

Plant hormones

OM9.1 SIGNAL PERCEPTION AND TRANSDUCTION

The ordered growth of a plant is completely dependent upon communication between its cells. Extracellular signals perceived at the plasma membrane by individual cells are essential for coordination of their programs of growth, development, and differentiation to produce tissues and organs. Extracellular signals adjust these programs in response to changes in the external environment such as fluctuations in light, temperature, water supply, nutrient availability, challenges from pests and pathogens, and so forth. The signals perceived at the plasma membrane of each cell must be transduced via signal transduction pathways into appropriate physiological, metabolic, developmental, and structural responses, usually involving adjustments in patterns of gene transcription that may involve many genes encoding proteins with diverse primary functions (**Figure OM9.1-1**). The perception of signals and their transduction into appropriate transcriptional responses requires a high degree of specificity. Hundreds of different signals – some transient and in tiny concentrations, others long-lasting and abundant – must be individually recognized by plasma membrane receptors, then each must be transduced via a unique signaling pathway to bring about a response specific to that signal.

Transduction of a signal is not a linear, stand-alone process. Networking of receptors and transduction pathways allows elements of core pathways and processes to be shared between signals, so that specificity of signaling is in part due to unique elements of a transduction pathway, and in part due to the precise path by which a given signal is propagated through a common signal transduction network. An overview of four features common to the perception and transduction of a multitude of different signals is presented here (see Figure OM9.1.1). Described first are the features of a class of plasma membrane sensors – called receptor-like kinases, receptor kinases, or RLKs

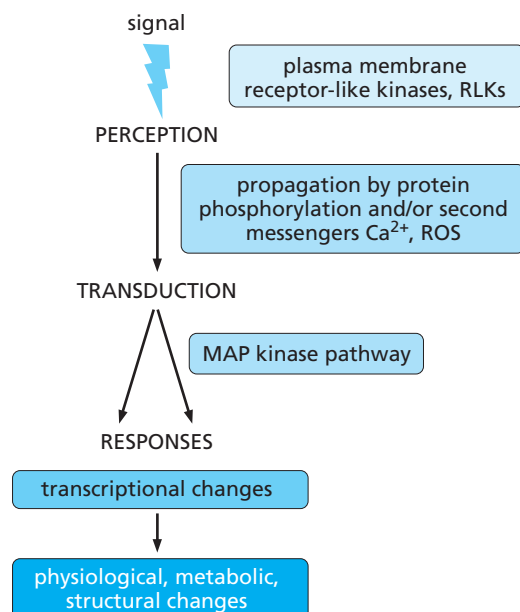


Figure OM9.1-1 Overview of signal perception and transduction.

- that perceive many different signals stemming from both growth and development processes and environmental factors. RLKs appear in many different roles throughout this book. Many other types of sensors exist and will be described elsewhere, but RLKs are the predominant sensors of extracellular signals. RLK genes are 2.5% of the genes in the Arabidopsis genome. Second, two, interlinked types of so-called **second messengers** are described: these are produced as an immediate response to signal perception at the plasma membrane and propagate the signal and transmit it to downstream elements of transduction pathways. These second messengers are the cation Ca^{2+} and reactive oxygen species (ROS). Finally, a major type of downstream signaling pathway is presented - the **mitogen-activated protein (MAP) kinase** (or MAPK) pathway - that modifies gene expression to bring about changes in the nature and function of the cell in response to the initial signal. Many other types of downstream signaling processes exist: the MAP kinase pathway is presented because it is common to many different signal transduction pathways and provides a general illustration of how receptors and second messengers can bring about cellular responses. It is important to note that while the description here relies heavily on research in Arabidopsis, analysis of signaling in other, diverse species (from plants to mammals) suggests that many basic components of signaling are shared and of ancient origin.

Plant receptor-like kinases

Plant receptor proteins constitute a large family of proteins involved in the perception of extracellular signals resulting in the activation of pathways that reprogram cellular development and/or responses to various environmental signals; they can activate signaling events directly or indirectly via other regulatory proteins. Many receptor proteins are located in the plasma membrane where they perceive extracellular signals, but others are in the cytoplasm or the nucleus, where they detect signals that are translocated to, or generated within, the cell.

The largest group of these receptor proteins is the RLKs that are located in the plasma membrane. They have an extracellular ligand-binding domain, a transmembrane domain, and a cytoplasmic protein kinase domain. Hundreds of these RLKs are encoded in plant genomes and their diverse extracellular domains enable them to detect and respond to a wide range of different signals. The roles of RLKs include perception of plant steroid hormones (brassinosteroids) and small signaling peptides that guide and control a diverse range of plant processes and environmental responses (for example the LURE peptides that guide the pollen tube into the ovule (see Section 11.3), the EFP peptides that control stomatal positioning on the leaf (see Section 10.4), and the CLE peptides involved in controlling nodulation of legume roots (see Section 16.2)). RLKs are also involved in responses to loss of cell turgor (see Section 7.2) and in the determination of **self-incompatibility** during pollination (see Section 11.3), and they play an essential role in the detection of pathogens and facilitating symbiotic interactions (see Chapters 15 and 16).

RLKs usually function in plasma membrane protein complexes that contain another RLK (**Figure OM9.1-2**). A related type of protein called a receptor-like protein (RLP), primarily implicated in plant responses to pathogens, can also function in complexes with one or more RLKs. Like RLKs, RLPs have an extracellular ligand-binding domain and a transmembrane domain, but unlike RLKs, they lack an active kinase domain. There are hundreds of RLK and RLP proteins encoded in the Arabidopsis genome and

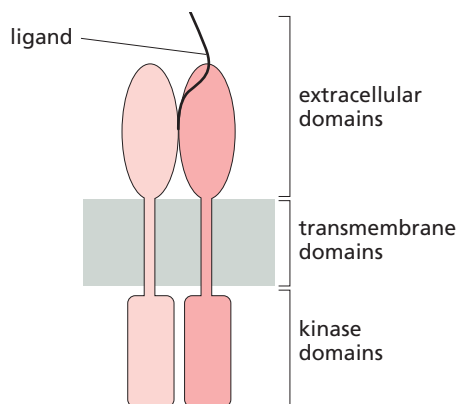


Figure OM 9.1-2 Plant receptor-like kinases. Receptor-like kinases (RLKs) have an extracellular domain, a transmembrane domain, and a kinase domain and, as illustrated, often form complexes with coreceptors of similar structure. They can also interact with receptor-like proteins (RLPs) that have related extracellular domains but lack a kinase domain (e.g. see Figure 15.12). As described in the main text, there are several different types of extracellular domain structures. Different complexes enable highly specific ligand binding, resulting in activation of the kinase domains. This can bring about direct activation of downstream regulators or their indirect activation, e.g. via an interacting receptor-like cytoplasmic kinase (RLCK), as illustrated in Figure 15.10.

their different extracellular sensing domains enable plants to detect and respond to a diverse array of different ligands including proteins, lipids, nucleotides, hormones, and glycans or polysaccharides.

The extracellular domains of RLKs and RLPs fall into broad groupings based on their types of extracellular domain structures. These include: (i) leucine-rich repeat (LRR) domains (discussed in Section 15.4); (ii) malectin-like glycan-binding domains (for example the FERONIA RLK involved in pollen tube reception during fertilization; see Section 11.3); (iii) various types of **lectin**-like domains (lectins are carbohydrate-binding proteins; some RLKs with lectin-like domains also recognize specific hydrophobic ligands); (iv) epidermal growth factor-like domains that monitor cell wall integrity through detection of ligands derived from, for example, pectin; and (v) LysM-like domains that detect chitin-like oligomers and function in defense (see Section 15.3) and signaling with endosymbionts (see Sections 16.2 and 16.3). As illustrated in Figure OM9.1-2, the RLKs and RLPs that come together to form complexes have similar extracellular domain structures. The resulting diverse arrays of complexes containing RLKs and/or RLPs enable differential recognition of many ligands, some very similar to each other in structure. In addition to playing roles in development, meristem maintenance, and environmental signaling, these complexes play major roles in plant defense.

RLKs are often associated with another class of protein kinases called **receptor-like cytoplasmic kinases (RLCKs)**, which are phosphorylated by activated RLKs, and go on to phosphorylate, and thus influence the activity of, downstream regulators that control different biological functions. RLCKs lack the extracellular and transmembrane domains that characterize RLKs but have similar kinase domains. Evolutionary selection and interactions with pathogens have resulted in the large repertoires of plant RLKs and RLCK gene families. RLCKs function in responses to pathogens, insect pests, symbionts, and nutrient deficiency, in embryonic patterning, and in plant reproduction.

Many RLKs and associated RLPs and RLCKs involved in plant defense (see Chapter 15) recognize **pathogen-associated molecular patterns (PAMPs)**, also called microbe-associated molecular patterns (MAMPs)). Examples include domains within bacterial proteins such as elongation factor Tu (Ef-Tu) and flagellin, chitin and chitin-related compounds, peptidoglycan, and plant cell wall fragments and peptides. Such recognition triggers **pattern-triggered immunity (PTI)**; described in Chapter 15). This differs from the defense response triggered by nucleotide-binding leucine-rich receptors (NLRs), which are cytoplasmically located and recognize specific pathogen effectors, leading to **effector-triggered immunity (ETI)**. In defense responses, RLKs, RLPs, and RLCKs work synergistically with NLRs to mount integrated responses to pathogens at multiple levels.

Calcium signaling

Ca²⁺ is the most ubiquitous secondary messenger in plants and promotes secondary signaling in a wide range of physiological, developmental, and stress-responsive pathways. In general terms, this role requires four sequential steps: (i) a low level of Ca²⁺ in the cytoplasm; (ii) regulated channels that can allow Ca²⁺ to flow into the cytoplasm; (iii) Ca²⁺-binding proteins that sense the Ca²⁺; and (iv) proteins that are subsequently targeted by these Ca²⁺-binding proteins. Many of these targets then go on to activate other proteins thereby influencing gene regulation, metabolism, stress responses, and development.

Pumps and ion exchangers maintain low levels of cytoplasmic Ca²⁺

The concentration of Ca²⁺ in the cytoplasm (and nucleoplasm) of resting (unstimulated) plant cells is maintained at about 100 nM or less, which is several orders of magnitude lower than the extracellular levels of Ca²⁺ or the levels of Ca²⁺ in the vacuole. This low level of cytoplasmic Ca²⁺ is crucial: high levels can be toxic due its tendency to precipitate inorganic phosphate and because many enzymes that are normally activated by transient increases in Ca²⁺ or Ca²⁺-binding proteins would be constitutively active. Low levels of cytoplasmic Ca²⁺ are maintained by active transport mechanisms that move Ca²⁺ out of the cell across the plasma membrane, into the vacuole across the tonoplast, and into the endoplasmic reticulum and the contiguous compartment bounded by the double nuclear membranes. Different Ca²⁺ ATPases (ACA) pump Ca²⁺ out of the cell, into the vacuole, and into the endoplasmic reticulum. Ca²⁺/H⁺ ion exchangers (CAX) pump Ca²⁺ into the vacuole (**Figure OM9.1-3**).

There are also Ca²⁺ **uniporters** (mitochondrial calcium uniporters (MCU)) that are energized by the membrane potential to pump Ca²⁺ into mitochondria and chloroplasts, but these uniporters have a lower affinity for Ca²⁺ than ACA and CAX.

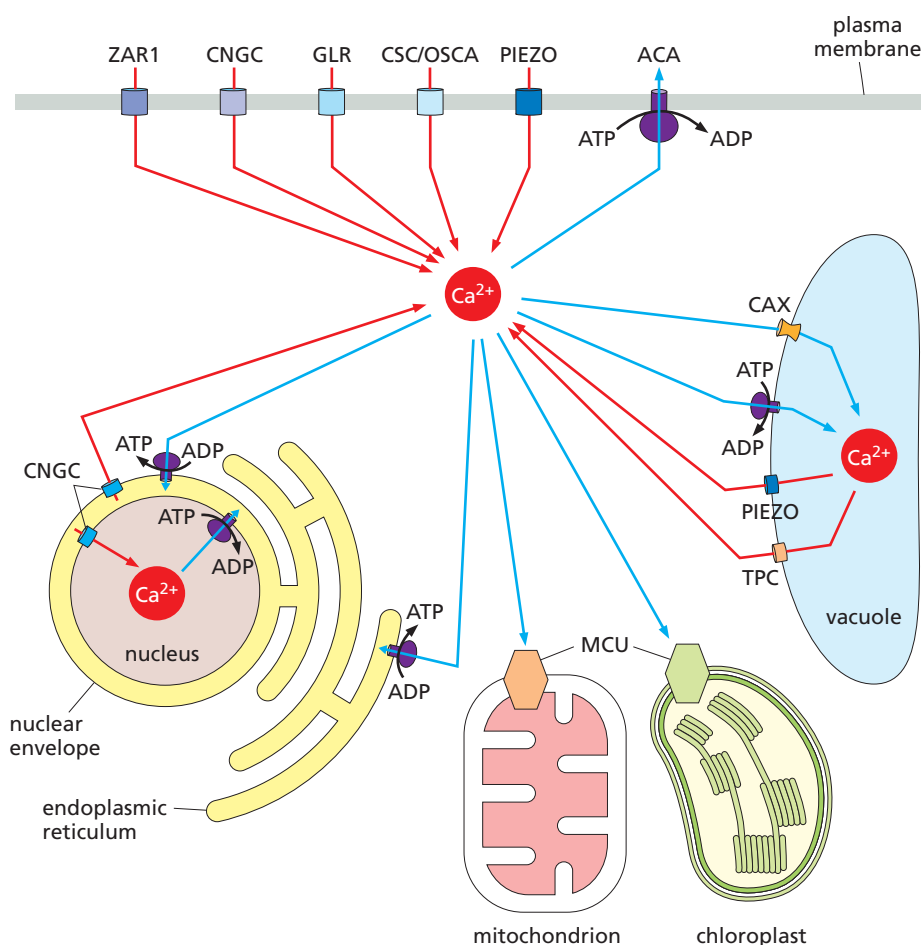


Figure OM 9.1-3 Calcium signaling pathways. Ca²⁺ is pumped out of the cytoplasm (blue arrows) by ATP-dependent Ca²⁺ pumps (ACAs) in the plasma, vacuolar, and endoplasmic reticulum membranes, and from the nucleoplasm and cytoplasm by a nuclear membrane-localized Ca²⁺ pump (MCA8). A Ca²⁺/H⁺ ion exchanger (CAX) pumps Ca²⁺ into the vacuole. Ca²⁺ uniporters (mitochondrial calcium uniporters, or MCU: uniporters transport a single type of molecule or ion without exchange for other substances) pump Ca²⁺ into mitochondria and chloroplasts. Ca²⁺ can flow down its ion gradient (red arrows) by opening of several gated ion channels in the plasma, vacuolar, and nuclear membranes. The plasma membrane channels include PIEZO mechanosensitive channels, Ca²⁺-permeable stress-gated cation channels (CSCs; also known as reduced hypo-osmolarity-induced Ca²⁺ channels (OSCs)), glutamate receptor-like channels (GLRs), cyclic nucleotide-gated channels (CNGCs); and ZAR1, a large hetero-oligomeric protein complex termed the ZAR1 resistosome that functions in defense against pathogens. There is also a CNGC in the nuclear membrane that acts in symbiosis. In the vacuolar membrane, a mechanosensitive PIEZO channel and a two-pore channel (TPC), which functions as a voltage-dependent cation-permeable channel, allow Ca²⁺ to flow from the vacuole into the cytoplasm.

Gating of ion channels allows rapid transient increases in cytoplasmic Ca²⁺

The net effect of the various Ca²⁺ pumps, ion exchangers, and sinks (apoplast, vacuole, and endoplasmic reticulum) is to poise the cells to allow a rapid Ca²⁺ influx into the cytoplasm or nucleoplasm by activation (opening) of ion channels through which Ca²⁺ can flow down a concentration gradient. Depending on which channels are activated, Ca²⁺ can flow into the cytoplasm from the apoplast, the vacuole, and the endoplasmic reticulum, or into the nucleoplasm from the compartment bounded by the double nuclear membranes. This means that there is the potential to have some degree of localization of the main increases in cytoplasmic Ca²⁺. The magnitude and duration of increases in cytoplasmic Ca²⁺ levels are determined by the gating (opening and subsequent closure) of the Ca²⁺ channels, the opening of additional ion channels to allow compensating charge balance, and the rate of pumping the Ca²⁺ out of the cell or into the intracellular stores to return the cytoplasm to the resting state level of Ca²⁺. The coordinated activity of the various Ca²⁺ pumps, channels, and other ion channels can produce distinct calcium signatures, such as a relatively slow increase in cytoplasmic Ca²⁺ or a rapid oscillation in Ca²⁺ in and around the nucleus. These different types of Ca²⁺ changes have the potential to differentially activate Ca²⁺-responsive binding proteins such as calmodulin, calmodulin-like proteins, kinases, transcription factors, and other ion channels.

Many different environmental changes can induce opening of the different types of Ca²⁺ channels. Examples include: (i) abiotic stresses such as heat or cold; (ii) water deficit, which can influence activity of channels that regulate stomatal opening and closing and/or water retention; (iii) biotic stresses such as PAMPs or MAMPs; (iv) perception of signals (such as nodulation or mycorrhizal fungal signals) from beneficial microbes; (v) perception of plant hormones such as ABA in stomata (see Online Material 8.2); (vi) mechanical stress induced for example by wind-induced movements or barriers encountered during root or shoot growth; and (vii) toxicity caused by extracellular

chemical stresses including toxins and various ions such as Na⁺. In addition to these environmentally imposed changes, the normal growth of cells requires changes in cytosolic Ca²⁺, for example in promoting cell division, pollen tube elongation, and apoptosis (programmed cell death).

Many channels contribute to the elevation of Ca²⁺ levels in response to internal signals during growth and development and/or external signals that emanate from environmental fluctuations. They fall into different families, namely: glutamate receptor-like (GLR) channels, cyclic nucleotide-gated channels (CNGC), Ca²⁺-permeable stress-gated cation channels (CSCs), PIEZO mechanosensitive channels, and complexes formed by activated NLRs in response to pathogen effectors (such as ZAR1 that forms a large pentameric complex, termed the ZAR1 resistosome, which functions as a plasma membrane Ca²⁺ channel: see Figure 15.17). Members of all these families of channels are found in the plasma membranes of at least some cell types. There is a specific CNGC in the nuclear membrane that releases Ca²⁺ from the store between the nuclear membranes, and in the vacuolar membrane there is a PIEZO channel and a two-pore voltage-gated vacuolar channel (TPC) that can activate Ca²⁺ efflux from the vacuole to the cytoplasm.

Ca²⁺-binding regulatory proteins and their targets

There are four major multigene families of Ca²⁺-binding proteins that function in signaling: (i) calmodulin (CaM); (ii) calmodulin-like proteins (CMLs); (iii) Ca²⁺-dependent protein kinases; and (iv) calcineurin B-like proteins (CBLs) that act together with CBL-interacting protein kinases. Members of these families of proteins modulate the activity of downstream target proteins by binding to them in the case of CaM or CML, or by phosphorylating them, and sometimes both. Typically, the downstream targets are transcription factors, protein kinases (including MAP kinases; see later), enzymes used in the production of ROS (described later), Ca²⁺ ATPases, and ion channels. Most of the members of the families of Ca²⁺-sensing proteins bind Ca²⁺ via motifs called EF hands – distinctive helix-loop-helix domains with high affinity for Ca²⁺ – but there are also other Ca²⁺-binding motifs.

- 1 **Calmodulins.** CaMs, encoded by a few genes (usually three to six) in plants, are small, highly conserved proteins of about 150 amino acids that have two pairs of EF hands and are relatively abundant in plant cells. Ca²⁺ binding to CaMs induces a conformational change that enables CaM-mediated activation of a diverse range of proteins involved in stress responses associated with light, gravity, and mechanical stress, heat shock, osmotic stress, low temperatures, and pathogens. Activated CaMs also play roles in phytohormonal signaling, metabolism, ion transport, cytoskeletal changes, protein folding, and transcriptional regulation.
- 2 **Calmodulin-like proteins.** CMLs are related to CaMs but are more diverse (with 50 genes in Arabidopsis) and more variable in sequence and size (80–330 amino acids, 1–6 EF hands). CMLs have been implicated in defense against pathogens, and in salt, drought, oxidative, and UV-light stress; they also play roles in development of pollen tubes, flowers, fruit, seed, roots, and root hairs.
- 3 **Calcium-dependent protein kinases.** These fall into three broad families depending upon whether they are activated by Ca²⁺ independently of CaM (calcium-dependent protein kinases, CDPKs), by Ca²⁺ bound to CaM (CaM-dependent kinases, CaMKs), or by both (calcium- and calmodulin-dependent kinases, CCaMKs).
 - **Calcium-dependent protein kinases.** Arabidopsis has 34 genes encoding CDPK proteins, all of which have a CaM-like domain with four EF hands. Ca²⁺ binding to these motifs brings about conformational changes that activate the kinase. When activated, different CDPKs are involved in: control of the cell cycle; pollen development; hormone signaling; light-regulated gene expression; gravitropism; thigmotropism (directional growth in response to touch); cold adaptation; tolerance to salinity and drought; and responses to pathogens.
 - **Calmodulin-dependent kinases.** Several distinct CaMKs have specific roles in the transition to flowering, formation of flower primordia, gravitropism, and responses to salt and oxidative stress. Some CaMKs directly affect the activity of transcription factors related to development and environmental stress, and some can also affect the activity of protein phosphatases, e.g. in the MAP kinase pathway (see later) involved in responses to pathogens, wounding, and salt stress.
 - **Calcium- and calmodulin-dependent kinases.** CCaMKs are nuclear localized proteins involved in signaling associated with plant symbioses with arbuscular mycorrhizal fungi and with nitrogen-fixing rhizobia (see Section

16.2). CCaMK has also been shown to be required for ABA signaling in rice leaves.

- 4 Calcineurin B-like proteins. Arabidopsis has at least ten genes encoding CBLs and at least 25 encoding CBL-interacting protein kinases (CIPKs). These were first identified in relation to responses to salt. They are involved in: maintaining ion homeostasis in plant cells by promotion of Na⁺ efflux by an Na⁺/H⁺ exchanger; the transport of Na⁺ into vacuoles; control of the balance between ammonia and nitrate uptake through phosphorylation of transporters (see Section 7.4); regulation of K⁺ uptake in roots; modulation of ion changes associated with stomatal movements and leaf transpiration; and tolerance of anoxia in germinating rice seeds (see Section 13.1).

Specificity in Ca²⁺-mediated signaling

In view of the huge diversity of Ca²⁺ signaling pathways described, an obvious question is how changes in Ca²⁺ levels can induce specific downstream responses. There are several potential explanations. One, mentioned earlier, is the specificity in the types of Ca²⁺ signatures produced by a specific stimulus, determined by the spectrum of ion channels and coordinated pumps activated by the stimulus. Another aspect is that different cell types may express only specific members of each family of Ca²⁺ channels. An additional level of specificity is conferred by cell type; there is cell-specific expression of proteins that respond to the Ca²⁺ signature and induce downstream responses. These may be enzymes that bind Ca²⁺ directly, or enzymes that bind it indirectly, e.g. by binding calmodulin after it has bound Ca²⁺. Additionally, a key aspect of signal specificity is that the Ca²⁺ signature associated with induced responses and developmental changes is only part of the signaling pathway. Often, additional inputs are required to ensure a full response.

Signaling mediated via reactive oxygen species

Reactive oxygen species

Reactive oxygen species (ROS) are derivatives of oxygen that are more reactive than molecular oxygen (O₂). They include singlet oxygen (¹O₂), the **superoxide** radical (O₂⁻), hydrogen peroxide (H₂O₂), and the hydroxy radical (OH[•]). ROS are unavoidable by-products of aerobic metabolism. About 1–2% of O₂ consumed in plants may form ROS, the main sources of which are chloroplasts (photosynthesis), mitochondria (respiration), peroxisomes (photorespiration), and the apoplast via oxidase reactions catalyzed by NADPH oxidases and other oxidases such as oxalate oxidase.

High ROS concentrations in the cytoplasm can cause oxidative damage to membranes (via lipid peroxidation), proteins, RNA, and DNA and can lead to cell death via a process called oxidative stress. Lower levels of ROS can affect the redox state of proteins, including enzymes, receptors, and small molecules, thereby influencing diverse stress-response signal transduction pathways. Levels of ROS are controlled in the plant via the activities of many ROS-detoxifying enzymes including superoxide dismutases, catalases, ascorbate peroxidases, glutathione peroxidases, and other peroxiredoxins, and by the production of antioxidants such as ascorbate and glutathione (see Section 7.5). To this end, plants have sensitive ROS detection systems that induce enzymes and antioxidants that control ROS levels.

Activation of ROS-mediated signaling

The focus here is on the functions of ROS as signal transduction molecules, or second messengers (**Figure OM9.1-4**). Plant cells maintain relatively low levels of ROS in the cytoplasm and have many ROS-sensitive regulators. They are thus able to detect quickly the increases in ROS levels induced by stimuli, and to restore normal basal levels rapidly via the activities of ROS-detoxifying enzymes and the production of antioxidants. There are clear parallels with the mode of action of the other major second messenger, Ca²⁺ (discussed earlier). Both ROS and Ca²⁺ increase rapidly in response to stimuli, and both are rapidly restored to a normal basal state when the stimulus ceases.

The elevation of ROS in response to imposition of environmental stimuli – abiotic and biotic stresses – is mainly due to activation of plasma-membrane **NADPH oxidases** (called respiratory burst oxidase homologs, **RBOHs**). RBOHs have been called the “engines of ROS signaling.” The activities of different RBOHs can be regulated in several ways, including binding of Ca²⁺ to EF hands in the amino terminal cytosolic domain, binding of phosphatidic acid, and numerous post-translational modifications including phosphorylation, ubiquitination, glutathionylation, and nitrosylation. Receptors that

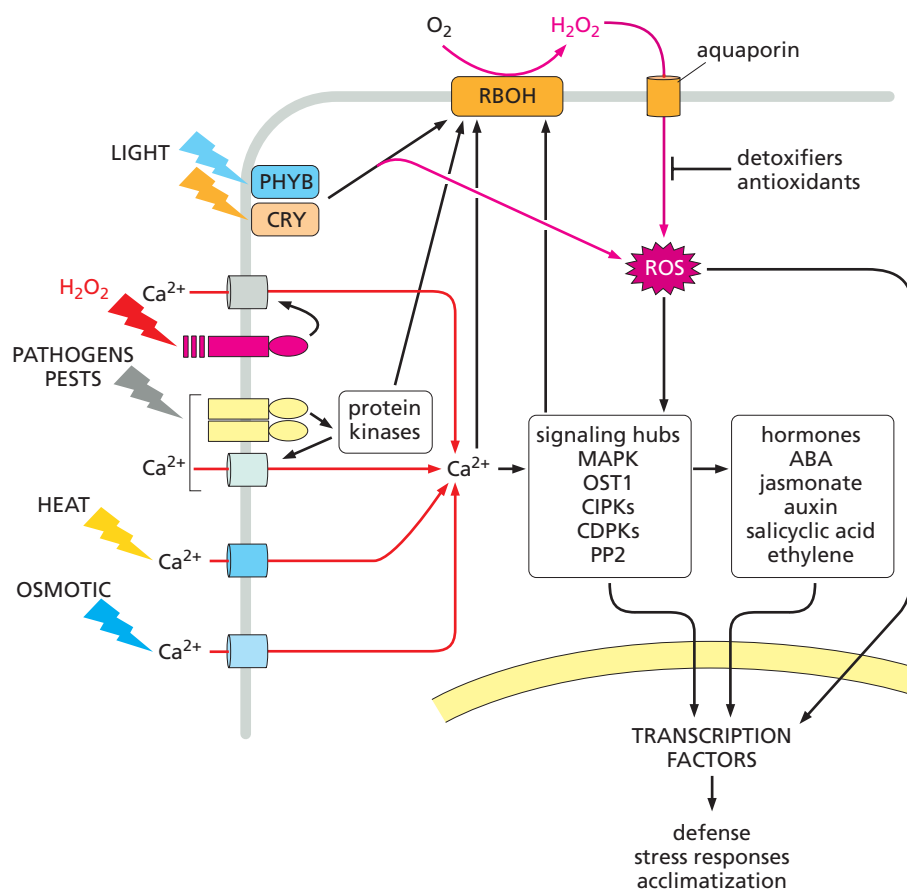


Figure OM9.1-4 Stress-induced activation of ROS signaling and its integration with other signaling networks. The diagram shows examples of how external stimuli generate complex, intersecting Ca²⁺ and ROS signaling networks that can bring about diverse and dynamic responses in the cell. The plasma membrane (gray border) and the nuclear envelope (yellow border) are represented as in Figure OM9.1-3. Pathogens, heat, osmotic stress, and H₂O₂ can induce the opening of diverse ion channels (blue/grey cylinders) that allow influx of Ca²⁺ into the cytoplasm (red arrows). Some ion channels are acted on directly by external stimuli (e.g. heat and osmotic stresses). In other cases, external stimuli act indirectly to open ion channels – for example the plasma membrane receptor HPAC1 (hydrogen peroxide-induced calcium increases 1; pink shape) is activated by H₂O₂, which oxidizes (cross-links) thiol groups in its extracellular LRR domain, and other RLKs (yellow shapes) are activated by detection in their extracellular domains of pathogen-associated molecules (PAMPs). In both cases the activation of external domains of these receptors activates their intracellular protein kinase domains that act to open ion channels. The elevation of cytoplasmic Ca²⁺ concentration brought about by ion channel opening in response to external stimuli directly and indirectly acts to activate plasma membrane NADPH oxidases (respiratory burst oxidase homologs; RBOHs, orange). RBOHs produce H₂O₂ from O₂ in the apoplast, which diffuses via aquaporins (orange cylinder) into the cytoplasm, elevating cytoplasmic ROS (pink star) concentration. Light-induced signaling via cryptochrome (CRY) and phytochrome (PHYB) can also activate RBOHs and ROS production. Pathways of ROS production are shown by pink arrows. Downstream Ca²⁺ and ROS signaling activates transcriptional changes that result in responses to external stimuli and stresses. Both signals converge on various intracellular protein kinases and protein phosphatases that constitute signaling hubs responsible for modulating expression of a host of transcription factors and also the concentrations of and sensitivity to various plant hormones. Proteins that constitute signaling hubs include components of the mitogen-activated protein kinase (MAPK) signaling cascades, OST1 (OPEN STOMATA 1), CIPK (calcineurin B-like-interacting protein kinase), CDPK (Ca²⁺-dependent protein kinase), and PP2 (protein phosphatase). Modulation of the activity of the signaling hubs by Ca²⁺ and ROS signals leads to dynamic changes in expression of many genes directly necessary for adaptation to external stimuli.

feed into RBOH-mediated ROS signaling via Ca^{2+} activation of RBOHs include various receptors for changes in light, osmotic pressure, and the presence of pathogens. Examples are: (i) plant pathogen elicitors (PAMPs), which can activate RBOH via Ca^{2+} -dependent and/or phosphorylation-dependent pathways; (ii) heat stress, which can activate CNGC (see earlier); (iii) osmolarity-induced changes that activate Ca^{2+} -permeable channels and RLKs; and (iv) changes in light quality and intensity. Reoxidation of the flavin cofactor of the blue-light sensor cryptochrome during light to dark transitions produces ROS, and high light activates RBOHs via signaling from the light sensor phytochrome B.

H_2O_2 produced as a result of RBOH activity is released into the apoplast, from where it rapidly diffuses into the cytoplasm via aquaporins. Increases in ROS levels are mostly detected by oxidation of a protein cysteine (-SH) thiol forming a disulfide link (S-S) with another cysteine thiol; this cross-linking induces conformational changes in proteins thereby changing their activity. The cysteine SH group can also be oxidized to sulfenic acid (-SOH) and methionine can be oxidized to methionine sulfoxide, both reactive forms of sulfur that can react with small molecules, other parts of the protein, or other proteins, again inducing conformational changes. Importantly for effective signaling, all of these changes are reversible when ROS levels fall.

Outputs of ROS-mediated signaling

The activities of many proteins are altered by ROS-induced conformational changes. ROS signaling is integrated with other signaling pathways because some of the proteins affected by ROS signaling are components of major signaling hubs (Figure OM9.1-4). These include components of: (i) the MAP kinase cascade (described later); (ii) the OST1 (or SnRK2.6) protein kinase that modulates ABA-induced stomatal closure, responses to cold stress, and flowering and root growth during drought (see later); (iii) Ca^{2+} -responsive protein kinases (see earlier) such as CIPKs and CDPKs; and (iv) protein phosphatase 2 (PP2). Many other proteins are also affected, including the LRR receptor kinase HPAC1 (hydrogen peroxide-induced calcium increases 1), which detects apoplastic H_2O_2 directly via oxidation of thiol groups in its LRR extracellular domain. HPAC1 is a ROS receptor that feeds into many aspects of responses to ROS (see later); this results in activation of Ca^{2+} channels in guard cells and is required for stomatal closure.

The signaling hubs can enhance the ROS response by increasing the activity of RBOHs, either by phosphorylation of RBOHs or by activating ion channels allowing Ca^{2+} influx into the cytoplasm. This increase in cytoplasmic Ca^{2+} can directly activate RBOH (see earlier) and can also change the activity of kinases and phosphatases in the signaling hubs. Some of these kinases can enhance RBOH activity by phosphorylation. Components of the signaling hubs can also be activated directly by interaction with ROS. These activated kinases can have diverse effects including direct activation of transcription factors; they can also influence production of plant hormones, leading to induction and activation of transcription factors. The activated transcription factors then induce expression of genes involved in defense, and responses to various environmental stresses.

Propagation of ROS signaling between cells

Reactive oxygen species such as H_2O_2 cannot diffuse over long distances because they are scavenged by detoxifying enzymes or react with antioxidants. However, following localized ROS production resulting from a biotic or abiotic stress at a particular location, a ROS wave can be propagated from cell to cell by a process called ROS-induced ROS production. ROS generated by RBOH activity in one cell can move to the cytoplasm of an adjacent cell via the apoplast and plasmodesmata. Elevation of ROS in the adjacent cell can trigger ROS production, an effect that is then propagated to further surrounding cells. Spread of ROS-induced ROS production in this way can, in effect, propagate ROS signals as a wave across many cells, resulting in elevation of ROS in tissues and organs remote from the original site at which the stress was perceived. This systemic production of ROS triggers ROS signaling that results in enhanced tolerance to various stresses in cells and organs not directly affected by the original stress. The phenomenon has been named “systemic acquired acclimation.”

The primary player in ROS-induced ROS production in response to local stress caused by high light, salt, or bacterial infection is the LRR receptor kinase HPAC1 (introduced earlier). This kinase acts as a ROS receptor that both mediates ROS-induced responses to stress at local sites at which stress occurs, and enables propagation of ROS signals that lead to systemic acquired acclimation. In brief, HPAC1 detection of ROS in the apoplast results in activation of the plasma membrane mechanosensitive channel MSL3, allowing entry of Ca^{2+} from the apoplast and elevation of cytoplasmic Ca^{2+} . Signaling mediated by the Ca^{2+} -binding regulatory proteins CBL4 and CIPK26 results in activation of the

protein kinase OST1 (also called SnRK2.6). OST1 phosphorylates and thus activates RBOHs in the plasma membrane, resulting in further H₂O₂ production. Stress defenses are activated in the cell, and the ROS signal is propagated to surrounding cells.

The rate of spread and eventual steady-state levels of ROS will be determined by the levels of ROS scavenging and presumably level of sensitivity of each cell to ROS. This sensitivity could be affected by the precise complement of ROS-sensing regulatory proteins, channels, and kinases in each cell. Although many ROS-induced signaling pathways are illustrated in Figure OM9.1-4, it is important to note that only some of the signaling components shown will be expressed in each cell type. Specificity in, and outputs of, ROS-mediated signaling in a given cell will depend on its complement of ROS-generating and ROS-sensitive proteins.

MAP kinase cascades

Mitogen-activated protein kinases are a family of proteins that transduce signals from extracellular stimuli into cellular responses. MAP kinase cascades are involved in normal growth and development and also in adaptive responses to environmental changes: MAP kinase signaling coordinates these two functions. In Arabidopsis, there are 20 different MAP kinases that can use ATP to phosphorylate transcription factors, protein kinases, enzymes, and structural proteins, modulating their activities.

Activation of MAP kinases is by ATP-dependent phosphorylation by a family of MAP kinase kinases (referred to as MAPKKs or MAP2Ks), of which there are ten in Arabidopsis. Modulation of MAPKK activity also requires ATP-dependent phosphorylation, by MAPKK kinases (referred to as MAPKKKs or MAP3Ks), of which there are about ten in Arabidopsis. This cascade of activation of MAP kinases can function via single members of each of the three groups of MAP kinases, but can also integrate different inputs via multiple MAP kinase components at each tier of the cascade. This multiplicity of inputs can incorporate redundancy or partially overlapping functions, enabling diverse and dynamic responses to multiple environmental changes. For sensitive responses to changing conditions it is important that MAP kinase activities can be switched off when the activating stimulus disappears: this is achieved by dephosphorylation by MAP kinase phosphatases.

The MAP kinase cascade (**Figure OM9.1-5**) usually starts with activation of MAPKKKs by phosphorylation. This can occur as the direct result of extracellular stimuli activating a variety of plasma membrane-located RLKs. Alternatively, some RLKs can phosphorylate cytoplasmic kinases (RLCKs), which can in turn phosphorylate MAPKKKs, or sometimes MAPKKs. Some RLCKs that activate the MAP kinase cascade can be activated by NLRs (nucleotide-binding leucine-rich receptor proteins).

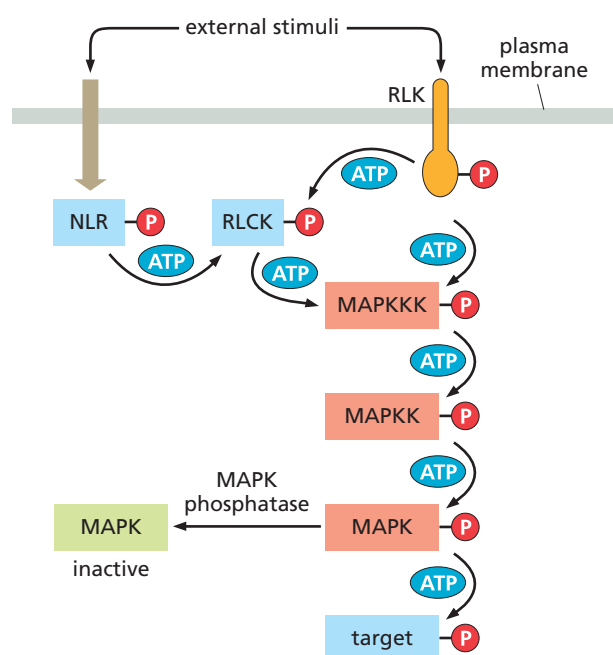


Figure OM9.1-5 MAP kinase signaling. Activation of plasma membrane receptor-like kinases (RLKs) by external stimuli can activate mitogen-activated protein kinase kinases (MAPKKKs) directly by phosphorylation. RLKs can also activate MAPKKKs indirectly via phosphorylation-dependent activation of receptor-like cytoplasmic kinases (RLCKs). External stimuli can also induce nucleotide-binding leucine-rich (NLR) receptor proteins to activate RLCKs that in turn activate MAPKKKs by phosphorylation. MAPKKKs phosphorylate and activate MAPKKs, which in turn phosphorylate MAP kinases. The resulting activated MAP kinases can then phosphorylate and activate target proteins such as transcriptional regulators, enzymes, and structural proteins. The activity of MAP kinases can be reversed by dephosphorylation via the activity of MAP kinase phosphatase.

A wide range of extracellular stimuli activate MAP kinase cascades. This is seen in the induction of defense responses by, for example, PAMPs and pathogen effectors (see Chapter 15). Abiotic stress responses via MAP kinase signaling can be induced by, for example, cold, salt stress, hypoxia, nutrient deficiency, and toxic heavy metals. Growth and development require MAP kinase pathways that promote cytokinesis, cell division, stomatal development, ovule and pollen production as well as pollen–pistil interactions, embryogenesis, abscission and cell separation, and root and root-hair development. The MAP kinase cascade regulates hormone production and promotes signaling via various hormones, wound-induced jasmonic acid production, abscisic acid, and salicylic acid.

Further reading

Plant receptor-like kinases

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Signaling mediated via reactive oxygen species

Fichman Y, Zandalinas SI, Peck S, Luan S, Mittler R (2022) HPCA1 is required for systemic reactive oxygen species and calcium cell-to-cell signaling and plant acclimation to stress. *Plant Cell* 34:4453–4471.

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MAP kinase cascades

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OM9.2 KARRIKINS ARE STRUCTURALLY RELATED TO STRIGOLACTONES AND PERCEIVED BY A RECEPTOR RELATED TO THE D14 STRIGOLACTONE RECEPTOR

Karrikins (KARs) were first identified as small molecules in smoke that stimulate germination of the dormant seeds of a few plant species. KARs have a similar chemical structure to strigolactone (SL). Accordingly, the KAR receptor (KARRIKIN INSENSITIVE 2 (KAI2) in Arabidopsis) is a protein closely related in amino acid sequence and structure to the SL receptor D14. Nevertheless, the ligand specificities of KAI2 and D14 are different, and D14 cannot perceive KARs.

As with SL signaling, KAR signaling requires the F-box protein MAX2. KAI2 contains structural domains that are highly similar to D14 and which mediate interaction with the MAX2. It is thought that D14-MAX2 and KAI2-MAX2 complexes, respectively, mediate SL and KAR signaling via the recruitment and ubiquitination of distinct members of the SMXL family. For example, while SMAX1 is involved in KAR signaling, SMXL6, 7, and 8 mediate SL signaling. However, there are also functional overlaps: while Arabidopsis SMXL2 mediates both (D14-dependent) SL and (KAI2-dependent) KAR signaling, rice SMAX1 suppresses arbuscular mycorrhizal symbiosis and negatively controls SL biosynthesis. Thus the SL and KAR signaling pathways are both highly similar to one another, and closely interactive. One significant difference between the two pathways, however, is that while D14 is degraded in a MAX2-dependent manner, KAI2 is not.

OM9.3 APICAL DOMINANCE IS IMPOSED BY A DOWNWARD FLOW OF AUXIN FROM THE SHOOT APEX

The critical importance of auxin flux down the stem in maintaining apical dominance was first demonstrated in 1933 by Kenneth V. Thimann and Folke Skoog, in experiments in which they explored the effects of removal of the shoot apex (decapitation) on shoot growth in the bean *Vicia faba*. Decapitation resulted in rapid outgrowth of axillary branches, suggesting that a substance (a “growth hormone”) produced by the apex normally prevented axillary bud outgrowth. This idea was proved correct in experiments in which excised buds were placed on blocks of agar to collect substances that exuded from them. Placing blocks of agar containing substances from apical buds onto the top of a decapitated plant restored apical dominance, whereas blocks of agar containing substances from axillary buds did not. The substance that promoted apical dominance was subsequently identified as auxin. Placing blocks of agar containing auxin onto the top of a decapitated plant restored apical dominance.

OM9.4 THE DISCOVERY AND FUNCTIONS OF DELLA PROTEINS

The *gai* mutant of *Arabidopsis thaliana* exhibits a dwarf phenotype resembling that of gibberellin (GA)-deficient mutants. However, unlike GA-deficient mutants, the dwarf phenotype of *gai* cannot be restored to normal by exogenous GA (i.e. the *gai* mutant is GA-insensitive). Molecular cloning of the gene (*GAI*) mutated in *gai* enabled the discovery of GAI, the founding member of what are now known collectively as the DELLA proteins (or DELLAs). The mutant *gai* gene contains an “in-frame” deletion and encodes a mutant *gai* protein that lacks a 17 amino acid “DELLA” domain, named (in the single-letter amino acid code) after the first five missing amino acids. Because it lacks the DELLA domain, the *gai* protein cannot interact with the GID1 (Gibberellin Insensitive Dwarf1) GA receptor, and thus is resistant to GA-mediated destruction (see main text), in turn causing the GA-insensitive dwarf phenotype of the *gai* mutant. As will be seen in Chapter 18, mutations affecting the DELLA domain, and hence impairing interaction with GID1, are characteristic of the semi-dwarf *Rht* wheat varieties of the Green Revolution. Intriguingly, while many plant species possess multiple DELLAs (e.g. there are five in *Arabidopsis*: GAI, RGA, RGL1, RGL2, and RGL3), others (e.g. the diploid cereals barley and rice) possess only a single DELLA. DELLAs are nuclear proteins, and are the GA-opposable growth inhibitors whose existence had been inferred in classic experiments in the 1950s, which showed that some mutations in pea (mutations now known to cause loss of DELLA function) could restore tall growth to GA-deficient peas, while not restoring the GA-deficiency itself. As detailed in the main text, the molecular function of DELLAs is to modulate the activity of a diverse range of gene transcription factors, thus inhibiting growth and influencing a wide range of plant biological processes.