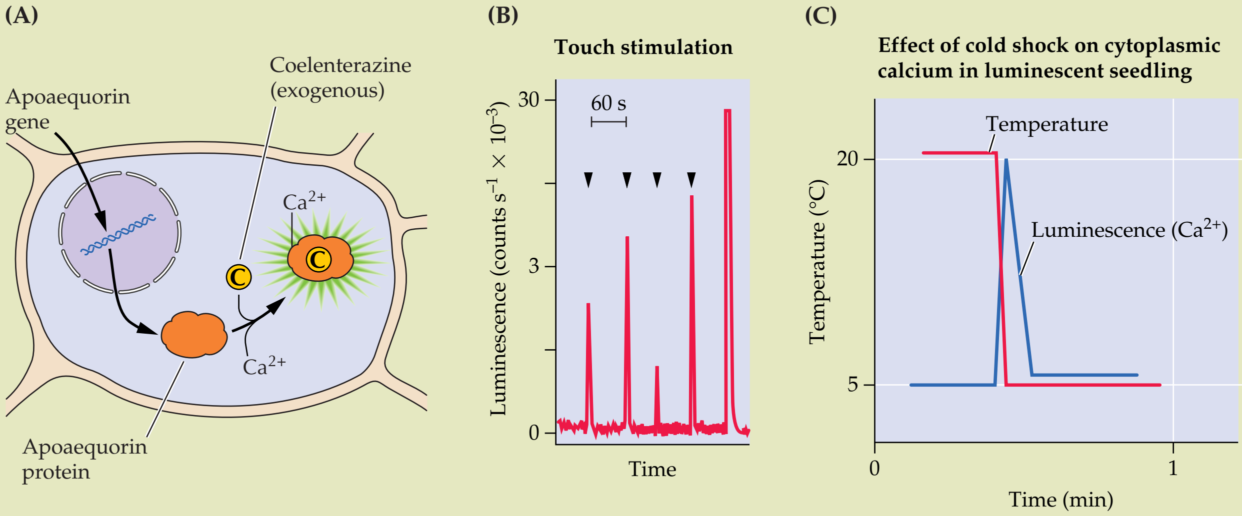


Figure 18.48
Pollen tubes maintain a standing gradient of cytosolic Ca^{2+} in their tip region. The standing gradient of $[Ca^{2+}]_i$ is essential for growth and results from a tip-associated cluster of Ca^{2+} channels. Pollen tubes can be loaded with Ca^{2+}-sensitive fluorescent ratio imaging dyes such as indo-1 or fura-2 for quantification of free Ca^{2+} by fluorescence microscopy.

Box 18.6 A luminescent Ca^{2+}-sensitive protein can help measure $[Ca^{2+}]_i$ directly.



The jellyfish *Aequorea victorea* contains a calcium-sensitive luminescent protein, aequorin. Aequorin consists of two constituents, an apoprotein (apoaequorin) and coelenterazine, a hydrophobic luminophore. When reconstituted, aequorin binds Ca^{2+} atoms with low affinity but high specificity. The Ca^{2+}–aequorin complex undergoes a conformational change that results in oxidation of the bound coelenterazine and an accompanying emission of luminescent light. Transformation of plants with the cDNA for apoaequorin and reconstitution with coelenterazine (shown in panel A above as circled C) generates luminous plants, the luminescence of which directly reports $[Ca^{2+}]_i$ (panel A). This method for measuring $[Ca^{2+}]_i$ is very simple. Many coelenterazines are available that yield aequorins with different properties, including some useful for ratio measurements. Aequorin can be targeted to different cell compartments and attached to cell membranes. Luminescence can be measured continuously for many weeks. This novel method offers a broad scope for obtaining very precise and significant information on $[Ca^{2+}]_i$ in plant cells.

Transgenic plants containing reconstituted aequorin have been used to detect the effects of touch, wind, cold, oxidative stress, hyperosmotic stress, auxin, blue light, and anaerobiosis—among many other signals. When touched, for example, transgenic seedlings exhibit rapid luminescence spikes (panel B, arrowheads). If the temperature is lowered rapidly, the resulting Ca^{2+} spike induces changes in gene expression (panel C).

Special cameras, originally developed for astronomers, can be used to image luminescent light. Panel D shows tissues of transgenic aequorin-containing seedlings induced to luminesce by cold-shock treatment. The images include a whole seedling (magnification × 2) and a whole cotyledon (magnification × 20; note spottiness in response).

18.6.5 Advanced fluorescence and luminescence technologies allow the imaging of free calcium concentrations inside living cells.

$[Ca^{2+}]_i$ can be measured and imaged by the use of fluorescent dyes. In addition, cells can be transformed to express the protein component of a luminescent compound from jellyfish. When exposed to a second, light-reactive component, the protein luminesces and can be used to monitor Ca^{2+} concentrations for extended periods in vivo (see Box 18.6).

Two types of fluorescent dyes can be distinguished (Fig. 18.51). **Ratio dyes** undergo shifts in wavelength of the fluorescence spectrum when binding Ca^{2+}. In contrast, Ca^{2+} binding increases the fluorescence

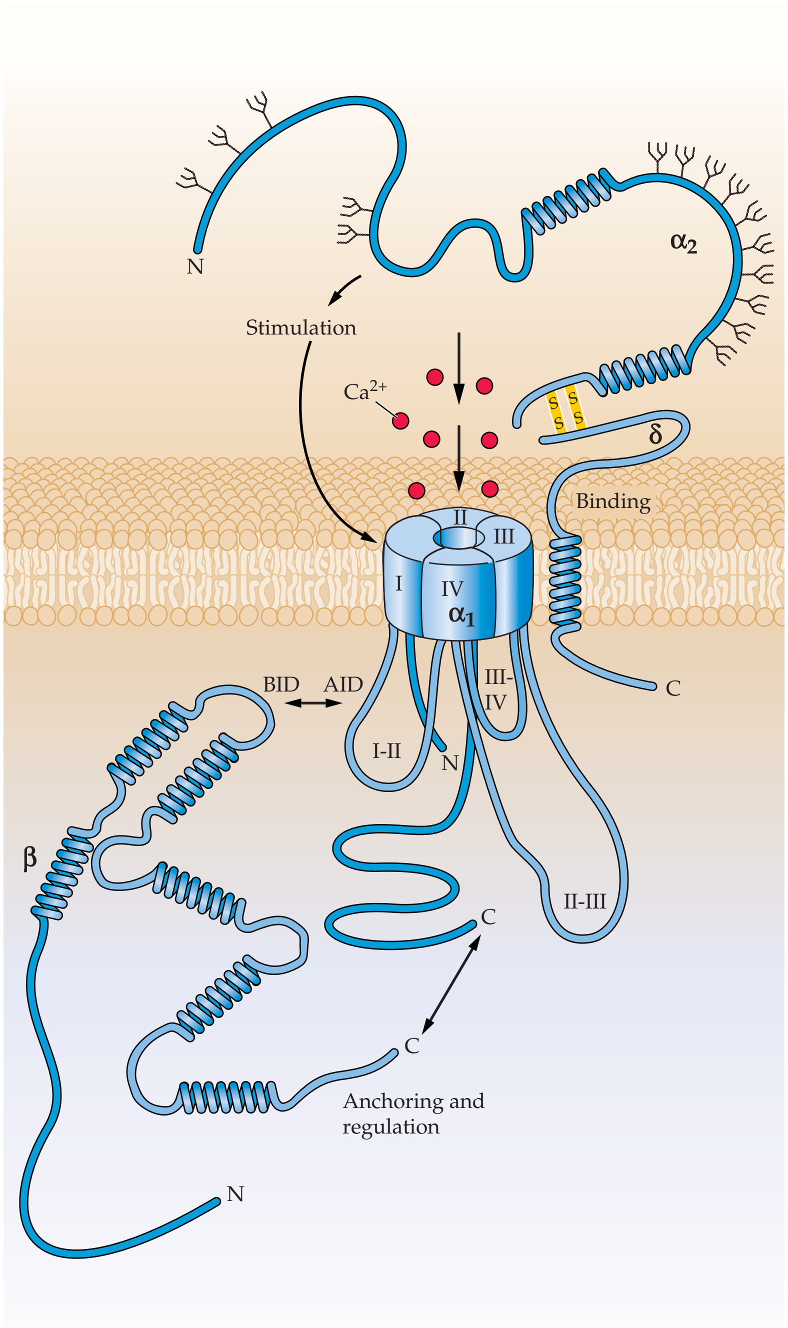


Figure 18.49

Structural organization of voltage-gated calcium channels in animals. The calcium channel regulated by membrane potential shares common features among many different organisms. The basic channel is composed of four different proteins (α_1, α_2, β, and δ), of which the sites of interaction are indicated by double-headed arrows. The major membrane-spanning subunit (α_1) consists of four homologous domains (I–IV), each composed of six transmembrane segments (not shown). Cytoplasmic loops are labeled according to the domains they connect. The α_2 protein is linked by disulfide bridges to δ, which in turn interacts with α_1. Inside the cytoplasm, the β-subunit (through BID [beta-subunit interacting domain]) interacts with the I–II domain of α_1 (AID [alpha-subunit interacting domain]). The extracellular regions of the channel may be glycosylated and the internal regions phosphorylated by protein kinases. Anchorage of the calcium channel to the cytoskeleton and further regulation take place through the C termini of β and α_1. Why such a large structure is necessary to transmit Ca^{2+} is not understood.

intensity of **single-wavelength dyes** but does not induce a spectral shift. Dyes are characterized by either their **excitation spectrum** (determined by varying the wavelength of the exciting light and assaying the fluorescence intensity at specific, defined longer wavelengths) or their **emission spectrum** (determined by using a constant source of light excitation and assaying fluorescence intensity at several longer wavelengths).

A valuable property of ratio dyes is that either their excitation spectra or their emission spectra contain two wavelengths, the ratio of which is unique to each Ca^{2+} concentration. Thus, the measurement of $[Ca^{2+}]_i$ is independent of the dye concentration. This is particularly useful for cellular imaging studies because cells vary in thickness, which means the dye concentrations are rarely uniform. Two common ratio dyes used to monitor Ca^{2+} in fluorescence-ratio imaging experiments are fura-2 and indo-1. Figure 18.52 illustrates the use of fluorescence-ratio imaging to demonstrate the involvement of Ca^{2+} in stomatal closure and, in particular, the role of the vacuole.

The common single-wavelength dyes are calcium green and fluo-3, which are excited by visible light and can be used in conjunction with the confocal microscope. Single-wavelength dyes are usually much brighter than ratio dyes; consequently, the irradiance needed to excite single-wavelength dyes is less, thus reducing photodamage to the cell and photobleaching of the dye. Comparison of successive fluorescence images of a cell during signaling can ameliorate the lack of exact quantitation with single-wavelength dyes. Moreover, dyes can be coupled to dextran to prevent their accumulation in the vacuole or other organelles.

Imaging the distribution of Ca^{2+} in single living cells has been a major achievement of research, requiring fluorescence microscopes coupled to powerful computers. Use of these technologies has enabled the demonstration of permanent gradients of $[Ca^{2+}]_i$ in growing pollen tubes and root hairs, the production of waves and oscillations in guard cells and pollen tubes, and the release of Ca^{2+} from vacuoles inside guard cells. They have also been used to demonstrate that specific modification of the $[Ca^{2+}]_i$ gradient in the pollen tube tip initiates reorientation (see Box 18.5).

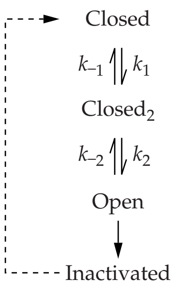


Figure 18.50
At any one instant, a Ca^{2+} channel may exist in various closed, open, or inactivated states. Like all channels, Ca^{2+} channels are only open for very brief periods of time and the behavior of individual channels is stochastic. Thus the channels are better described in terms of the probability of their opening or closing rather than their being open or closed. Opening conditions merely ensure the channel has a high probability that it will be found in the open state; the converse is true for the closed state. The variety of states ensures the presence of a diverse set of channel activities, rather than a uniform population, all open or all closed.

18.6.6 Signaling through $[Ca^{2+}]_i$ may involve waves, cascades, oscillations, capacitative calcium entry, and pacemaker cells.

Calcium signaling can take place through simple transients or through more complex patterns. In some cells (e.g., guard cells, pollen tubes), oscillations of $[Ca^{2+}]_i$ can be observed—apparently resulting from a sequential filling and emptying of the Ca^{2+} stores in the ER and perhaps the vacuole. The emptying and filling are thought to be regulated by a capacitative Ca^{2+} signaling mechanism involving Ca^{2+}-induced Ca^{2+}-release (CICR), probably by way of Ca^{2+}-dependent calcium channels (Fig. 18.53; see also Chapter 3). Oscillations may also enable cells to distinguish genuine $[Ca^{2+}]_i$ signals from noise. Organelles such as mitochondria might initiate metabolic changes only in

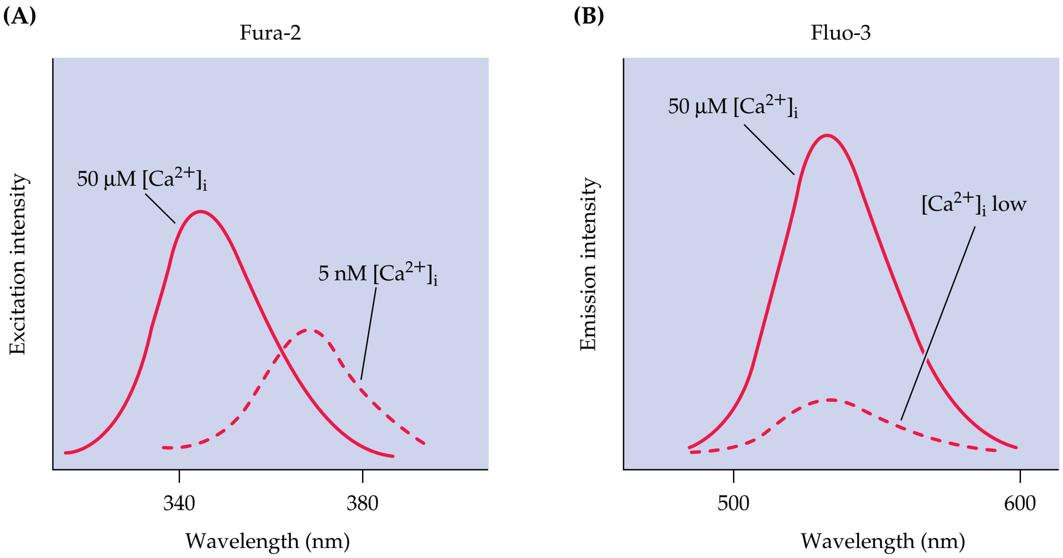


Figure 18.51
Fluorescence spectra for fura-2, a ratio dye, and fluo-3, a single-wavelength dye. All fluorescent dyes respond to wavelengths of exciting light by emitting fluorescent light at longer wavelengths. Dyes can be characterized by subjecting them to different wavelengths of exciting light to generate an excitation spectrum, with fluorescence measured at defined longer wavelengths. Alternatively, a spectrum of emitted fluorescent light can be constructed by using an invariant source of exciting light. The Ca^{2+}-sensitive fluorescent ratio dye, fura-2 (A), exhibits a shift in its excitation spectrum when it binds Ca^{2+}. The ratio of fluorescence emitted after excitation at two wavelengths, 340 and 380 nm, is unique for each Ca^{2+} concentration. The value of ratio imaging is that it obviates variations in dye concentration, dye bleaching, and cell thickness. Single-wavelength dyes, e.g., fluo-3 (B), do not exhibit a spectral shift but are frequently used because they are much brighter than dual-wavelength dyes. Further, single-wavelength dyes, which require less time for resolution can be useful for detecting changes in cytosolic calcium by comparing images taken close together in time.

response to oscillations rather than to sustained or transient $[Ca^{2+}]_i$ changes.

IP_3, which may also participate in the oscillations discussed above, produces Ca^{2+} waves that regulate the growth and orientation of pollen tubes. IP_3 is thought to function as a relay between intracellular Ca^{2+} stores (Fig. 18.54) and sites on the plasma membrane and thus overcome the constraint on diffusion of Ca^{2+} described in Section 18.6.3. Both waves and oscillations can pass between cells in a form of intercellular communication (Fig. 18.55) and may give rise to more complex forms of Ca^{2+} signaling such as circadian oscillations.

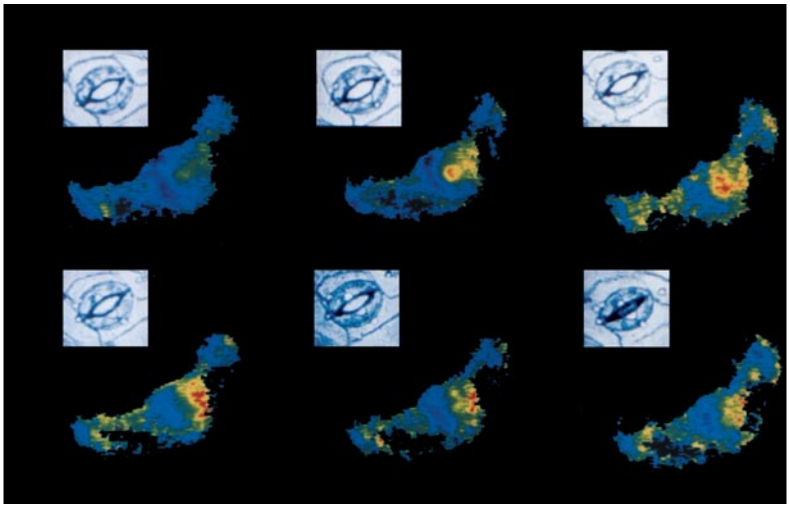


Figure 18.52
Fluorescence-ratio imaging of $[Ca^{2+}]_i$ in single guard cells of *Commelina communis* after changing the concentration of Ca^{2+} in the bathing medium from 20 μM to 1 mM at time zero. Individual guard cells were loaded with the ratio dye, indo-1. Ratio images were taken (left to right) 0, 2, and 5 (top row) and 10, 20, and 30 (bottom row) minutes after the medium change. Bright field images are shown as insets to indicate guard cell apertures.

18.6.7 Wherein lies the specificity of calcium signaling?

Few signals do not involve changes in $[Ca^{2+}]_i$, raising the question of how such a simple molecule can give rise to so many

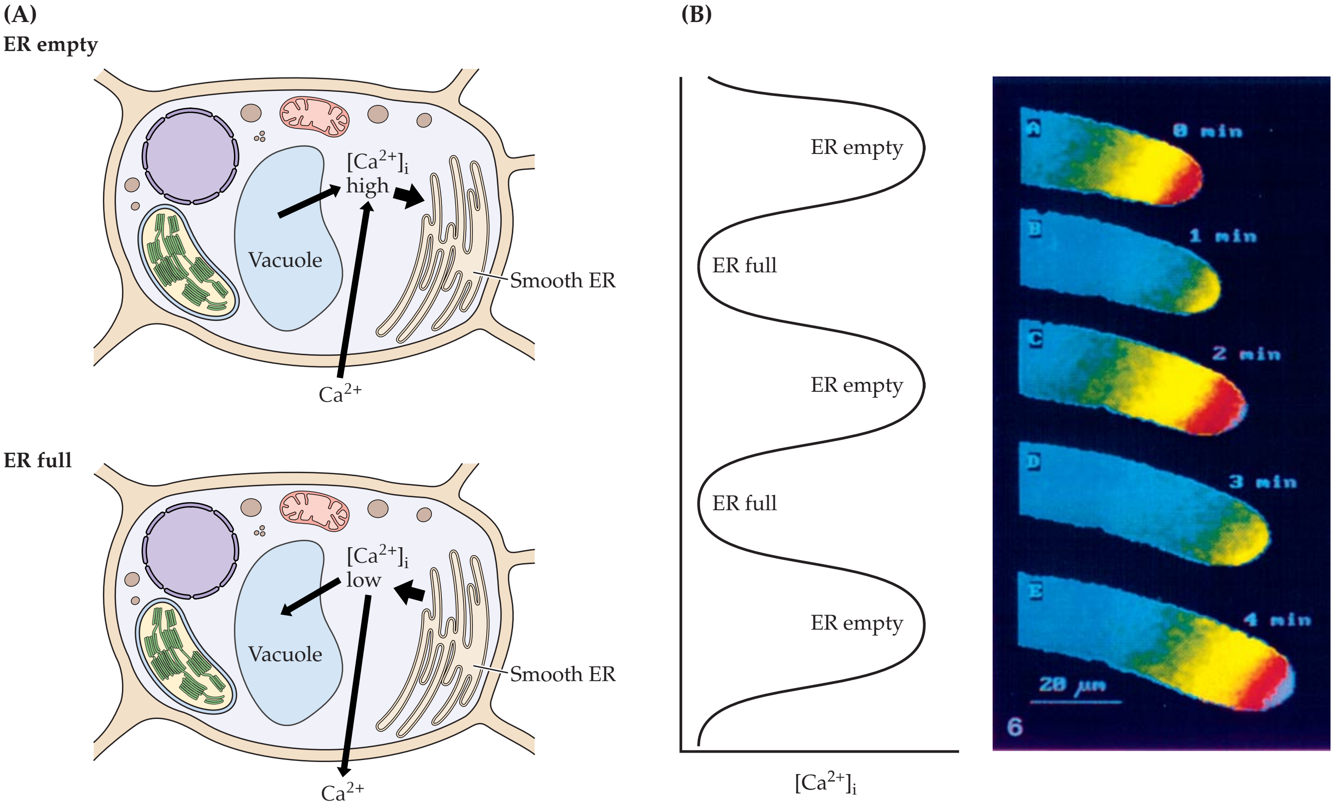


Figure 18.53
(A) Oscillations in cytosolic Ca^{2+} may arise from a filling and emptying of the Ca^{2+} stores in the endoplasmic reticulum (ER). The size of the arrows indicates flux rates. When the ER store is empty, Ca^{2+} is sequestered into the store; when the store is full, Ca^{2+} is released. The basis of the mechanism may operate through alternation of the activities of channels and ATPases in the organelles involved. (B) Oscillations in $[Ca^{2+}]_i$ accompany pollen tube growth. Fluorescence-ratio images taken at 1-minute intervals reveal oscillations in $[Ca^{2+}]_i$ in the tip.

different physiological responses. One aspect of this specificity involves the duration of the signaling itself. Some transient increases in Ca^{2+} concentration last longer than others. The longer-lasting influxes can be expected to penetrate farther into the cytoplasm and therefore encounter more centrally located Ca^{2+}-dependent enzymes (Fig. 18.56). Spatial specificity can also play a role in determining the physiological response to a given Ca^{2+} signal. Just as Ca^{2+} channels are concentrated in the tips of growing pollen tubes, so can receptors be clustered such that only certain parts of the cytoplasm receive signals. Cells may also "read" the frequency of oscillations or the speed of waves.

A useful analogy for the specificity associated with ubiquitous signaling molecules such as calcium is electrical wiring. In any house one switch will turn on a light, another will activate a TV. Identical switches can electrify any appliance. The factor determining the result of toggling a switch is the wiring that links switches to components. The cytoskeleton/scaffold is analogously hard-wired with Ca^{2+}-sensitive proteins. All are subject to control by the same switch—Ca^{2+}—but spatial differentiation of the receptors ensures that only some of the circuitry is activated in response.

18.6.8 The eukaryotic Ca^{2+}-based signaling systems may have evolved as detoxification mechanisms.

The use of $[Ca^{2+}]_i$ as a signaling molecule seems to be largely limited to eukaryotic cells, raising questions about the evolution of signal transduction. One suggestion is that eukaryotic cells experienced a Ca^{2+} catastrophe during early evolution, which resulted in the elaboration of detoxification mechanisms to remove Ca^{2+} and thereby avoid its toxic effects on ATP metabolism: These detoxification mechanisms later developed into signaling pathways. The precedent here is the hypothesis that sees the evolution of aerobic respiration as resulting from an early attempt to detoxify O_2 produced during photosynthesis. An alternative view sees $[Ca^{2+}]_i$ signaling as evolving naturally from the very limited signaling systems in bacteria that regulate aspects of chemotaxis by utilizing $[Ca^{2+}]_i$. According to this hypothesis, eukaryotes learned to recognize perturbations in the plasma membrane because those gave rise to transient increases in $[Ca^{2+}]_i$. With the evident evolutionary need to sense more signals, a primitive $[Ca^{2+}]_i$-based system was elaborated and various divergent mechanisms evolved independently,

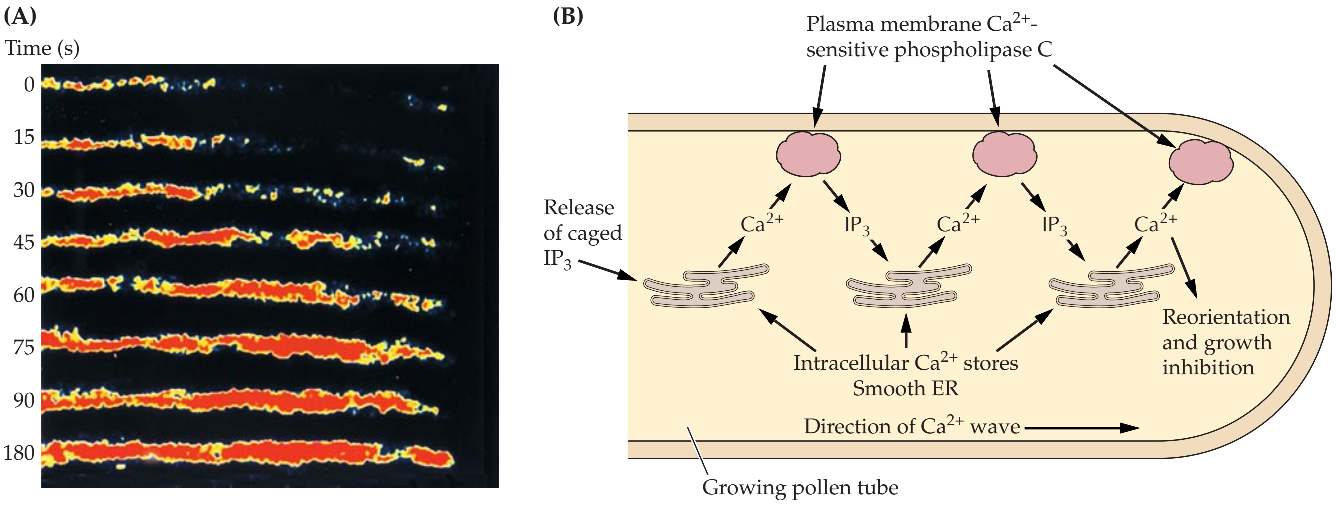


Figure 18.54
Calcium waves in growing pollen tubes. (A) The color image of the Ca^{2+}-sensitive fluorescent dye, fluo-3, in poppy pollen tubes is shown after photolysis of loaded caged inositol 1,4,5-trisphosphate (IP_3) at time zero (at top of figure). An induced calcium wave is initiated in the vicinity of the nucleus (the middle of the cytoplasmic region of the pollen tube). The wave reaches the pollen tube apex in about one minute and may oscillate during its progress. Images are taken at defined time intervals after photolytic release. (B) Deduced mechanism of Ca^{2+} wave observed in pollen tubes after photolysis of caged IP_3. The wave is most likely propagated by a Ca^{2+}-dependent phospholipase C in the plasma membrane, because the synthesis of IP_3 mobilizes Ca^{2+} from intracellular stores. When the wave reaches the tip, growth is inhibited; on recovery, growth is reoriented.

acquiring different signal specificities. Elaboration of the downstream signaling coupled to spatially separate $[Ca^{2+}]_i$ changes would generate a chain of signaling and transduction components specific to each signal, much as seems to occur in present-day yeast.

18.6.9 Calmodulin is the primary calcium receptor, and there are many calmodulin-binding proteins.

Calmodulin, a small (15 to 17 kDa), highly conserved, Ca^{2+}-binding protein, is the primary calcium receptor in both plant and animal cells. Sequences of plant and animal calmodulins differ by as few as 12 to 13 amino acids among about 150. The molecule has four Ca^{2+}-binding regions or loops (Fig. 18.57A), which contain 12 amino acids each and are rich in aspartate and glutamate. Two helices found on either side of the loops have given rise to the terminology of helix–loop–helix proteins, because such Ca^{2+}-binding structures may be found in many other proteins. Sometimes the loop is called an EF hand, in reference to the prominence of glutamate and phenylalanine in the beginning and end of the loop.

Calmodulin is found in both the cytoplasmic and nuclear compartments and can

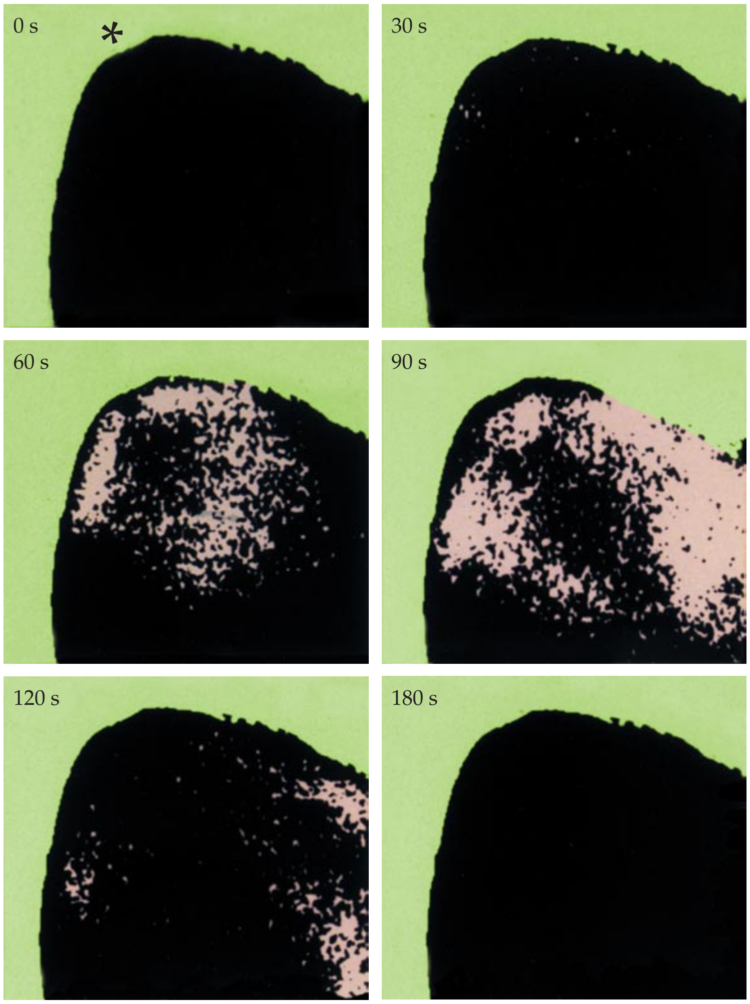


Figure 18.55
A tissue Ca^{2+} wave in tobacco. Transgenic tobacco seedlings containing aequorin can be cold-shocked by placing a tiny block of ice adjacent to the cotyledon (asterisk). Luminescence is imaged here at 30-second intervals. A tissue $[Ca^{2+}]_i$ wave that takes about 30 seconds to develop is clearly visible as it traverses the cotyledon.

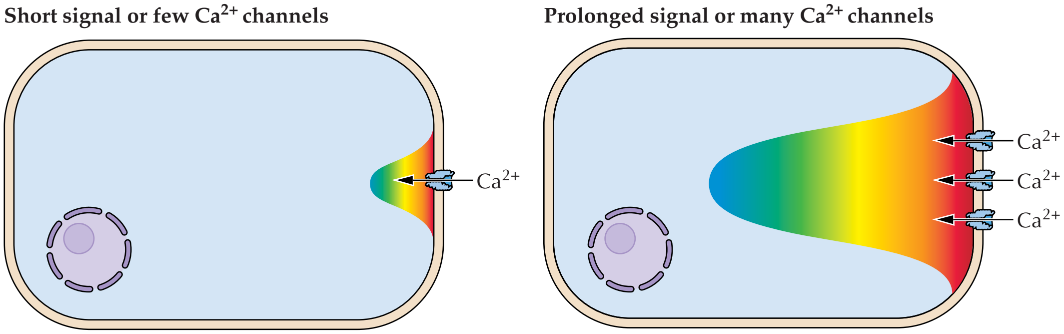


Figure 18.56
The duration of a transient influx of Ca^{2+} determines the distance that a Ca^{2+} signal penetrates from the plasma membrane to the cytoplasm because Ca^{2+} diffuses slowly in cytoplasm. Clustering of Ca^{2+} channels ensures a greater distance of penetration of the signal by limiting Ca^{2+} buffering by the cytoplasm. The slow diffusion of the Ca^{2+} signal is therefore partially offset by the numbers of channels involved in responding to the signal.

be attached to the plasma membrane. The cellular concentration of calmodulin varies greatly among plant cell types and developmental stages: As mentioned in Section 18.2.8, root statocytes contain calmodulin concentrations an order of magnitude greater than that in meristematic cells and thus are probably more sensitive to small changes in $[Ca^{2+}]_i$. In polarized cells (e.g., the polarizing *Fucus* zygote), calmodulin may be concentrated in regions of active growth and metabolism.

On binding Ca^{2+}, calmodulin undergoes a substantial change in tertiary structure, exposing a very hydrophobic patch rich in methionine, leucine, and phenylalanine. Specific regions on target proteins recognize this patch and combine with the calmodulin, resulting in activation of the target proteins. Unlike calmodulin itself, the sequences of the target peptides are not conserved. The prominence of methionine in the calmodulin-binding region is thought to ensure flexibility in the interaction.

Calmodulin-binding proteins or peptides (e.g., M13; Fig. 18.57B) may be detected in several ways. Isotopically labeled calmodulin can be used to probe expression libraries or even separated proteins on gel electrophoresis. The characterized calmodulin-binding proteins in plants are myosin V, kinesin, NAD^+ kinase, glutamate decarboxylase, protein kinases, and Ca^{2+}-ATPases. Plant calmodulin is also important in formation of actin filaments and cytoplasmic streaming (see Chapter 5), polarized growth, and the cell division cycle (at both S phase and M phase; see Chapter 11). Many more calmodulin-binding proteins have been identified and await characterization.

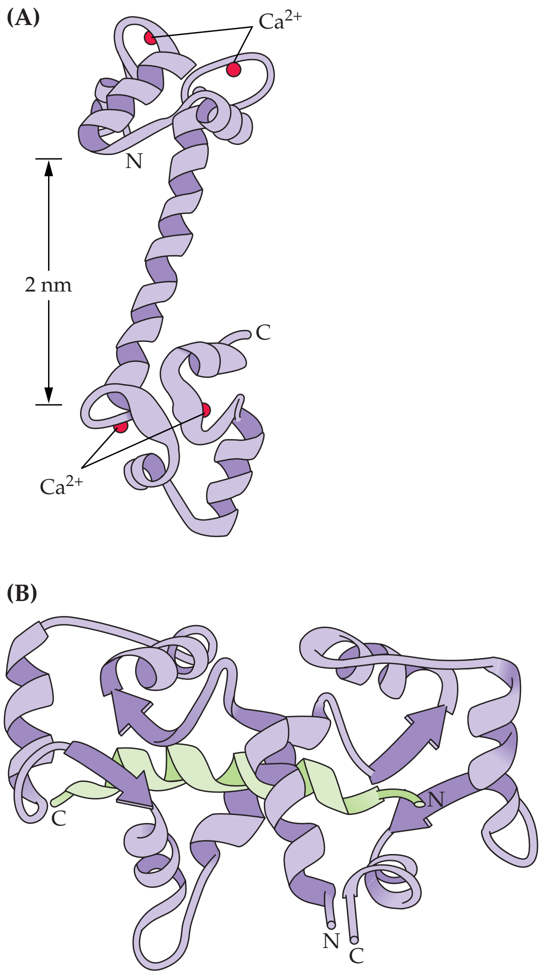


Figure 18.57
Structures of calmodulin. (A) The uncomplexed, calcium-loaded form is a dumbbell-shaped structure with two Ca^{2+}-binding domains at each end. Binding to Ca^{2+} exposes hydrophobic patches on each end that are rich in methionine, leucine, and phenylalanine residues. These hydrophobic interactions play a key role in the binding of calmodulin to target proteins. (B) The Ca^{2+}/calmodulin complex bound to M13, a calmodulin-binding peptide (Ca^{2+} ions not shown). Calmodulin is shown in purple, the M13 peptide in green.

18.7 Protein kinases: primary elements in signal transduction

18.7.1 Protein kinases are ubiquitous enzymes, and many are signal specific.

Members of the protein kinase superfamily catalyze the reversible transfer of the γ-phosphate from ATP to serine, threonine, or tyrosine amino acid side chains on target proteins. Protein kinase activity is counterbalanced by the action of specific protein phosphatases that remove the phosphate from proteins. In most cases phosphorylation modifies target protein activity. One protein kinase molecule can phosphorylate many hundreds of target proteins, thereby greatly amplifying weak signals.

Activation of protein kinase has been implicated in responses to light, pathogen attack, growth regulators, temperature stress, and nutrient deprivation. Several important protein kinases are concerned with regulation of metabolic pathways. Hundreds of plant genes for different protein kinases have been identified but at least a thousand must exist. Figure 18.58 illustrates the various groups of protein kinases that have been identified in plant cells.

18.7.2 RLKs represent a complex family of protein kinases with diverse functions in signaling.

The mechanisms used by plants to transmit extracellular signals into the cytoplasm require receptors located in the plasma membrane. RLKs (see Section 18.2.2) are an important group of protein kinases with direct functions in transmission of signals across the plasma membrane. They undergo autophosphorylation on the intracellular kinase domain in a reaction thought to result from homologous dimerization of the receptors in the plasma membrane when the ligand binds. Four major groups of RLKs have been described in plants.

The **S-domain RLKs** have an extracellular domain similar to that of the S-locus glycoproteins concerned with *Brassica* sporophytic incompatibility (see Chapter 19). Incompatibility occurs as the result of a cell-to-cell interaction between pollen tube and stigma (Fig. 18.59). This intercellular communication results in a rapid cessation of pollen tube growth. The proteins involved in this communication are the S-locus glycoproteins and an S-locus glycoprotein receptor kinase located in the stigma tissue. Even though its members are expressed in many different tissues and in different species, signaling that leads to incompatibility reactions might be the primary function of this group.

The **leucine rich-repeat (LRR) RLKs** contain a recognition core of leucine and asparagine, which is thought to engage in protein–protein interactions. LRR RLKs are found in many different tissues, including the shoot apex. One example is RLK5, which is found in association with a protein phosphatase (Fig. 18.60). The *Cf-9* gene encodes a putative transmembrane protein with an extracellular domain of 28 LRRs and a short cytoplasmic tail. The extracellular domain exhibits considerable similarity to the LRR class of RLK. *Cf-9* confers resistance against tomato mold (see Chapter 21).

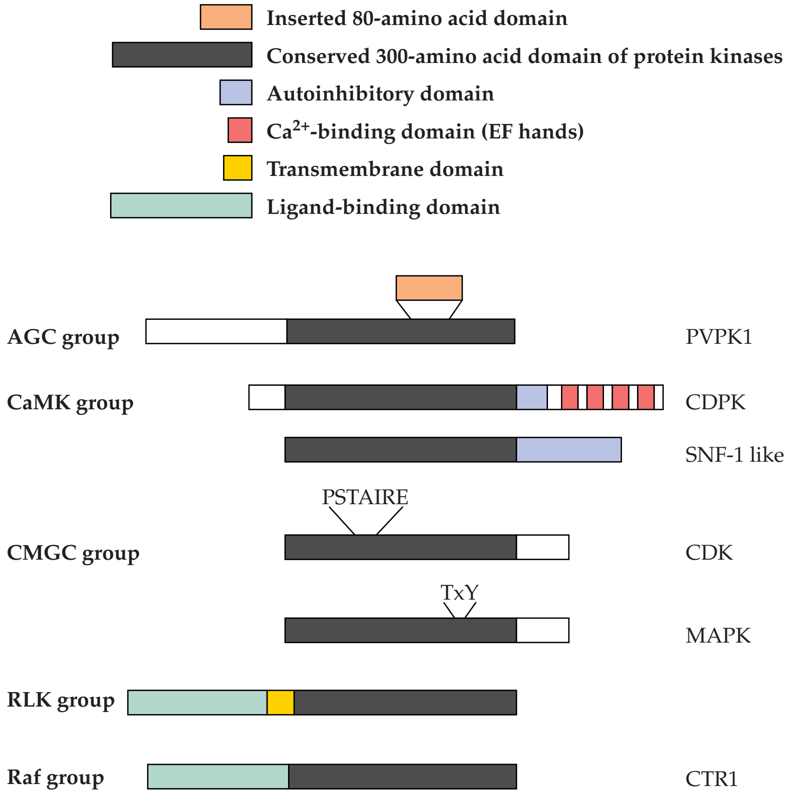


Figure 18.58
The various classes of protein kinase (PK) found in plant cells. The catalytic domain of most PKs consists of a 300-amino acid region. This conserved catalytic domain has various insertions and modifications that enable the classification of kinases into distinct groups. The AGC group is represented by cyclic nucleotide PKs (e.g., PKA; see Section 18.5.1) and calcium- and phospholipid-dependent PK (e.g., PKC; see Section 18.4.5). A plant protein member of this group, PVPK1, has an insert of approximately 80 amino acids within the kinase catalytic domain. The CaMK group contains PKs that are activated by or dependent on calcium and calmodulin. CDPK, a prominent member of this group in plant cells, has a C-terminal calmodulin-like region containing four (or fewer) calcium-binding EF hands. This enzyme also contains an autoinhibitory region involved in the regulation of CDPK activity. The CMGC group includes the CDK (cyclin-dependent PK), MAPK (mitogen-activated PK), GSK-3 (glycogen synthase kinase), and CKII (CaM kinase II) families. CDK has a conserved PSTAIRE region. MAPK has a conserved Thr-X-Tyr (TxY) motif. RLKs (receptor-like kinases) have a ligand/signal-binding domain joined to a conserved catalytic sequence by a membrane-spanning domain; a generic RLK structure is shown here. CTR1, a Raf-like PK active in ethylene transduction, also has a putative ligand-binding domain.

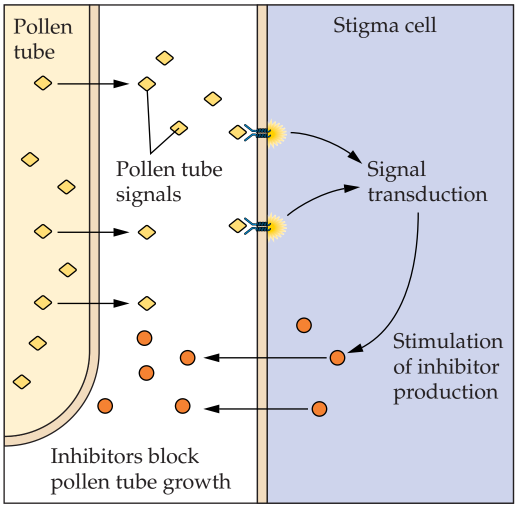


Figure 18.59
General model for pollen tube incompatibility as a result of two-way signaling between pollen tube and stigma in the Brassicaceae. Signals are derived from the growing pollen tubes of genetically incompatible pollen grains and induce a response in the stigma cells, leading to the secretion of growth inhibitors. S-domain receptor-like kinases are located in the stigma cells, suggesting that proteins secreted by pollen tubes might represent the pollen tube signal (see Chapter 19).

The **epidermal growth factor-like (EGF-like) RLKs** contain EGF-like repeats in the extracellular domain. A particular protein kinase in this group has been found attached to the cell wall (Fig. 18.61A). The binding domain located in the cell wall is linked through a single membrane-spanning domain to an internal kinase catalytic site. This kinase may transduce mechanical signals, which are sensed at the wall/plasma membrane junction.

Only one **lectin receptor kinase** has been identified in plant cells (Fig. 18.61B). The large extracellular domain of this protein kinase might bind important carbohydrate signals such as oligosaccharides and other wall fragments. Because many lectins are glycoproteins, there may be some similarity with the S-locus class of RLKs.

Identification of downstream elements of these RLKs has begun. If RLKs are similar to the well-characterized growth receptor-like tyrosine kinases, a cascade of protein kinases should link signal reception to regulation of gene expression. As noted above, an important aspect to regulation by phosphorylation is the large-scale amplification built into the mechanism.

18.7.3 Plants have an unusual Ca^{2+}-dependent protein kinase with a calmodulin-like domain.

The calmodulin kinase (CaMK) group of protein kinases includes the Ca^{2+}/calmodulin–dependent protein kinases and SNF1/AMP–activated protein kinase families. CDPK (see Section 18.6.1) was originally purified from soybean but has now been found in many plant cells. The N-terminal half shares homology with other members of the CaMK group, whereas the C-terminal region shows homology to calmodulin with four helix–loop–helix Ca^{2+}-binding sites (Fig. 18.62). A junction that joins the kinase and calmodulin-like domains may function as an autoinhibitory site. In the absence of Ca^{2+}, the junction covers the active site; in the presence of Ca^{2+}, the site is exposed and catalytic activity can commence.

CDPKs have been found attached to the cytoskeleton or the plasma membrane and in the cytoplasm. These proteins may phosphorylate the plasma membrane H^+-ATPase and membrane transporters. CpCK1 is a CDPK variant that contains a consensus myristylation site at its N terminus. This type of CDPK is thought to use a Ca^{2+}/myristylation switch to associate reversibly with the plasma membrane. Ca^{2+} binding alters the conformation of the protein, allowing myristylation to occur and thus activating the kinase domain. Several other Ca^{2+}/calmodulin–dependent protein kinases that most likely function in Ca^{2+}-activated signal transduction pathways have also been identified in plant cells.

SNF1-like (sucrose-nonfermenting) kinases are required in yeast for catabolite repression (see Chapter 8); equivalent enzymes from plants have been identified by complementation of yeast *snf1* mutants. These kinases may act as metabolic sensors of the ATP/AMP ratio and may control the metabolic flux between anabolism and catabolism by regulating the transcription of genes encoding enzymes that function in carbohydrate metabolism. In plants, some

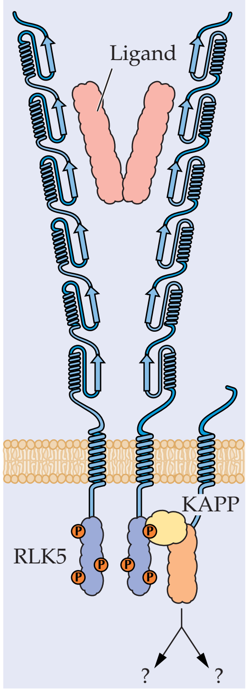


Figure 18.60
Model of a homodimeric receptor-like protein kinase, RLK5, binding its ligand and undergoing autophosphorylation. RLKs are located in the plasma membrane. The RLK shown here, a member of the LRR (leucine rich-repeat) class, contains a recognition core of leucine and asparagine residues. The extracellular region of such a kinase has extended numbers of LRRs, suggesting that the ligand may be another protein. On binding of the ligand, the receptor dimerizes, undergoing intramolecular phosphorylation and activation. A kinase-associated protein phosphatase (KAPP) controls the duration of signaling, thereby regulating the degree of signal amplification.

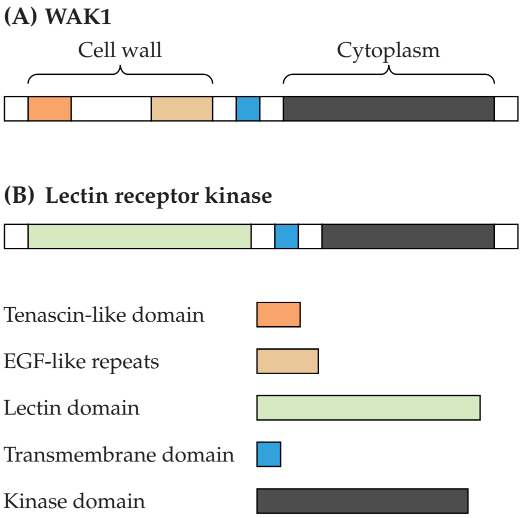


Figure 18.61
Potential signaling molecules at the plasma membrane–cell wall continuum. (A) WAK1, a wall-associated kinase, spans the plasma membrane so that its extracellular domain can interact with the wall and its cytoplasmic kinase domain can relay this interaction to proteins within the cell. Some wall-associated kinases have sequences that suggest similarity with signaling molecules in animals, such as epidermal growth factor (EGF), collagen, and neurexin, or with plant extensins (see Chapter 2). (B) A lectin-like receptor kinase traverses the plasma membrane. Lectins may interact with carbohydrate molecules or glycoproteins.

members of this group of kinases are synthesized when cells are water-stressed or exposed to high concentrations of ABA.

18.7.4 Growth factor kinases and mitogen-activated protein kinases are critical elements in the transduction of numerous signals, many of which affect growth.

Members of the CMGC (i.e., CDK/MAPK/GSK-3/CKII) group include some of the kinases most important for growth and development. Mitogen-activated protein (MAP) kinase (MAPK), responsible for the direct regulation of transcription factors, is in turn activated by a protein kinase cascade consisting of MAPKK (MAPK kinase) and MAPKKK (MAPKK kinase, sometimes called Raf) (Fig. 18.63). MAPKKK can be activated in animal cells by an important small GTP-binding protein called Ras or by other GTP-binding proteins that participate in parallel cascades (e.g., Rho or Rac). These cascades are controlled by growth factors and may mediate the action of growth regulators.

In plants, ABA may regulate MAPK activity by removing an inhibitory phosphate on a Raf-like kinase by way of the putative ABA receptor/phosphatase; auxin may regulate MAPKK. MAPK cascades are implicated in the transduction of mechanical signals such as touch or wind and in signal transduction cascades triggered by gibberellin, ethylene, osmotic stress, wounding, and fungal elicitors. In pollen, a MAPK is synthesized within 1 or 2 minutes after imbibition. These enzymes will phosphorylate serine, threonine, and tyrosine residues in target proteins.

Members of one important class of kinases in the CMGC group regulate cell division and are activated by cyclins, proteins that control progression through the cell cycle (see Chapter 11). These cyclin-dependent protein kinases (CDKs) are themselves regulated by phosphorylation.

Proteins belonging to another important subgroup of CMGC kinases are homologous to members of the GSK-3 (glycogen synthase kinase) group. This subgroup is referred to as shaggy/zeste white 3, named for gene products that specify cell fate and polarity in *Drosophila.* GSK-3s are encoded by a family of genes in plant cells, different members of

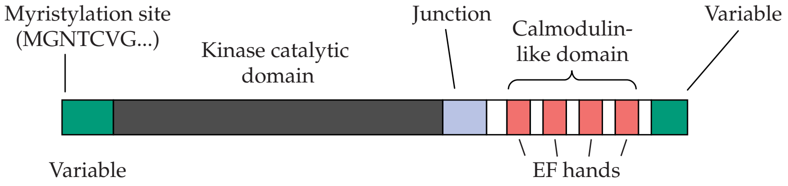


Figure 18.62
Calmodulin-like domain protein kinases (CDPKs) are ubiquitous protein kinases in plant cells. All the plant CDPKs cloned thus far contain a calmodulin-like sequence that binds Ca^{2+} at four (or fewer) EF hands. A junction region contains an autoinhibitory sequence that binds to the active site in the absence of Ca^{2+}. Some forms of CDPK may bind to the plasma membrane by way of a myristyl moiety that modifies the N terminus. Perhaps a combined Ca^{2+} and myristylation signal is responsible for the movement of soluble CDPK to the plasma membrane.

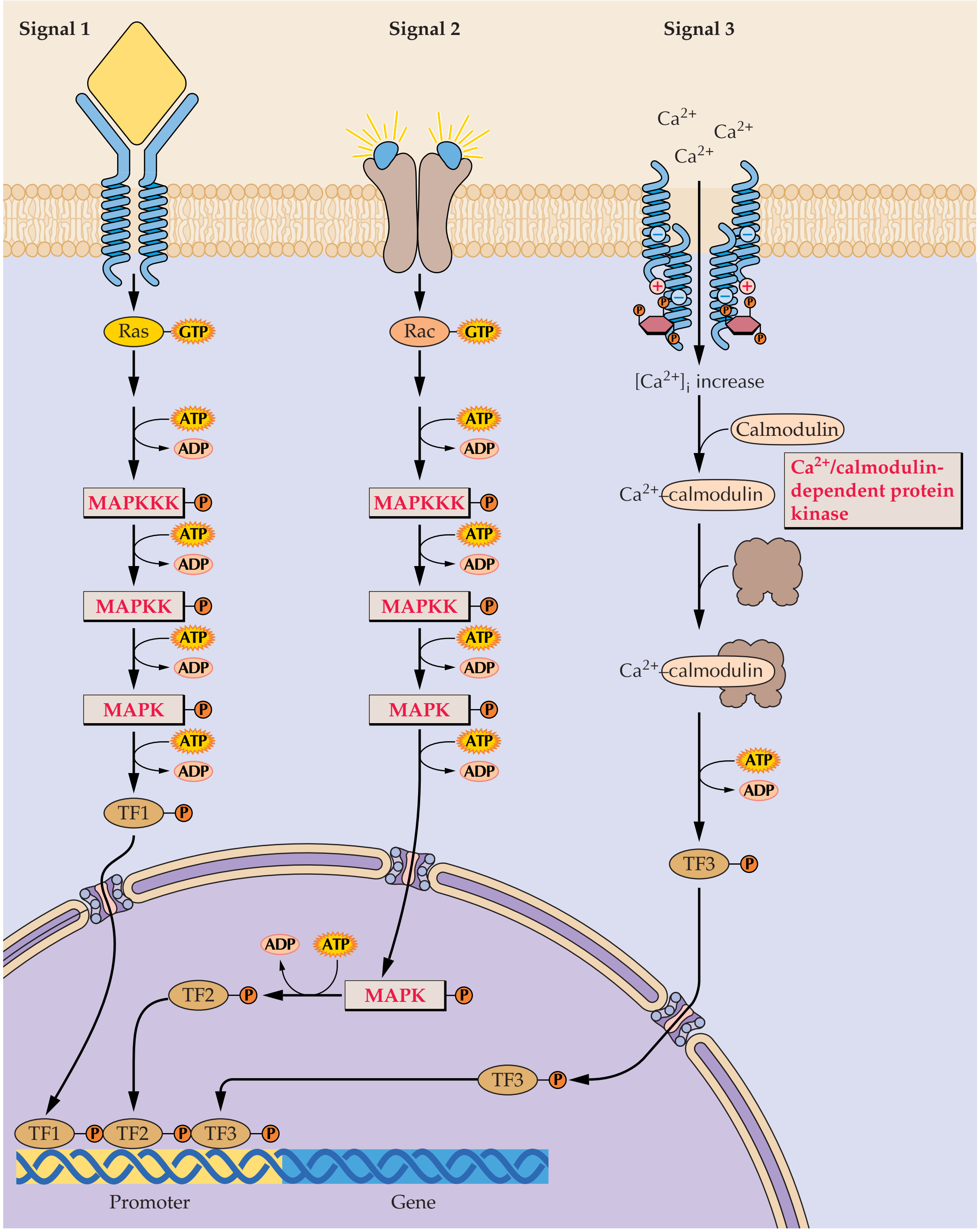


Figure 18.63
Many signals are transduced by protein kinase cascades that regulate gene expression. One such transduction sequence is believed to underlie regulation of gene expression involving a small GTPase (Ras- or Rac-like protein) and mitogen-activated protein kinase (MAPK) cascade leading to transcription factor phosphorylation. Transduction chains from different signals using a MAPK cascade (e.g., signals 1 and 2) or through alteration of $[Ca^{2+}]_i$ (e.g., signal 3) can all affect the same gene through the phosphorylation of different transcription factors (TFs). The TFs may move through the nuclear membrane when they are phosphorylated (signals 1 and 3); alternatively, MAPK may move into the nucleus after phosphorylation by MAPKK (signal 2).

which are differentially expressed, indicating their significance in the regulation of development. In *Arabidopsis,* at least five genes encode homologs of GSK-3 that can autophosphorylate serine, threonine, and tyrosine residues. In animals, these protein kinases are important for specifying cell fate and polarity; they may have a similar function in plants.

CKII (CaM kinase II) is discussed in Section 18.9.3.

18.7.5 Kinases can regulate transcription through phosphorylation of transcription factors.

Expression of a gene depends on the binding of transcription factors to the promoter region of the gene. Phosphorylation (e.g., by MAPK or CKII) represents a primary means for regulating transcription. Many transcription factors are unable to bind DNA unless phosphorylated, although some are inhibited by phosphorylation. Chromatin in which genes are actively transcribed is also rich in protein kinase activity and phosphorylated proteins. A single gene can be regulated by several transcription factors that interact and activate each other, and a single transcription factor can have multiple phosphorylation sites. In some cases, transcription factors are phosphorylated in the cytoplasm, which is a signal for the protein to move to the nucleus; phosphorylation of most transcription factors, however, takes place in the nucleus itself.

18.7.6 Raf-like kinases, members of an important group of receptor-mediating kinases, may also transduce signals in plants by way of MAPK cascades.

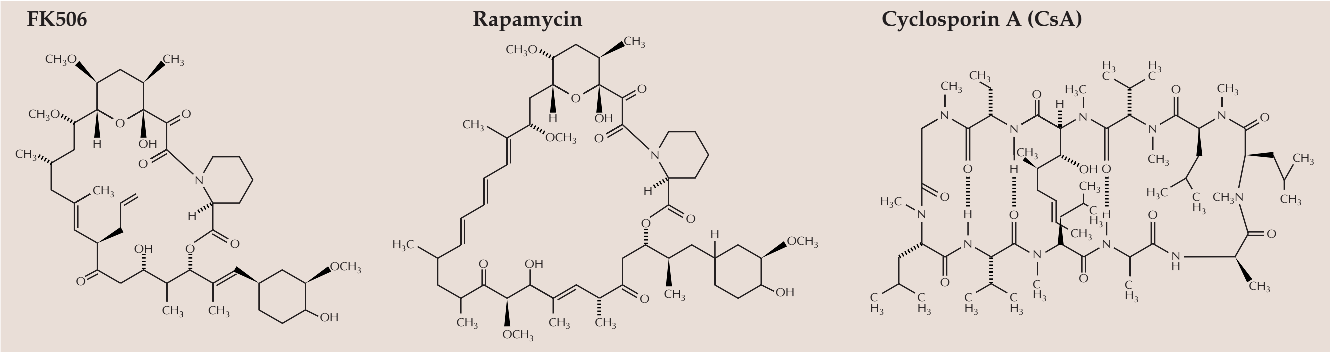
The Raf group of kinases mediates a signaling cascade thought to be initiated at receptors with seven membrane-spanning domains. One such kinase, CTR1 (constitutive triple response), was the first identified from molecular studies on an ethylene-insensitive mutant. Named for the phenotype of its air-grown seedlings, which resemble wild-type seedlings exposed to ethylene, CTR1 is a serine/threonine kinase thought to activate MAPKK and other components of this type of transduction system.

18.7.7 Protein phosphatases control numerous processes in plant cells.

The protein kinase signal is regulated by protein phosphatases acting to dephosphorylate target proteins. At any one time, the extent of protein phosphorylation represents a balance between the activities of the protein kinases and the protein phosphatases. Changes in either can modify the steady-state concentrations of phosphorylated and dephosphorylated proteins.

Of the several classes of protein phosphatases identified in animal cells, two types (1 and 2A) have been purified from plant cells. Protein phosphatase 2B (calcineurin) is Ca^{2+}/calmodulin–activated and inhibited by the immunosuppressants cyclosporin A and FK506 (Fig. 18.64), macrocyclic lactones that suppress the immune response by blocking the activation of T-lymphocytes. Cyclosporin also blocks the Ca^{2+}-induced inactivation of K^+ channels in guard cells, suggesting that a calcineurin-type protein phosphatase may act to regulate the K^+ channel function in guard cells. In animal cells, calcineurin is a heterodimeric enzyme consisting of a catalytic phosphatase subunit and a regulatory subunit. Recently, calcineurin-like regulatory subunits have been identified in plant cells.

The importance of protein phosphatase activity in plant cells has also been deduced from the inhibitory effects of the well-characterized protein phosphatase inhibitors calyculin, okadaic acid, and microcystin. The putative identification of a protein phosphatase involved directly in the ABA transduction pathway has also emphasized the importance of protein dephosphorylation. These inhibitors can either prolong an apparent stimulation of protein kinase activity or prevent the transduction of signals that require target protein phosphorylation turnover. Protein phosphatase inhibitors function at very low concentrations and are able to prevent the operation of protein kinase cascades initiated by red light, proliferation, growth regulators, and pollen tube signaling.


FK506

Rapamycin

Cyclosporin A (CsA)

Figure 18.64
Structures of FK506, rapamycin, and cyclosporin—compounds used medicinally to suppress the activity of the immune system, which they do by inhibiting lymphocyte signal sensitivity. All three are thought to inhibit protein phosphatase activity. Remarkably, they all block Ca^{2+}-induced inactivation of specific K^+ channels in guard cells.

18.8 Particular pathways of signal transduction associated with plant growth regulators

18.8.1 Ethylene transduction uses protein kinase cascades.

Several classes of mutants obtained by using the seedling bioassay as a screen (Fig. 18.65A) affect a broad range of ethylene responses throughout the life cycle of the plant. These observations indicate that the responses to ethylene share a primary signal transduction pathway. Multiple mutant alleles at the *ETR1* and *EIN* (ethylene-insensitive) loci confer global insensitivity to ethylene. *CTR1* encodes another transduction protein that, when mutated, constitutively activates three ethylene-regulated processes in seedlings: hook development, hypocotyl elongation, and root growth (Fig. 18.65B). CTR1 is therefore a negative regulator of the ethylene response. Double-mutant analysis involving *etr1*, *ctr1*, and *ein2* indicates that CTR1 is downstream from ETR1 and upstream from EIN2. CTR1 has sequence similarity to the Raf group of protein kinases (i.e., MAPKKK; Fig. 18.66). CTR1 activation might specifically regulate ethylene-dependent processes exclusively, especially if it is linked to downstream elements through direct structural interaction.

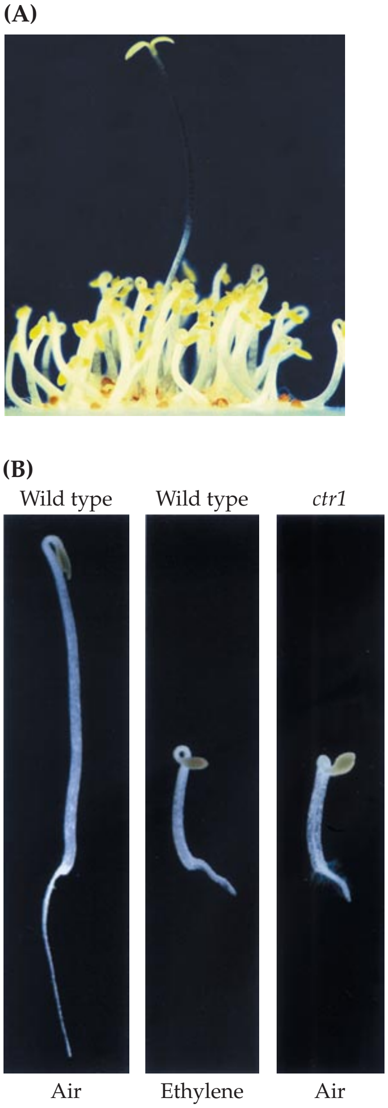


Figure 18.65
Ethylene-signaling mutants have striking phenotypes. (A) Mutation in the *ETR1* gene prevents ethylene-dependent hypocotyl shortening in early germination. (B) The constitutive triple response mutation (*ctr1*, right panel) has an air-grown seedling grown in the presence of 10 ppm ethylene (middle panel). Wild-type CTR1 (left panel) functions as a negative regulator of the ethylene response.

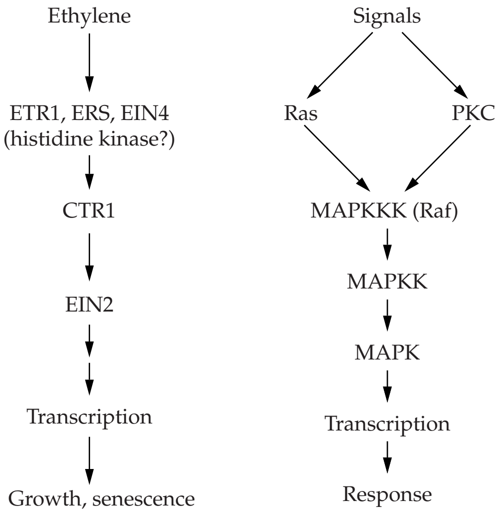
18.8.2 Gibberellin signal–response pathways indicate the involvement of transcription factors.

Numerous mutants defective in gibberellin (gibberellic acid; GA) signaling have been identified (Fig. 18.67). Most mutants fall into either of two classes: those that resemble GA-deficient plants but do not respond to GA, and those that resemble plants in which a GA response pathway has been constituitively activated. The *rht* (reduced height) mutants are in the first class. Mutants in GA biosynthesis have been exploited by breeders to create "green revolution" cereals, including short-length wheat varieties commonly grown worldwide.

In *Arabidopsis*, SPY (spindly) is a negative regulator of GA signal transduction. *spy* plants are tall but capable of some increased height response to applied gibberellin.

Sequence analysis of *SPY* suggests that it encodes an *O*-glucosyl-*N*-acetyltransferase. *gai* (gibberellic acid–insensitive) was identified during characterization of mutant plants with a GA-deficient phenotype. *gai* plants are unresponsive to applied GA but mimic all other aspects of GA deficiency. *GRS* (GAI-related sequence) and *GAI* have been sequenced and most likely encode transcription factors.

Figure 18.66
Comparison of ethylene-signaling pathway and animal Ras-signaling pathway. CTR1 has distinct sequence similarity to Raf (MAPKKK). A protein kinase cascade is indicated for transduction of the ethylene signal, but the physiological function of many of the proteins in the ethylene pathway is currently unknown.



Finally, RGA (repressor of GAI3) does not suppress as many aspects of the GA-deficient phenotype as *spy* does. Double-mutants of *spy* and *rga* exhibit an additive phenotype, which suggests these two genes are in separate GA signal transduction pathways. Figure 18.68 suggests one possible outline of GA signal transduction.

GA-dependent transduction pathways in barley cells involve cytosolic Ca^{2+} and cGMP. These aleurone cells secrete α-amylase in response to gibberellin. MAPK pathways have also been implicated in GA signal transduction.

18.8.3 Auxin signal transduction may involve a protein kinase cascade, 14-3-3 proteins, and ubiquitin-degradation pathways.

Auxin is often regarded as a “master” hormone because cell division, growth, maturation, and differentiation are all associated with auxin regulation. At least some auxin-mediated processes (e.g., acidification of the apoplast during cell wall expansion; see below) appear to be mediated by 14-3-3 proteins, a recently discovered group of proteins. 14-3-3 proteins facilitate phosphorylation of other molecules and may discriminate between phosphorylated and nonphosphorylated target proteins. Therefore, 14-3-3 proteins act as intracellular messengers that can cross-link signal transduction chains.

The plasma membrane–localized H^+-ATPase (P-type ATPase) is an important target for regulation of growth by auxin, because the enzyme is able to increase wall extensibility by acidifying the apoplast. In addition, the establishment of a proton gradient between the plasma membrane and the wall provides the necessary energy for active uptake of the potassium ion, which is required to maintain turgor pressure as the cell expands. The toxin fusicoccin (see Chapter 3, Fig. 3.8) binds to and increases the activity of the electrogenic ATPase. Fusicoccin induces stomatal opening, enhances growth rates, and breaks seed dormancy. Because the fusicoccin receptor is a 14-3-3 protein, it is likely that auxin modifies ATPase activity through a 14-3-3 protein, probably through activation of a protein kinase.

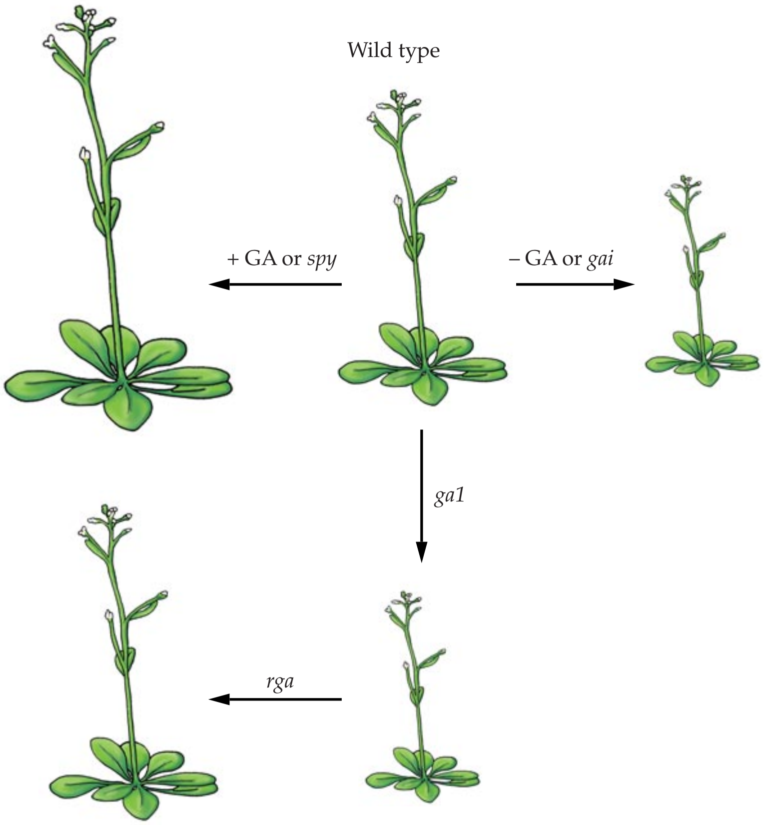


Figure 18.67
Cartoon of the various types of *Arabidopsis* mutants with altered responses to gibberellic acid (GA). The *spy* mutation phenocopies the effect of treating wild-type plants with GA. The *gai* mutation phenocopies the effect of treating plants with a GA biosynthesis inhibitor. The *ga1* mutation disrupts GA biosynthesis. The *rga* mutation suppresses the GA-deficient phenotypes associated with the *ga1* mutation.

Several auxin-resistant mutants have been isolated and analyzed. Sequence analysis of *AXR1* indicates that it encodes a protein with homology to a ubiquitin-activating enzyme, which prepares proteins for ubiquitin-mediated degradation (see Chapter 9). The auxin-resistant mutant *tir1* is defective in synthesis of a protein having a conserved amino acid domain similar to that found in a ubiquitin protein ligase. These observations suggest an unusual mechanism to explain auxin action (Fig. 18.69).

Auxin is generally abundant in shoot tips, which contain cells that progress through a defined developmental program: division → expansion → maturation → differentiation. The continued presence of auxin is necessary for these processes to occur. Each stage of development is associated with a unique set of proteins, some of which must be degraded by coupling to ubiquitin before the subsequent stage can be initiated. The critical proteins of one stage are proposed to repress by feedback the transcription of genes necessary for the next stage. Only if the critical proteins are degraded will the next stage of the developmental program be enabled. Regulation of the ubiquitin-conjugating pathway by auxin is thus indicated, but the mechanism for this is not yet clear. Direct interaction with enzymes in this pathway might explain, for example, the rapidity with which growth diminishes when auxin is removed. Another interesting possibility is that progress through development might be accelerated to some extent if the pathway is enhanced (Fig. 18.69).

18.8.4 ABA transduction involves Ca^{2+}-dependent pathways and protein kinases.

The control of guard cell aperture has provided substantial information on the transduction of ABA signals. Both Ca^{2+}-dependent and Ca^{2+}-independent pathways are involved, the latter pathway being known to require changes in intracellular pH, i.e., uses H$^+$ ions as a second messenger. The discovery of mutants that are ABA-insensitive has enabled identification of ABI1, a type-2C protein phosphatase. A transduction sequence using phosphorylation and Ca^{2+} is thus indicated. Patch clamp investigations and the use of immunosuppressors that inhibit protein phosphatase activity (e.g., cyclosporin) indicate that the potassium channels that open to permit turgor loss during stomatal closure are regulated by phosphorylation.

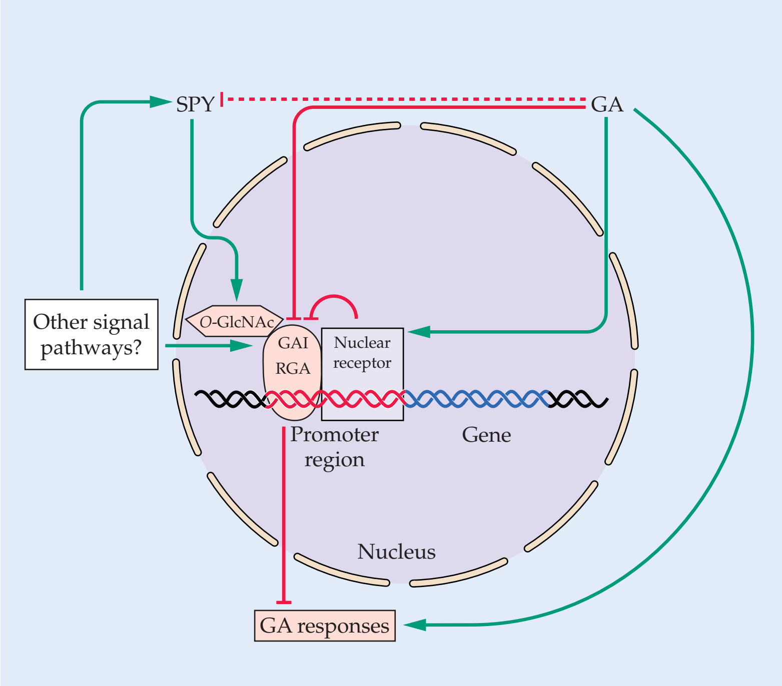


Figure 18.68
Model of how SPY, GAI, and RGA may act in GA signal transduction. SPY is predicted to be a *N*-acetylglucosamine (*O*-GlcNAc) transferase, whereas GAI and RGA are predicted to be transcription factors.

The mobilization of Ca^{2+} by ABA may involve the second messenger cADP-R (Fig. 18.70), which is synthesized from NAD$^+$. cADP-R can mobilize Ca^{2+} from intracellular stores. Microinjection of cADP-R into tomato cells mimicks the effect of ABA on gene expression. Reporter constructs containing the promoter *LTI78,* an ABA-dependent gene that also responds separately to water stress and low-temperature signals, have shown that coinjection of cADP-R and protein phosphatase inhibitors into individual cells induces expression of *LTI78,* thus circumventing the normal requirement for ABA induction. Coinjection of cADP-R and either of two kinase inhibitors, staurosporine or K252a, blocks *LTI78* expression. Again, a transduction pathway involving Ca^{2+} and protein kinase is implicated.

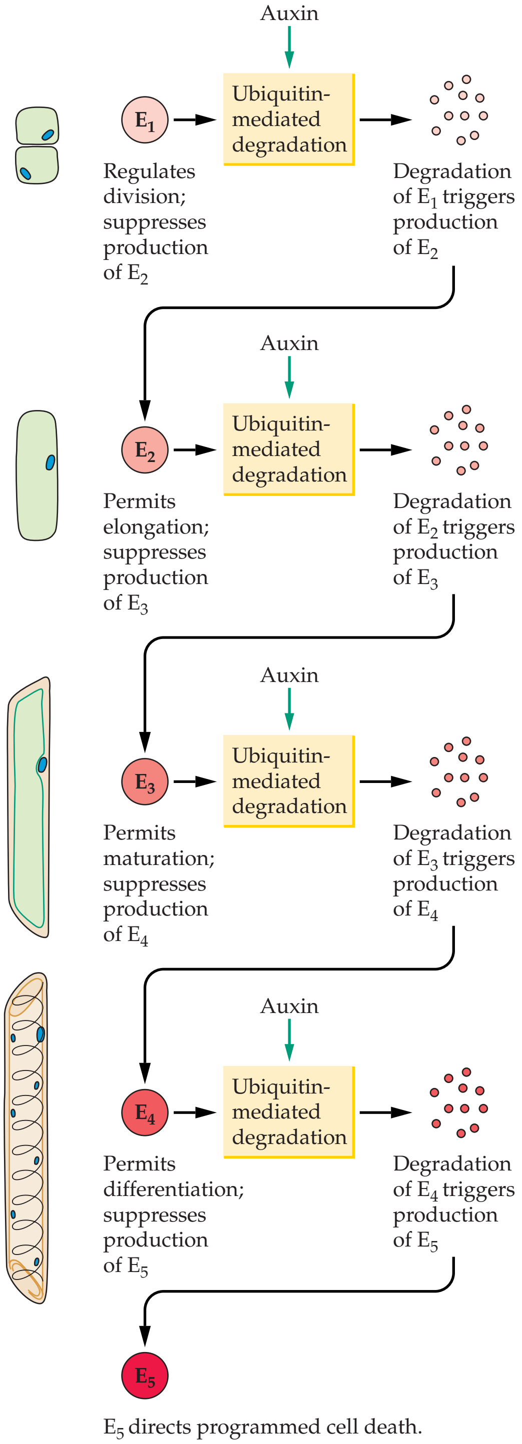


Figure 18.69
Isolation and sequencing of auxin-resistant mutants suggest that auxin might regulate protein degradation through ubiquitination. The development of a tracheid is used as an example. It has been suggested that the progress of cells through their developmental schedule requires degradation of the specific proteins associated with one developmental phase before the next stage can occur. Thus, the presence of specific proteins concerned with cell division represses the genes encoding specific proteins required for cell growth and so on. At each stage ubiquitination of these critical proteins may be required for degradation (see Chapter 9). Auxin acts to maintain the activity of this developmental pathway. Removal of auxin disrupts the developmental schedule, arresting development at whatever stage has previously been reached. Restoring auxin concentrations enables completion of the entire program.

18.9 The future of plant cell signal transduction research

At present, knowledge of plant cell signal transduction is in its infancy. There is considerable room for filling in details of the transduction map, and there are many uncharted directions in which the field will soon expand. Only some can be predicted.

18.9.1 The main signaling pathways cross-talk with each other.

Until recently, the molecular switches that set signaling into action were neatly divided into a few discrete groups, each comprising a distinct set of protein families that receive a particular set of extracellular stimuli and mediate distinct cell responses. One such chain uses G-proteins and $[Ca^{2+}]_i$ as its primary transduction pathway; the other uses protein kinase and leads to growth and proliferation. We now know that such division is simplistic, because the two pathways are linked by many connections, generally referred to as **cross talk.**

We can expect the number of signaling pathways that have been identified to proliferate. In addition, each pathway bifurcates, presumably providing partial or full redundancy in each pathway. For example, guard cells can be closed by an increase in $[Ca^{2+}]_i$ but do not necessarily require changes in $[Ca^{2+}]_i$ for closure to occur. With environmental fluctuation, the flux through each pathway can be expected to vary substantially. Complex integration processes lead to the physiological response. Various different Ca^{2+}-regulated protein kinases clearly cross-talk along the two established pathways. In animals, protein kinases can be activated by small GTPases of the Ras family, which can

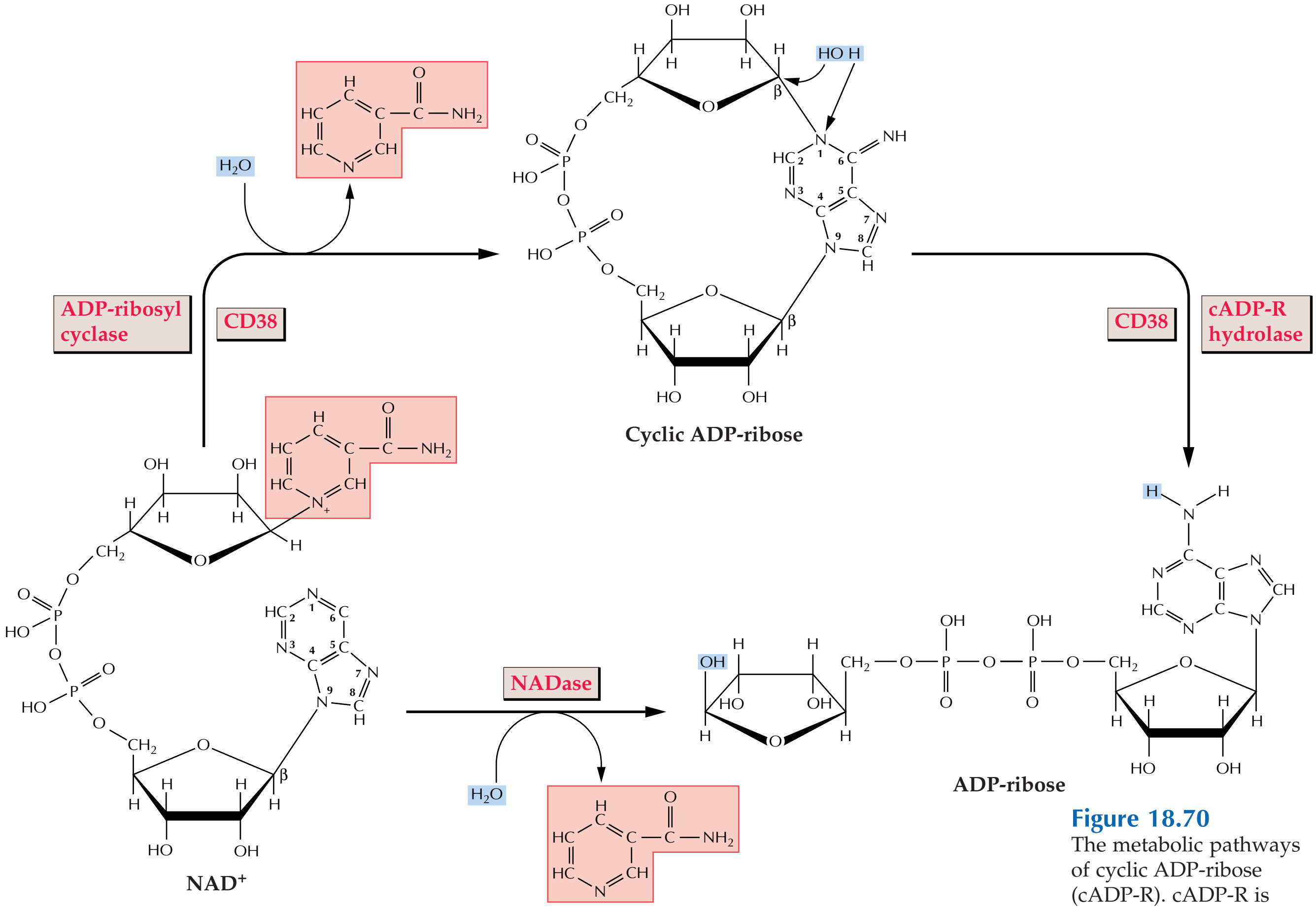


Figure 18.70
The metabolic pathways of cyclic ADP-ribose (cADP-R). cADP-R is produced by cyclization of NAD$^+$, a reaction catalyzed by ADP-ribosyl cyclase, and is degraded by cADP-R hydrolase. Enzymes such as CD38 (from animal sources) can catalyze both reactions. NADase catalyzes the breakdown of NAD$^+$ to ADP-ribose and is involved in cADP-R metabolism.

also increase $[Ca^{2+}]_i$ and various other adapter proteins, thereby establishing crosstalk between the two pathways. Ras can also modify $[Ca^{2+}]_i$, but the β/γ-subunits from G-proteins can activate Ras. Clearly, our expanding base of knowledge indicates that this system is very complex. Figure 18.71 maps some of the known connections that form the basis of a complex transduction network.

18.9.2 Specificity in signaling arises from the specific spatial locations of the signaling elements.

Nature has generated a remarkable array of amino acid sequence motifs to ensure that the right enzymes are in the right place at the right time. Precise spatial and temporal activation of signal transduction pathway components is particularly critical because biochemical "noise" that might obscure signaling must be kept to a minimum. As the second messengers relay signals through cellular compartments that contain many different kinases and phosphatases, it is critical to activate only the appropriate signal transducers; uncontrolled activation would be fatal to the cell. A mechanism for targeting involves anchoring specific components of a signaling cascade to scaffold proteins, thereby producing a large protein complex termed a **transducon** (Fig. 18.72). Several well-characterized protein kinases (e.g., PKC and PKA, MAPK) and phosphatases are coupled to the cytoskeletal scaffold by means of specific tethering subunits. Calmodulin binds to specific structures during cell division and attaches to putative growth poles during polarization; CDPK binds to actin microfilaments. Clustering of Ca^{2+} channels is in some way directed by microfilaments. Crucial research goals include understanding the spatial distribution of transducons, the possibility of spatial overlapping, the sharing of individual constituents, and the importance of mobile second messengers in

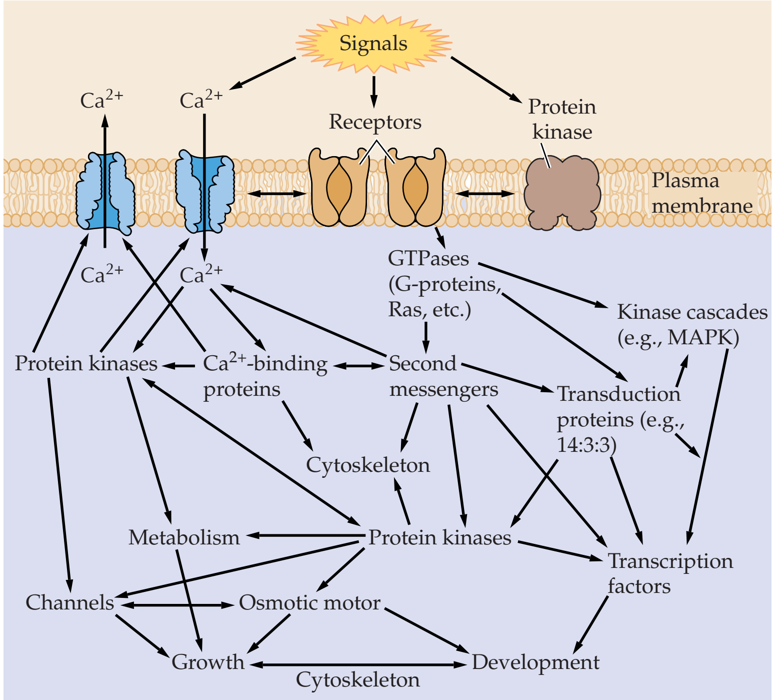


Figure 18.71
Some of the known interactions in the plant cell signal transduction network. The complexity of these interactions can be expected to increase as research progresses. Figuring centrally in the network are the protein kinases, many of which remain to be described. MAPK, mitogen-activated protein kinase.

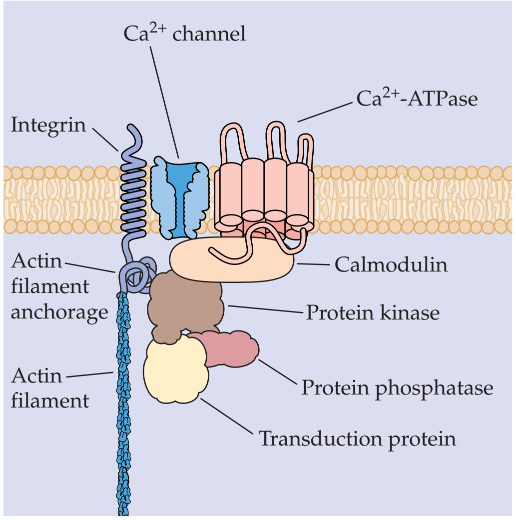


Figure 18.72
Suggested constituents for a transducon, a complex of proteins that acts to transduce signals. The Ca^{2+} channel and Ca^{2+}-ATPase are concerned with regulation of the Ca^{2+} signal; calmodulin, protein kinase, and phosphatase deal with the interpretation of the Ca^{2+} signal. The closeness of the actin filament suggests that a major target of Ca^{2+} signaling involves cytoskeletal changes.

integrating the activities of limited numbers of transducons. Signaling complexes have already been discovered in yeast.

18.9.3 Integration of many signals might result from multiple phosphorylation sites or from integrating enzymes such as CKII.

CKII is a calcium/calmodulin–activated protein kinase originally detected in brain. An equivalent form appears to be present in plant cells. Part of its cycle of activation by Ca^{2+} leads to autophosphorylation and, once a threshold is exceeded, this activation is irreversible and becomes independent of Ca^{2+}. Moreover, the enzyme depends for activation not only on the size and number of Ca^{2+} spikes it perceives but also on their frequency (Fig. 18.73). This enables integration of all the signals that contribute to the $[Ca^{2+}]_i$ signal.

Integration may occur in other ways. Many nuclear proteins have multiple phosphorylation sites that may be phosphorylated by several separate protein kinases. Multiple phosphatases may also participate in dephosphorylation. The phosphorylation states and biological functions of these proteins therefore represent the integrated activity of various different signals transduced through different kinases. Protein kinase activation may also signal the cell without additional phosphorylation. For example, a receptor protein kinase can dimerize in the plasma membrane and undergo autophosphorylation. The two subunits can then separate and their phosphorylation status can be perceived by other proteins, which are then activated and continue transduction of this active state.

18.9.4 Adapter proteins may help cross-link signaling pathways or act as phosphorylation receptors.

Members of the highly conserved and abundant eukaryotic family of 14-3-3 proteins interact with many important signaling proteins in animal cells (e.g., PKC). In plant cells, they interact with and regulate nitrate reductase (see Chapter 16) and act as the receptor for fusicoccin, a fungal toxin that activates the plasma membrane H^+-ATPase (see Chapter 3 and Section 18.8.3). There are at least 10 isoforms of 14-3-3 in *Arabidopsis*, which have been proposed as potential adapters between pairs of different signaling components, thereby controlling signal transduction. One of the important 14-3-3–binding molecules in animal cells is Raf.

Phosphorylation of the target protein seems to be an essential signal for 14-3-3 binding, and a peptide motif containing a serine phosphate is shared by most 14-3-3–binding proteins. Thus, the 14-3-3 proteins may act as receptors for the phosphorylated state of proteins and activate downstream constituents as a consequence. Plants probably contain many proteins that bind 14-3-3 proteins but their identification and characterization are difficult because they must be phosphorylated to bind to a 14-3-3 protein. Proteins involved in cell cycle induction and cell cycle checkpoints may also be likely targets for 14-3-3–binding proteins.

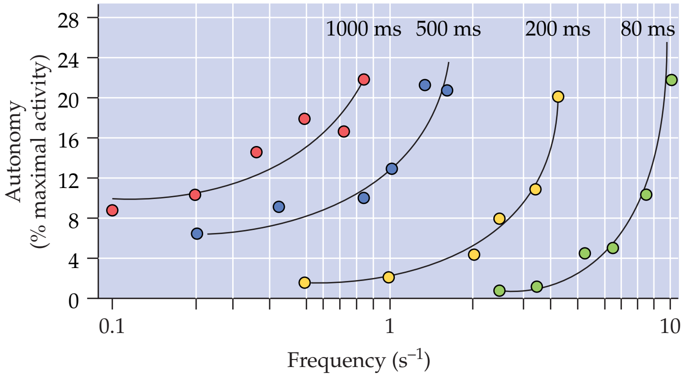


Figure 18.73
Modulation of the frequency response of CKII, a complex Calcium/calmodulin (CaM)–activated protein kinase found in substantial quantities in brain tissue. This unusual enzyme responds to activation by Ca^{2+}/CaM by becoming autonomous of these two activators, using autophosphorylation to provide a stable activated state. Remarkably, CKII can interpret oscillations of Ca^{2+}. Autonomy, as a percentage of maximum activity (i.e., the extent to which the enzyme is activated and no longer needs Ca^{2+}/CaM), has been measured against the frequency with which particular activating pulses are applied. Short pulses (80 milliseconds) must be applied with greater frequency than long pulses (1000 milliseconds) to achieve comparable effects.

18.9.5 Cells communicate by means of integrins and plasmodesmata.

The identity of each cell is specified by signals from neighboring cells. Cells may respond to mechanical signals from the cell wall, to chemical signals diffusing from other cells, or to signals that arrive through the plasmodesmata. Mechanical signals are probably important in the specification of cell form and may be determined by the tensions and compressions resulting from wall- and turgor-based interactions with the surrounding cells. Cellular receptors in the wall/extracellular matrix probably bind to **integrins** that span the plasma membrane and then connect to the cytoskeleton through other proteins (see Chapter 5). Animal integrins include vinculin, actinin, and talin; equivalent receptors and integrins are probably present in plant cells as well. Interaction with integrins can be inhibited by peptides such as RGD (Arg-Gly-Asp). Transduction through integrins is initiated by integrin clustering and results in activation of the $[Ca^{2+}]_i$ pathway, probably to ensure microfilament rearrangements in response to signaling. Protein kinases are almost certainly involved as well.

The passage of signals through the plasmodesmata (see Chapter 15) is thought to be regulated directly by $[Ca^{2+}]_i$, which can cause plasmodesmatal closure. Although it might be difficult for Ca^{2+} waves to move directly from one cell to another, Ca^{2+} could generate action potentials as an alternative form of communication capable of moving from cell to cell, increasing $[Ca^{2+}]_i$ sequentially. IP_3 might also carry a Ca^{2+} wave from cell to cell through the plasmodesmata. Recent studies on plasmodesmata have indicated that this intercellular communication route may also permit the direct exchange of transcription factors, thereby allowing the formation of a supracellular network of interactions controlled, in turn, by signal transduction.

Summary

Signal transduction is an actively expanding topic of research in plant biology. Signals, which include a wide array of external and internal stimuli, are amplified and communicated by complex signal transduction networks, most of which initiate with the activation of receptor proteins. Bacterial receptor and transduction systems provide models for plant receptors, including proteins that sense ethylene and phytochrome. Among the various plant signal transduction pathways that have been identified are other components common to many signal transduction networks in animals, such as GTPases and phospholipid derivatives. Investigations into the roles of GTPases in plant signal transduction are still in their infancy, but already a strong relationship is implicated between GTPase activity and phospholipid signaling. Phospholipases A, C, and D influence many aspects of plant development and signaling. Cyclic nucleotides also appear to act as second messengers in plant cells and most likely interact with another second messenger, cytosolic calcium. Calcium channels and other calcium transporters form the basis of a complex Ca^{2+} signaling network in plants. Protein kinases are the most common transduction components interpreting signals in plant cells. Various classes of protein kinase act in concert with protein phosphatases to mediate plant cell signaling and control metabolism. Plant hormones are important elements in controlling plant growth and development, and progress is being made in understanding how cells transduce these signals. Advances in signal transduction research are rapidly expanding our understanding of how plant cells communicate and cooperate.

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