

the perception of brassinosteroids, bacterial flagellin, CLE peptides, ABA, and mechanical forces. Section 18.3 then considers the functions of second messengers and kinase cascades in signal transduction and integration.

Sections 18.4 to 18.7 consider the perception and transduction of individual signals; they discuss how cells respond to the hormones ethylene, cytokinin, and auxin and how signal transduction follows light perception by phytochromes. These are among the best understood transduction pathways in plant cells. Signaling by gibberellins is discussed in Section 18.8 in relation to signal integration by DELLA proteins during seedling responses to light. Section 18.9 then considers the integration of light, ABA-, and CO₂ signaling in the control of stomatal aperture. This is far from an exhaustive survey of signal transduction in plant cells, but hopefully it provides a framework for understanding related areas of research.

18.2 Overview of signal perception at the plasma membrane

18.2.1 The plasma membrane is a major site of signal perception

Plant cells have two routes by which they are able to communicate with one another: the **apoplast**, formed by the interconnected cell walls of the plant, and the **symplast**, the cytoplasmic continuum created by strands of cytoplasm connecting neighboring cells via plasmodesmata. Molecular transport through the apoplast and symplast is discussed in detail in Chapter 15. With respect to intercellular signaling, the apoplast is associated with the transmission of signals such as the plant hormones and signal peptides, which require conventional receptor proteins. In contrast, signaling via the symplast allows regulated movement of RNAs and transcription factor proteins, which can induce responses in the receiving cell more directly.

For signals arriving at the cell through the apoplast, the plasma membrane is a major site of perception; indeed, large and hydrophilic molecules that lack a membrane import channel can only be perceived by plasma membrane receptors. Receptors at the membrane also perceive some physical signals, for example mechanical force (see Section 18.2.4) and blue light (via phototropins, see Box 18.2).

Plasma membrane receptors are divided into three major groups distinguished by how they interact with intracellular signaling components: receptor kinases, G protein-coupled receptors, and ion channel receptors. The receptor kinases are by far the largest group of membrane receptors in plants, and they transduce extracellular signals through phosphorylation of intracellular targets (see Section 18.2.2). G protein-coupled receptors regulate trimeric GTP-binding

proteins (**G proteins**) located on the inner surface of the membrane (see Section 18.2.3). Lastly, ion channel receptors regulate membrane ion channels and, thus, ion fluxes into or out of the cell (see Section 18.2.4).

Despite the importance of signal perception at the plasma membrane, it should be noted that small, lipophilic molecules can cross the membrane and be perceived in the cytoplasm or nucleus. This is the case for ethylene, which is detected by receptors on the endoplasmic reticulum membrane (see Section 18.4), and for the hormones auxin and gibberellin, which are perceived by soluble receptors that interact with the cell's protein degradation machinery (see Sections 18.6 and 18.8). Similarly, the plasma membrane is no barrier to many physical cues; the phytochrome and cryptochrome families of photoreceptors, for example, are intracellular, having both cytoplasmic and nuclear locations, depending on the light conditions and the particular family member (see Section 18.7, see Box 18.2).

18.2.2 Signaling by the largest group of membrane receptor kinases can involve both receptor heterodimerization and receptor endocytosis

Phosphorylation is the most common posttranslational modification used by cells to regulate protein activity. Catalyzed by **kinases**, phosphorylation can alter protein stability, subcellular location, binding properties, enzyme activity, and susceptibility to subsequent modifications. Furthermore, single proteins may be phosphorylated at multiple amino acid residues, sometimes by different kinases and with different effects, enabling integration of separate signal transduction pathways. Consequently, it is not surprising that kinases and **phosphatases** (which dephosphorylate proteins) play vital roles in signal transduction in all organisms.

In plants, signal perception appears to rely heavily on plasma membrane **receptor-like kinases** (RLKs), a family of over 600 proteins in *Arabidopsis* and over 1,100 in rice (*Oryza sativa*) that typically phosphorylate target proteins on serine and threonine residues. RLKs possess an extracellular domain that provides potential ligand-binding sites, a transmembrane domain, and an intracellular kinase domain. They act in a wide range of processes, including hormone perception, development, defense, symbiosis, and pollen tube germination and guidance. The best understood of the signal transduction mechanisms mediated by RLKs is the perception of brassinosteroids (BRs). RLKs also function in the perception of **CLE peptides** (CLAVATA3/ENDOSPERM SURROUNDING REGION, Box 18.1).

BRs are a group of steroid hormones that function in a variety of cellular and developmental contexts, such as promotion of cell elongation during etiolation of dark-grown seedlings. Research on the RLKs required for BR perception and on other, closely related RLKs has revealed multiple,

BOX
18.1

CLE peptide signals act in several developmental contexts

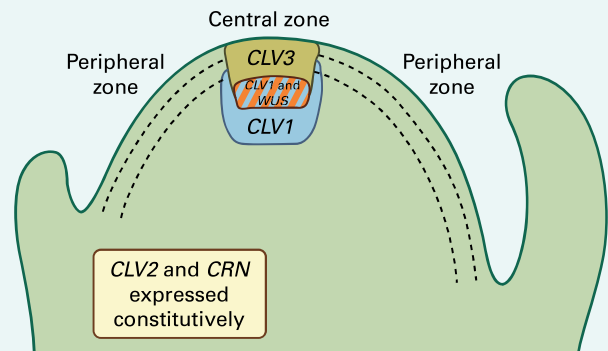
The **CLE peptides**, named after CLAVATA3 (CLV3)/ENDOSPERM SURROUNDING REGION (ESR), were discovered through research on the control of the size of the *Arabidopsis* shoot apical meristem (SAM). The SAM is composed of a central zone (CZ) and a peripheral zone (PZ) (panel A). Cells in the CZ act as stem cells for shoot development; they divide to supply cells to the PZ, in which cells both divide and differentiate to initiate the structures of the shoot.

The size of the CZ and, therefore, of the whole SAM is regulated by a feedback loop acting on the genes *WUSCHEL* (*WUS*) and *CLAVATA3* (*CLV3*). *WUS* is expressed in a small group of cells deep in the CZ and is required for the production of a signal that confers stem cell identity throughout the CZ. At least in part, this signal consists of the *WUS* protein—a transcription factor that is transported between meristem cells via plasmodesmata (see Section 11.5.1).

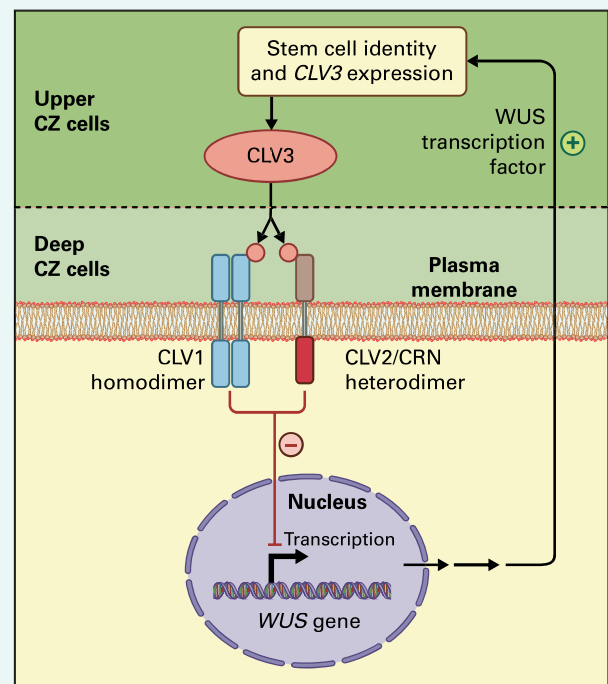
In the upper CZ layers, *WUS* activates transcription from *CLV3*, which encodes a small protein that is processed to produce a secreted, 13 amino acid CLE peptide. The *CLV3* CLE peptide acts as a short-range signal perceived by cells deeper in the CZ (panel B; cell wall not shown). In the current model, perception of the *CLV3* CLE peptide occurs at two receptors: a homodimer of the *CLV1* protein, and a heterodimer consisting of the *CLV2* and *CORYNE* (*CRN*) proteins. *CLV1* is a LRR-receptor-like kinase (see Section 18.2.2), and *CLV2* and *CRN* are related proteins. *CLV2* resembles *CLV1* but lacks an intracellular kinase domain. In contrast, *CRN* possesses a kinase domain but lacks a full extracellular domain. The *CLV2/CRN* heterodimer therefore has the necessary domains for signal perception and transduction. Signals from the *CLV1* and *CLV2/CRN* receptors converge to down regulate *WUS* expression. This completes the feedback loop and creates a mechanism in which SAM size is determined by the balance of *WUS* expression, which acts to promote CZ identity and hence SAM growth, and *CLV3* signaling, which limits *WUS* activity.

In total over 30 genes encoding proteins likely to produce secreted CLE peptides have been identified in *Arabidopsis*. The genes are expressed in a variety of tissues, and CLE peptides have been implicated in the regulation of root apical meristem activity and in vascular

differentiation. Although *CLV1* expression is confined to the SAM, *CLV2* and *CRN* are both expressed in a wide range of tissues. Therefore, the *CLV2/CRN* receptor may function beyond the CZ of the SAM and in the perception of other CLE peptides. Supporting this, both *crn* and *clv2* mutants have defects in stamen and anther development.



A



B

pairwise interactions between RLKs. The results of such research also indicate receptor endocytosis may have a key role in the regulation of signal transduction from RLKs.

BRs bind to the extracellular domain of the BR receptor BRASSINOSTEROID INSENSITIVE1 (BRI1) (Fig. 18.4). BRI1 is a member of the largest subgroup of RLKs, those in which

the extracellular domain contains leucine-rich repeats (LRRs), which consist of a series of repeated motifs of 20–29 amino acid residues rich in leucines, thought to function in protein–protein interactions. As steroid molecules, BRs are unlikely targets for a LRR; in BRI1, however, the LRR is interrupted by a 70-amino acid ‘island domain’ that is likely the site of BR binding.

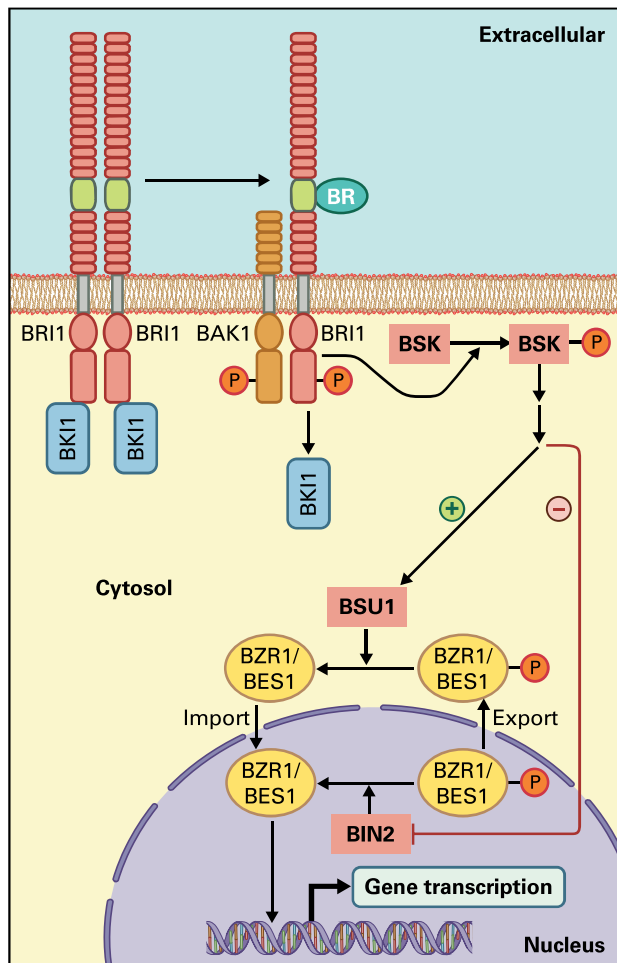


FIGURE 18.4 Brassinosteroids (BR) bind to the BRI1 receptor, causing dissociation of the inhibitor BKI1 and heterodimerization and reciprocal phosphorylation of BRI1 and BAK1. BRI1 phosphorylates BSKs, inducing signal transduction pathways that lead to accumulation of unphosphorylated forms of the BZR1 and BES1 transcription factors in the nucleus and consequent changes in gene transcription. Note that the cell wall is not shown for clarity.

BRI1 exists in the plasma membrane as a homodimer associated with the inhibitory protein BKI1 (BRI1 KINASE INHIBITOR1). BR binding induces the dissociation of BKI1 from the receptor, probably induced by phosphorylation of BKI1 by BRI1, and leads to the loss of BKI1 from the plasma membrane. Freed from BKI1 inhibition, BRI1 phosphorylates downstream targets. In addition, the BRI1 homodimer splits, and BRI1 forms heterodimers with another LRR-RLK called BAK1 (BRI1-ASSOCIATED KINASE1). Reciprocal phosphorylation within the heterodimer—of BAK1 by BRI1 and then of BRI1 by BAK1—subsequently enhances signaling from BRI1. BAK1 has a truncated extracellular domain containing only a short LRR domain and no island domain, and it does not appear to have a ligand-binding function.

The signal transduction pathway downstream of BRI1 is not fully known. In the current model, BRI1 phosphorylates and activates a small family of kinases (BSK1, BSK2, and BSK3) that induce downstream BR responses through their

own kinase activities. The targets of the BSKs are unknown; however, downstream responses are mediated through the regulation of a family of transcription factors that includes BZR1 and BES1. The activity of these transcription factors is downregulated through phosphorylation by the kinase BIN2 and upregulated through dephosphorylation by the phosphatase BSU1. Phosphorylation inhibits the activity of BZR1 and BES1 by multiple mechanisms, including the retention of the two transcription factors in the cytoplasm, an increase in their rate of degradation, and a reduction in their DNA binding ability. Following BR perception, BIN2 is inhibited and BSU1 is stimulated, leading to accumulation of unphosphorylated BZR1 and BES1 in the nucleus and regulation of BR-responsive genes (e.g., the inhibition of transcription from the *DWF4* gene).

The tomato (*Solanum lycopersicum*) homolog of BRI1 acts both as the BR receptor and as the receptor for the defense signal **systemin**, an 18 amino acid peptide produced by tomatoes and other Solanaceae in response to insect attack and that acts as a systemic signal capable of inducing defense-related genes. The ability of tomato BRI1 to bind two different signals raises the question of how specificity is maintained to differentiate responses to BRs and to systemin. One possibility is that systemin binding induces interaction with an LRR-RLK other than BAK1.

BRI1 is likely to signal from both the plasma membrane and endosomes (intracellular vesicles created by endocytosis; see Chapter 1). Both BRI1 and BAK1 are removed from the plasma membrane into endosomal membranes and then cycled back to the plasma membrane via transport vesicles budding off from recycling endosomes (Fig. 18.5). BRI1 cycling is constitutive and is not affected by either BR binding or the loss of BAK1 function. Treatment with brefeldin A, an inhibitor of endosomal trafficking, causes accumulation of BRI1 in endosomes and increases signaling through the BR pathway, suggesting a role for signaling from endosomal BRI1. The use of endosomes for signal transduction from plasma membrane receptors increases the membrane area available for signaling interactions. Furthermore, for typical elongated, differentiated plant cells in which the central vacuole occupies most of the cell volume, receptor endocytosis may help ensure even signaling strengths from different regions of the plasma membrane. As the resultant endosomes are circulated around the cell by cytoplasmic streaming, receptors activated at all parts of the plasma membrane are brought into the proximity of the nucleus.

Moving away from BR signaling, research on other functions of BAK1 suggests multiple interactions between pairs (at least) of plasma membrane RLKs. In addition to BRI1, BAK1 associates with the receptor for bacterial flagellin, an LRR-RLK called FLS2 (FLAGELLIN-SENSITIVE2). FLS2 induces a range of defense responses after flagellin binding. Similar to the case for BRI1, ligand binding to FLS2 induces association of FLS2 with BAK1, which promotes signaling by the receptor. Unlike BRI1, however, FLS2 is not constitutively cycled through endosomes. Instead, FLS2 endocytosis

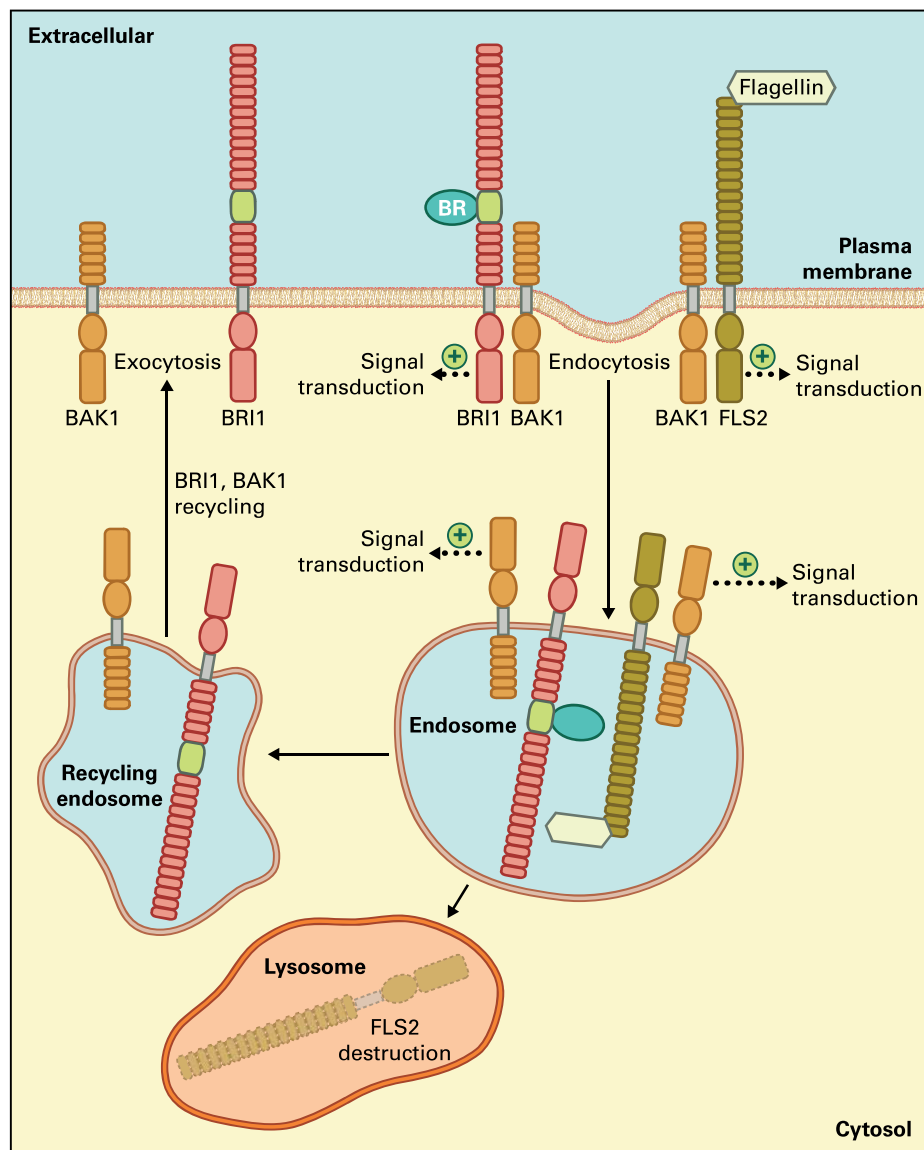


FIGURE 18.5 *BRI1* and *FLS2* receptors activated by binding to *BR* and flagellin, respectively, signal from both the plasma membrane and, after endocytosis, endosomal membranes. Following endocytosis of both the inactive (not shown) and activated forms of *BRI1*, endosomal *BRI1* is recycled to the plasma membrane. In contrast, only active *FLS2* undergoes endocytosis, and endosomal *FLS2* is targeted for destruction in lysosomes. Note that the cell wall is not shown for clarity.

is induced by flagellin binding, and although *FLS2* is also believed to signal from endosomes, the receptor is not recycled; instead endosomal *FLS2* is eventually destroyed (Fig. 18.5).

The functions of kinases closely related to *BAK1* demonstrate further levels of complexity (Fig. 18.6). *BAK1* is a member of a family of five LRR-RLKs called *SERKs* (SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASES). *BAK1* is *SERK3*. *SERK1* acts redundantly with *BAK1* as a co-receptor for *BRI1*. However, *SERK1*, together with *SERK2* also functions in the development of microspores in the anther, a process that does not appear to require *BAK1*. Lastly, mutational analysis shows that with *SERK4*, *BAK1* has a *BR*-independent role in controlling the cell death response. The functions of *SERK5* are unknown.

In addition to RLKs, plants possess histidine kinase receptors. These include the receptors for ethylene and cytokinin and constitute a separate group of kinases derived from bacterial two-component systems. Histidine kinase receptors are discussed in detail in Sections 18.4 and 18.5.

18.2.3 Plant cells possess a small group of putative G protein-coupled receptors

G protein-coupled receptors (GPCRs) regulate the activity of trimeric GTP-binding proteins (**G proteins**) (Fig. 18.7). These consist of $G\alpha$, $G\beta$, and $G\gamma$ subunits, and as trimers, they are inactive and bound at the $G\alpha$ subunit by a molecule

of GDP. GPCRs possess an extracellular ligand-binding domain, a transmembrane domain composed of seven hydrophobic helices, and an intracellular domain that interacts with inactive G protein trimers. Ligand binding by the receptor induces the $G\alpha$ subunit of the trimer to release its bound GDP molecule and bind a molecule of GTP instead. This activates the G protein, which uncouples from the receptor and dissociates into the $G\alpha$ subunit and a $G\beta/\gamma$ dimer.

$G\alpha$ and $G\gamma$ both have short, covalently attached lipid tails that anchor them to the inside of the plasma membrane so that $G\alpha$ and the $G\beta/\gamma$ dimer diffuse on the membrane to activate downstream signaling proteins. A single ligand-bound GPCR can amplify a signal by activating multiple G proteins. Signaling is terminated by the hydrolysis of bound GTP to GDP by $G\alpha$ (which has GTPase activity), and this process is stimulated by **GTPase activating proteins (GAPs)**. GDP-bound $G\alpha$ reassociates with the $G\beta/\gamma$ dimer to form an inactive trimer, which then binds a GPCR to complete the cycle.

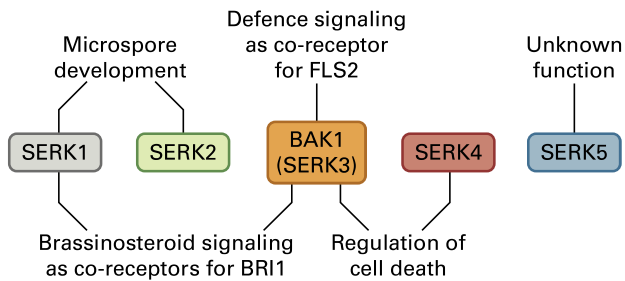
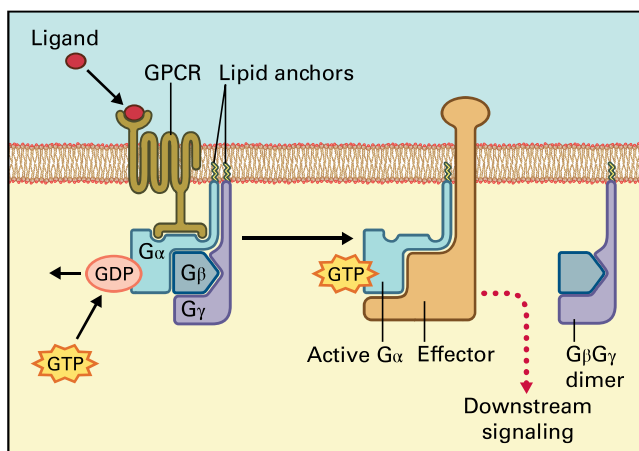
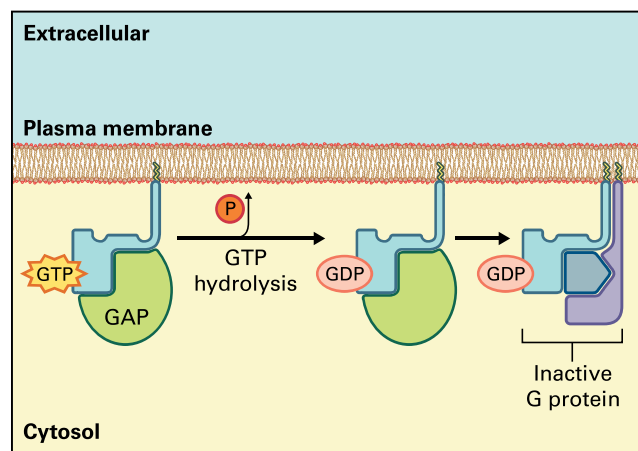


FIGURE 18.6 The SERK family of LRR-RLKs have multiple and overlapping functions.



A



B

FIGURE 18.7 Regulation of heterotrimeric G proteins. (A) Ligand binding by a G protein-coupled receptor (GPCR) induces the $G\alpha$ subunit of the heterotrimeric G protein to exchange GDP for GTP. This leads to the dissociation of the G protein into active $G\alpha$ and $G\beta/\gamma$ subunits that regulate the activity of downstream effector proteins ($G\beta/\gamma$ activity not shown). (B) $G\alpha$ is inactivated by the hydrolysis of bound GTP to GDP, which is stimulated by a GTPase activating protein (GAP). Inactive $G\alpha$ binds to the $G\beta/\gamma$ dimer, reconstituting the inactive heterotrimeric G protein. Note that the cell wall is not shown for clarity.

The alternation of $G\alpha$ between an active GTP-bound and an inactive GDP-bound form is a general feature of the GTP-binding protein superfamily. The superfamily includes GTP-binding initiation and elongation factors that act in protein synthesis, and also the **small or monomeric G proteins**, whose functions include signal transduction, organelar trafficking, and cytoskeleton assembly. The regulation of

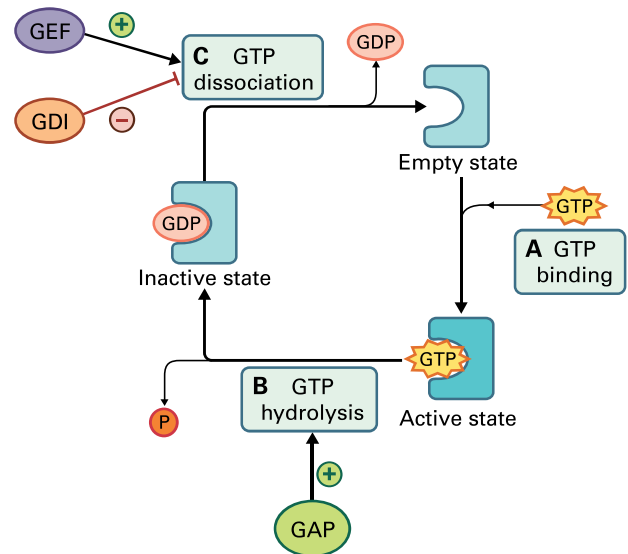


FIGURE 18.8 (A) Monomeric G proteins are converted from an empty state to an active state by GTP binding. (B) They are inactivated by GTP hydrolysis, which is stimulated by a GTPase activating protein (GAP). (C) GDP release, which is inhibited by GDP dissociation inhibitors (GDIs) and stimulated by guanine nucleotide exchange factors (GEFs), returns the G protein to its empty state and allows reactivation.

monomeric G proteins has parallels with the regulation of $G\alpha$ activity (Fig. 18.8). Monomeric G proteins are activated by **guanine nucleotide exchange factors** (GEFs), which induce the exchange of GDP for GTP, a function equivalent to that of GPCRs. As is the case for regulation of $G\alpha$ activity, the hydrolysis of GTP to GDP by monomeric G proteins is stimulated by GAPs. Lastly, **GDP dissociation inhibitors** (GDIs) prevent the spontaneous release of GDP by monomeric G proteins, a role played by the $G\beta/\gamma$ dimer in trimeric G proteins.

A sharp contrast exists between the extent of GPCR signaling in plants and animals. Mammals possess between 500 and 1,000 GPCRs, depending on the species, together with multiple copies of the G protein subunits and a wide range of G protein-regulated signal transducing proteins, including kinases, ion channels, and enzymes involved in the production or destruction of second messengers such as cyclic nucleotides. In contrast, analysis of the *Arabidopsis* genome has identified only a handful of putative GPCRs. Unequivocal roles for the function of these GPCRs are currently lacking. Furthermore, *Arabidopsis* possesses only one $G\alpha$ -, one $G\beta$ -, and two $G\gamma$ -encoding genes. The nature of the signaling proteins acting downstream of $G\alpha$ and the $G\beta/\gamma$ dimer in plants is uncertain.

18.2.4 Ion channel receptors can perceive mechanical stimuli

Ion channels are water-filled pores created by conformational changes in multisubunit, membrane-spanning proteins. They allow inorganic ions to flow across membranes, down their electrochemical gradients. Pore diameter and the charges on the amino acid residues that line the pore determine which ions are able to use a channel (see Chapter 3 for more details).

Ion channels can be regulated by binding either extracellular or intracellular ligands and by changes in voltage across the membrane or by mechanical force. In the plant plasma membrane, no identified ion channel proteins function as receptors for extracellular ligands; mechanosensing calcium ion channels, however, play a role in plant responses to physical forces.

Plants display developmental responses to gravity, touch, soil pressure, wind, and the stresses imposed by their own weight and tissue growth. Mechanical stimulation results in an immediate and transient increase in cytosolic calcium ion concentration. Though this suggests the existence of mechanosensing ion channels, the identity of such channels has yet to be confirmed. One candidate is the MID1 COMPLEMENTING ACTIVITY1 (MCA1) protein of *Arabidopsis*. MCA1 is believed to be a plasma membrane calcium ion channel that responds to mechanical stimulation, and the roots of *mca1* loss-of-function mutants are unable to penetrate harder agar growth media, implying an abnormal response to pressure.

18.3 Intracellular signal transduction, amplification, and integration via second messengers and MAPK cascades

18.3.1 Second messengers act as diffusible signals within the cell

Second messengers are diffusible, intracellular signals that are typically synthesized or released in a transient, but concentrated pulse following primary signal perception. They provide a means of rapidly relaying signals to downstream transduction components. Second messengers include small molecules and mineral ions that diffuse through the cytosol, as well as lipid-based signaling molecules that diffuse within membranes. Here, we consider two cytosolic second messengers: cyclic nucleotides and calcium ions (Ca^{2+}).

The term second messenger was first used to describe **cyclic 3',5'-adenosine monophosphate** (cAMP) after the discovery in the late 1950s that cAMP mediated signal transduction in response to a number of animal hormones. In the canonical pathway, hormone perception by an animal G protein-coupled receptor (see Section 18.2.3) leads to activation, via the G protein's $G\alpha$ subunit, of a plasma membrane **adenylyl cyclase**. This enzyme catalyzes the conversion of ATP to cAMP (Fig. 18.9), which in turn diffuses to activate cytosolic **cAMP-dependent protein kinase** (PKA). PKA phosphorylates downstream target proteins to activate appropriate transduction pathways. Animal cells also use **cGMP** as a second messenger; it is produced by **guanylyl cyclase** and activates **cGMP-dependent protein kinase** (PKG). In addition to activating PKA and PKG, cyclic nucleotides in animal cells act by opening plasma membrane **cyclic nucleotide-gated ion channels** (CNGCs), which allow nonselective influx of the Ca^{2+} , K^+ , and Na^+ cations. The cyclic nucleotide signal is terminated by the conversion of cAMP and cGMP into their noncyclic forms by phosphodiesterases.

Cyclic nucleotides have more restricted roles in plant cells than in animal cells. Importantly, no plant homologs of PKA or PKG have been found, and cyclic nucleotide signaling in plant cells may, therefore, be limited to the regulation of CNGCs. Like those found in animal cells, plant CNGCs are nonspecific cation channels. They have been implicated in various processes, including gibberellic acid (GA) and phytochrome signaling, pollen tube growth, cell cycle control, and stress and defense signaling. For example, pathogen infection induces rapid cAMP production and the influx of Ca^{2+} into plant cells, which is believed to occur through CNGCs. Spikes in cytosolic Ca^{2+} concentration are associated with a variety of defense responses, such as the expression of defense-related genes and the induction of programmed cell death.