Signal Perception and Transduction

23

Satish C Bhatla

While investigating the effect of unilateral light on the bending response of canary grass (*Phalaris canariensis*) seedlings, Charles Darwin (1881) observed that although light signal is perceived at the shoot tip, the bending of coleoptile due to differential growth occurs in the subapical region. This classic case of environmental signal perception and transduction resulting in growth response leads to the concept of signal transduction. It is now known that plant growth and development are modulated by a variety of environmental (external) and physiological (internal) signals. Some of the major signals (stimuli) to which plant cells are sensitive include light, mineral nutrients, organic metabolites, gravity, water status, soil quality, turgor, mechanical tensions, heat, cold, wind, freezing, growth hormones, pH, gases (CO_2, O_2, NO, C_2H_4), volatile compounds (e.g., jasmonates), electrical fluxes, wounding, and disease (Fig. 23.1). These signals can vary in quality and quantity over a period. Some signals penetrate across the plasma membrane, while others are carried over long transcellular distances through vessel elements (xylem) and sieve tubes (phloem). Plasmodesmata also facilitate symplastic migration of a number of signaling biomolecules. With the advent of molecular genetic studies on *Arabidopsis thaliana* in the current era of plant biology research, there has been a flood of information on signal perception and transduction mechanisms in plants. A variety of receptors for various plant hormones have been identified and characterized. Mutant analysis has facilitated the identification of many new signal transduction components which act downstream of receptors for various environmental and internal signals perceived by plants. Thus, an entirely new level of understanding of the complexities of signaling mechanisms in plants has been unfolded. New signaling molecules continue to be discovered, and sophisticated signaling mechanisms are being explained through the development of models which explain interactions between signaling pathways and modulation of various signaling networks (Fig. 23.2). The application of knowledge thus acquired on signaling

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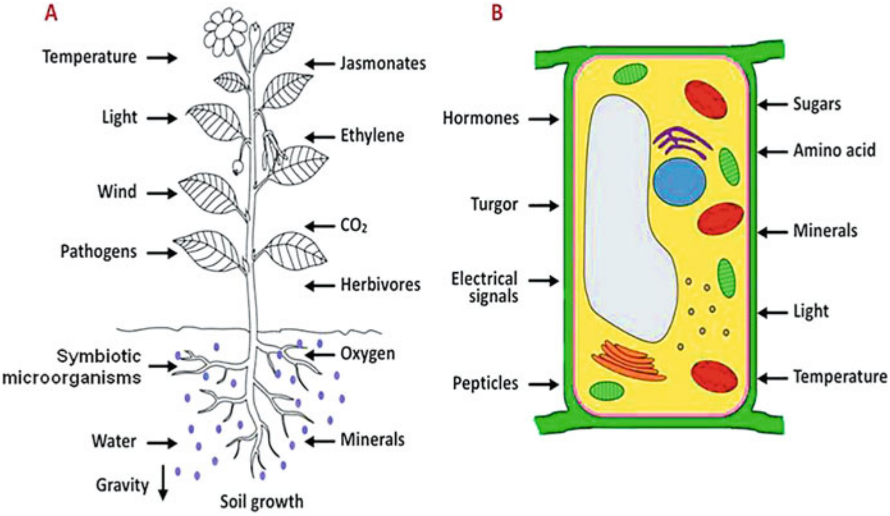


Fig. 23.1 External (**a**) and internal (**b**) signals which modify cell metabolism, defense, and development

mechanisms using model plant systems such as *Arabidopsis thaliana*, to agriculturally significant species, is likely to provide practical benefits in understanding plant responses to varied environmental stresses.

23.1 Routes of Signal Perception, Transduction, and Response in Plants

Any environmental or intracellular input, which initiates one or more responses in the cell/plant, is referred as a **signal**. Plant cells are sensitized to the signals (**perception**) by employing specialized sensor proteins, termed **receptors**. Receptors may be located on the plasma membrane, cytoplasm, endomembrane system, or in the nucleus. In quite a few cases, receptors can move from one cell compartment to another. Sensing of signal by the receptor is followed by the transfer of the information from the signal from one biochemical form to another (**transduction**) so as to amplify the impact of signal leading to a cellular response. Signal transduction is achieved by the receptor-mediated modifications of other proteins. The response to a signal is regulated by the nature of plant part experiencing the signal (root, leaf, meristematic tissue, etc.), the stage of tissue development (physiologically active cells or fully differentiated cells), the previous environmental sensing, and the circadian clocks. The response due to a signal can be biochemical, physiological, morphological, or developmental. Also, the responses due to more

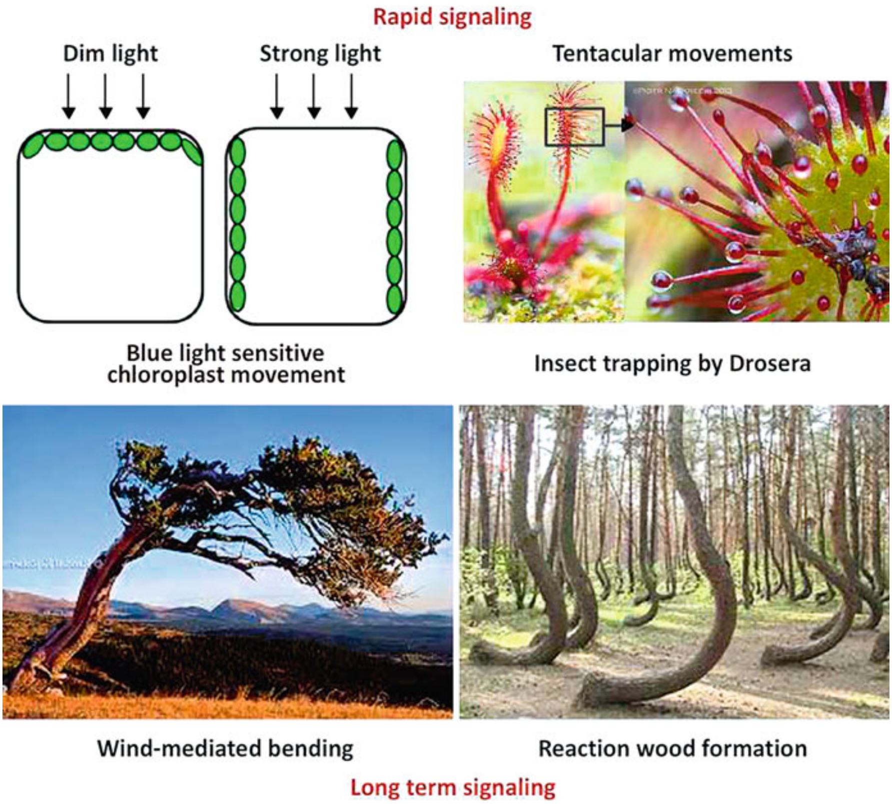


Fig. 23.2 Some examples of rapid and long-term signaling events in plants

than one signal can often integrate to determine the final response and multiple responses from any signal (Fig. 23.3).

Plants cells contain **genetic and epigenetic information systems**. Although phenotypic characteristics of plants are governed by the genetic system (DNA → RNA → Protein → Phenotype), quite a few phenotypic characteristics are strongly modified by the environment as well. This includes biomass production, duration of growth, branching, partitioning of photosynthate between reproductive and vegetative structures, and responses to stress. Such phenotypic characters are modulated by **epistatic genes**. The products of epistatic gene expression regulate the expression of other genes inherited independently. They (epistatic gene expression products) also regulate the expression of **pleiotropic genes** (single genes which influence multiple traits). Thus, epigenetic characters (heritable phenotypes resulting from changes in chromosomes without alterations in the DNA sequence) are resulting from a web of interacting gene products derived through different signal transduction networks regulated by plant environment.

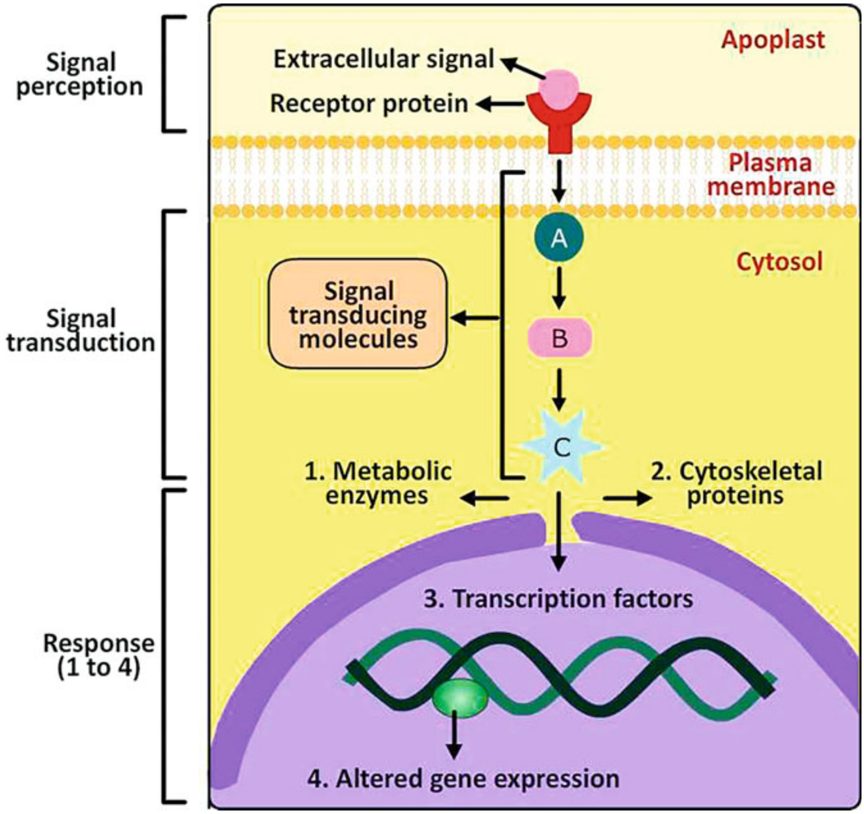


Fig. 23.3 Routes of signal perception, transduction, and response in plants

23.2 Spatial and Temporal Aspects of Signal Transduction (Table 23.1)

A number of signaling events in plants involve signal reception and response in the same cell. For example, opening of stomatal aperture involves activation of membrane-associated ion transporters in response to blue light, leading to swelling of guard cells via phototropin (blue light receptors). Such plant responses to environmental signals are called **cell autonomous responses**. Many other responses involve signal perception in one cell and response in distal cells, tissues, or organs. Such signal responses are called **non-cell autonomous responses**. Some examples of non-cell autonomous responses include:

1. Short-distance signaling response of radial patterning of *Arabidopsis* primary root is regulated by the **transcription regulator SHORT-ROOT (SHR)**. SHR gene is transcribed and translated in the cells of stele, and SHR protein moves to

Table 23.1 Spatial and temporal modulation of signal transduction events

Spatial modulation	Temporal modulation
I. Cell autonomous (intracellular) responses	I. Involving change in protein/enzyme activity (most rapid responses)
1. Stomatal opening and closing	1. Closing of leaf traps of Venus flytrap upon contact with insect
2. Chloroplast movement	2. Chloroplast movement in response to light
II. Non-cell autonomous (transcellular) responses	II. Rapid response involving electrochemical changes
1. Radial patterning in roots mediated by SHR transcription regulator	1. Stomatal opening
2. Gravitropic bending of roots modulated by differential auxin flow	
3. Floral induction through transmission of FT from leaves to shoot apex	
	III. Moderately fast responses involving gene expression modulation
	1. Insect attack on plants resulting in release of volatile compounds to attract insect predators
	IV. Long-term responses (involving chromatin remodeling)
	1. Vernalization
	2. Seed dormancy
	3. Modulation of root branching in response to nutrition availability
	4. Lateral bud outgrowth

endodermis via plasmodesmata where it activates the expression of cell plate regulator, such as SCARECROW (SCR). Thus, the transcription factor, SHR, can be considered as a short-distance signal transducer operating between adjacent cells and tissue (Fig. 23.4a).

2. **Auxins** can be considered as a short- to long-distance signaling molecules operating between adjacent cells, tissue, and organs. Thus, changes in the position of statoliths accompanying gravitropism result in rapid redistribution of PIN3 (auxin efflux protein) to create an auxin gradient at the root apex. Laterally located root cap cells facilitate rapid transport of auxin from columella cells to the zone of elongation by expressing uniform distribution of AUX1 (an auxin influx carrier) and asymmetric localization of PIN2 (auxin efflux carrier) (Fig. 23.4b).
3. At times, **transcription regulators** are able to act over long distances to control developmental programs. For example, during the transition from vegetative to reproductive phase (floral induction), a long-day inductive signal induces the expression of FLOWERING TIME (FT) gene in the companion cells of *Arabidopsis* leaves. The resulting FT protein is then transported to the shoot

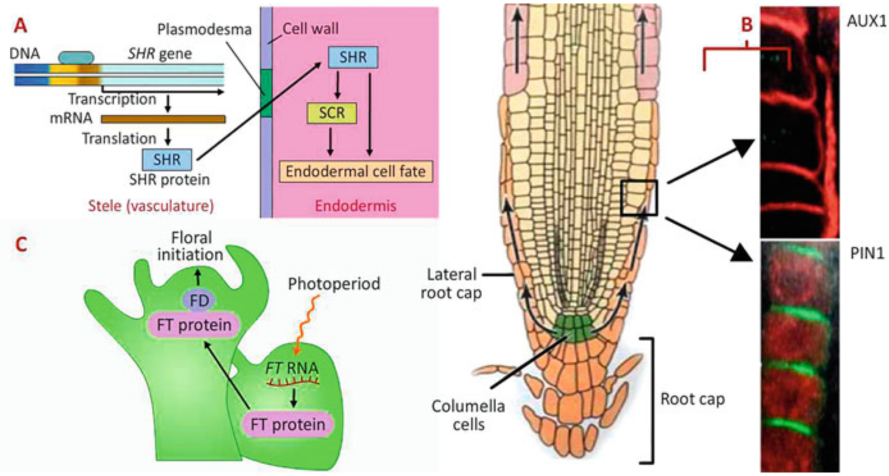


Fig. 23.4 (**a**) Short-distance signaling of SHR protein between stele and endodermal tissue regulates radial patterning in roots; (**b**) regulation of auxin transport by the modulation of AUX1 and PIN1 proteins in the gravity sensing cells of root; (**c**) long-distance transport of FT protein from leaf to shoot tip leading to floral initiation

apex to interact with another transcription factor, FLOWERING LOCUS D (FD). FT-FD protein interaction coactivates other targets genes, triggering flowering (Fig. 23.4c).

Signal transduction processes in plants operate over time scales ranging from few seconds to several days. Those involving just a **change in protein/enzyme activity** and **ion fluxes** are most rapid and take place in few seconds. For example, Venus flytrap (*Dionaea muscipula*) closes its modified leaf traps within seconds of touch by an insect. The same holds true for the folding of leaflets of *Mimosa pudica* upon being touched. Likewise, reorientation of chloroplasts is regulated by PHOTOTROPIN (PHOT) class of blue light photoreceptors located on the plasma membrane. Light-induced autophosphorylation of PHOT induces rapid changes in the cell's cytoskeleton, resulting in chloroplast reorientation within seconds. Quite a few rapid signaling responses involve **electrochemical changes** (e.g., stomatal opening). Signaling responses involving gene expression are moderately fast since plant cells take up to 30 min to transcribe a gene, process its mRNA, and then export mRNA to the cytoplasm. Additional time is further required for protein synthesis and intracellular trafficking. For example, plants attacked by insects are known to emit volatiles to attract insect predators within a few hours. Processes taking place in such a time scale often involve new transcription and translation activity. Long-term responses involving multiyear signaling mechanisms also affect plant development. They include vernalization in biennial species, flowering in trees, seed dormancy, modulation of root branching in response to nutrition availability, growth of sun or shade leaves, and activation of lateral bud outgrowth. **Chromatin remodeling** is often associated with such long-term signaling responses.

23.3 Signal Perception

Although some events of signal transduction in plants can be mediated by physical forces generated by tissue growth and also by electrical events at the membrane, most of the signaling events are mediated by specific molecules which result in a change in receptor protein activity (Box 23.1). Interactions between various signal-transducing molecules often result in covalent modifications and/or allosteric changes, ultimately leading to transfer of signal information from the site of perception to the site of response within the cell. Neighboring cells communicate with each other both through **apoplast** (formed by the interconnection of cell walls) and **symplast** (the cell-to-cell cytoplasmic continuum via plasmodesmata). Plasma membrane is the site for apoplastic sensing of environmental signals. Receptors located on the plasma membrane can perceive both physical signals (e.g., mechanical forces, such as touch and blue light) and chemical signals (such as hormones and signal peptides). Signaling via symplast permits regulated movement of RNAs and transcription factors, which induce response in the signal receiving cells. In addition to apoplastic and symplastic routes of signal perception, a third category of small lipophilic signaling molecules (e.g., ethylene, auxin) can cross the **plasma membrane** and is perceived in the cytoplasm or nucleus to evoke a response. Thus, ethylene is diffusible across the plasma membrane of plant cells and binds with its receptors located on the endoplasmic reticulum membrane. Likewise, auxins and gibberellins are perceived by the cytosolic (soluble) receptors which interact with intracellular components of protein degradation pathway to evoke a response.

Box 23.1: Contributions of Jagadis Chandra Bose (1858–1937) in Plant Electrophysiology

(continued)

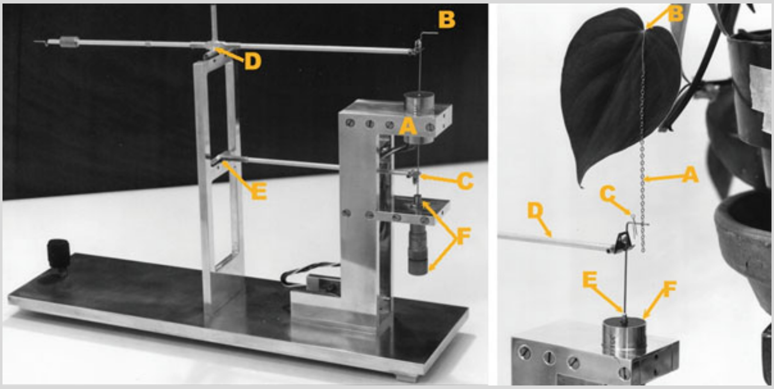
Box 23.1 (continued)

> *All creative scientists know that the*
> *true laboratory is the mind, where behind*
> *illusions they uncover the laws of truth.* J.C.BOSE

Jagadis Chandra Bose, who is generally acknowledged as the father of modern scientific research in India, began biophysical experiments in plants in 1897. The main focus of his investigations in plant physiology was to establish that all characteristics of responses to external factors exhibited by animal tissues are equally exhibited by plant tissues. He theorized that regular wave-like “pulsations” in cell electric potential and turgor pressure were an endogenous form of cell signaling. Bose selected plants which gave quick responses to external stimuli and displayed intrinsic rhythms. These include *Mimosa pudica*, which folds leaflets or dips the entire leaf upon stimulation. Likewise, Bose worked on the telegraph plant, *Desmodium*, which exhibits remarkable “spontaneous” gyration of the lateral leaflets as a result of rhythmic up and down movements. Bose devised an instrument called **crescograph** to measure mechanical responses, such as drooping of leaves at narrow time intervals (<1 to 2 s), in response to touch, heat, and chilling. He also experimented with the effects of changes in turgor pressure, sudden changes in temperature, and inhibitory poisons, such as KCN or anesthetics (e.g., chloroform and ether). Bose concluded that, like in animals, a stimulus was transmitted electrically to the motor organ, the pulvinus, both in *Desmodium* and *Mimosa*. The major conduction pathway (established with electric probe) was established to be phloem. Transmission of the response was strongly temperature-dependent and also influenced by light. He further observed that turgor decrease and cell contraction, accompanied by an electrical response, lead to leaf drooping in *Mimosa* and “downstroke” of *Desmodium* leaflet. Bose also used electric probes to measure periodic electrical “pulsations” in the inner cortical cells abutting endodermis of the herbaceous plant, *Impatiens*, *Chrysanthemum*, *Canna*, tomato, and potato. He reasoned that if all the inner cortical cells pulsed or contracted at the same moment, there would be no flow of water through xylem. Thus, there has to be a phase difference. J.C. Bose died in 1937, and in the current era of research in plant electrophysiology, his theories on electrical signaling and electromechanical oscillations in plant cells are being highly appreciated.

(continued)

Box 23.1 (continued)



Modern crescograph: The component which actually measures growth movement is a differential transformer (A). Its movable core is hinged between points (B) and (C). Two lever arms hinged at points (D), (B), (E), and (C) form a parallelogram that holds the core centered within the opening of the differential transformer as it moves up and down. A micrometer (F) is used to adjust and calibrate the system. Here a *Philodendron oxycardium* leaf is attached by a hook (b) and a gold chain to the plant movement detector. The lever arm (d) and transformer core (e) are held taut against the chain (a) by a 5 mg weight (c). A movement of the core (e) within the opening of the differential transformer (f) produces a change in the output voltage of the transformer which is proportional to the movement.

Selected publications of J.C. Bose:

1. Bose J.C. (1902) Response in Living and Non-Living. Longmans Green, London, UK.
2. Bose J.C. (1907) Comparative Electrophysiology. Longmans Green, London, UK.
3. Bose J.C. (1913) Researches on Irritability of Plants. Longmans Green, London, UK.
4. Bose J.C. (1918) Life Movements in Plants. B.R. Publishing Corp., Delhi, India.
5. Bose J.C. (1926) The Nervous Mechanism of Plants. Longmans Green & Co., London, UK.
6. Bose J.C. (1928) The Motor Mechanism of Plants. Longmans Green, London, UK.

23.3.1 Membrane Potential as a Receptor

Many signals have the ability to selectively discriminate migration of ions across the membrane, resulting in the establishment of a potential difference of −80 to −200 mV across the membrane. The plasma membrane potential, thus modified, activates voltage-gated ion channels which further activate a signal transduction sequence. Following the initial experiments conducted by Charles Darwin on Venus flytrap, action potential generation was first recorded in 1873 by Burdon Sanderson upon stimulation of *Dionaea* leaf. Subsequently in 1926, J.C. Bose isolated vascular bundles of a fern to show that excitation signal was transmitted as an electric disturbance that appeared to be operated by similar physiological processes as in animal nerves (Box 23.2). In 1930, action potential was recorded in *Nitella* cells using inserted microelectrodes (Fig. 23.5). Finally, in 1950s, Sibaoka was able to

Box 23.2: Sensitive Plants

A number of plants with compound leaves (e.g., *Mimosa pudica* and *Cassia fasciculata* from Leguminosae and *Biophytum sensitivum* from Oxalidaceae) are called as sensitive plants because they exhibit rapid folding of leaflets upon mechanical disturbance by touch or shaking. In nature, this behavior may protect leaves from being eaten by animals and also protect them from damage due to rain or wind. Such movements in the double pinnate leaves are regulated by motor cells in the pulvinules of individual leaflets (pinnules), in the pulvini at the juncture between pinnae and the rachis, and even in the pulvinus at the base of the petiole (the juncture with the stem). The motor cells in the lower (abaxial) sector of the pulvinus undergo immediate loss of water and turgor, and folding of leaves is completed within 1–2 s. Original configuration is recovered by the uptake of osmotically active solutes (K^+ and Cl^-) into the vacuole, followed by water uptake. The process of recovery is slow and is completed within 15 min. Recovery of pinnules and pinnae (but not of the base of petiole) takes place only in light, which is required to activate plasma membrane H^+-ATPase to enable K^+ and Cl^- influx into the cell. Sensors of mechanical perturbation (**motor cells**) are located in pulvinus, and their excitation spreads rapidly from one to the neighboring motor cell, probably through electric signals, leading to leaf folding. Such responses are called **seismonastic responses**. Insect-trapping movements of insectivorous plants are also driven by structural deformations caused by mechanical perturbation of specialized sensory organs, such as bristles (in *Dionaea muscipula*) or tentacles (in *Drosera tentaculata*).

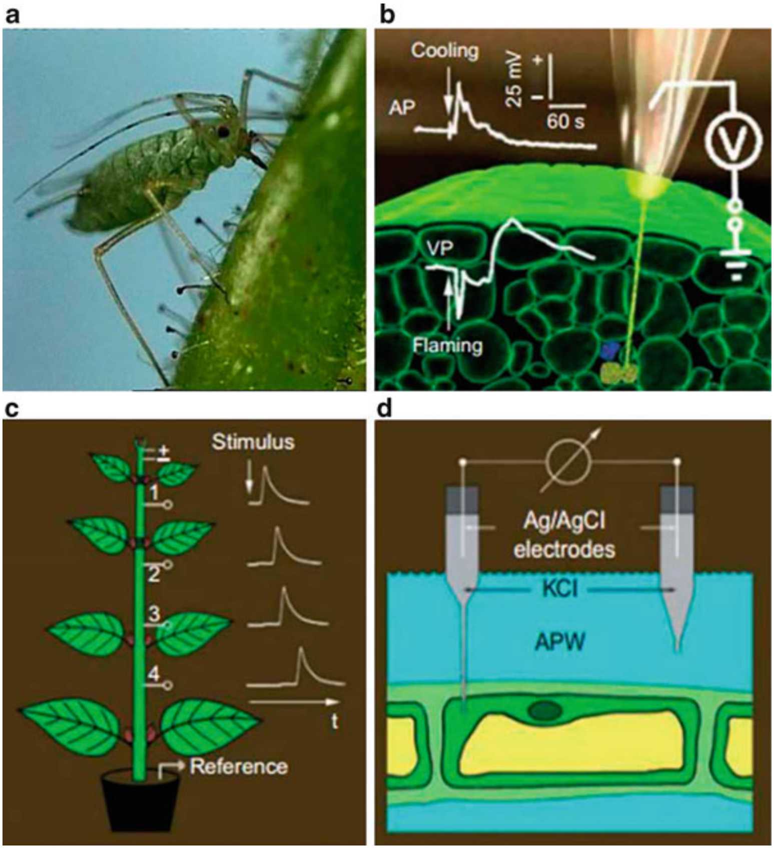


Fig. 23.5 Techniques for measuring electrical signals in plants. (**a** and **b**) Phloem potential measurement. (**a**) An aphid with its stylet inserted into a stump to obtain phloem exudate. (**b**) A tip of the microelectrode is attached to the stylet after separating it from aphid. (**c**) Extracellular recording of electrical signals with four channels and a reference electrode inserted in the soil ± electrical stimulation. An action potential generated by electrical stimulation appeared successively at electrodes 1–4. (**d**) Intracellular measurement of membrane potential with a microelectrode inserted into the cytoplasm of a cell while the reference electrode is in contact with bathing solution

demonstrate propagation of electrical signals in *Mimosa pudica*. Electrical signals not only trigger leaf movements in "sensitive" plants, such as *Mimosa pudica* or *Dionaea muscipula*, they also stimulate physiological processes in other plants through generation of action potential (Box 23.3). It is likely that plants have developed pathways for electrical signals leading to rapid response to external stimuli, such as environmental stress. In contrast to chemical signals, such as hormones, electrical signals are able to rapidly transmit information over long distances (Table 23.2). Touch-sensitized drooping of leaflets of *Mimosa pudica* is a classic example where membrane potential serves as a receptor (Fig. 23.6). Each leaf has a pulvinus at the base of its petiole. Many secondary pulvini are located at the bases of individual leaflets. When turgid, pulvini hold leaflets away from the stem and remain expanded. Loss of turgor in pulvini results in the folding of leaflets.

Box 23.3: Motor Cells and Tissues

Although plants are rooted in place, their specific regions in roots and in aerial parts are constantly in motion. Thus, some move their leaves or flowers and some twist their whole body. Likewise, roots grow deeper and penetrate soil to obtain nutrients. These movements are brought about by individual cells or a group of cells in a tissue through their contraction and expansion. Such cells/tissues are referred as **motor cells/tissues**. Single-cell motors are involved in movements of stomatal guard cells, root hairs, and pollen tube growth. Multi-cell motors are responsible for moving entire leaves or leaflets and other subterranean plant parts, apical buds, flowers or inflorescence, fruits, or entire shoots. Most movements are driven by turgor or hydrostatic pressure within the motor cells. When such turgor-driven movements occur in immature cells still capable of growth through cell wall and volume extension, they are called as **growth-mediated movements**. When these turgor-mediated movements occur in mature cells, which undergo reversible, elastic extension of cell wall, they are designated as **turgor-mediated movements**. Most plant movements are driven by multicellular motors such as pulvinus—a tissue found at the base of a leaf or petiole. As a rule, the distal part of multicellular motor is firmly attached to the base of the moving part, thereby allowing the motor to direct the movement of freely suspended portion. Most, but not all multicellular motors possess a central core of vascular tissue surrounded by multilayered cortical cells. Differences in growth are brought about by differential changes in cell volume in the opposite sectors of the motor tissue of the pulvinus. Solutes and water are lost from the contracting side and taken up by cells on the expanding side. The changes in volume of the pulvinar motor tissue, which result in change in curvature, are fully reversible. Cellulose microfibrils in the walls of motor cells are transversely oriented, thereby restricting volume changes in motor cells along the pulvinar axis. Furthermore, epidermis restricts radial expansion of the pulvinus. Veins in the pulvinus coalesce thereby allowing any change in the radius of curvature without changing length. Motor cells generate enough metabolic energy to execute these processes, as is evident from large nuclei, abundance of mitochondria, chloroplasts, and ER. Root hairs represent unicellular, growth-mediated motors, as are pollen tubes. On the other hand, stomata represent unicellular, turgor-mediated motors. Such motors bring about change in conformation (spatial architecture) of the cell.

Leaf stroking (touch) generates an action potential, i.e., membrane is depolarized to a voltage more positive than the threshold voltage. This results in massive movement of K^+ and Cl^- in the motor cells which causes loss of turgor in the pulvini and consequent folding of leaflets. It may be noted that the upper and lower halves of pulvinus exhibit differential changes in action potential, which facilitates leaflets folding and unfolding (Box 23.4).

Table 23.2 Some physiological effects of electrical signals in plants

Plant	Stimulus	Physiological effects	Signal
Mimosa pudica (Touch Me Not Plant)	Cold shock, mechanical (touch)	Leaf movement	Action potential
Drosera tentaculata (Sundews)	Mechanical	Movement of tentacles around insect	Action potential
Dionaea muscipula (Venus flytrap)	Mechanical	Release of digestive enzymes; trap closure	Action potential
Hibiscus sp. (Shoeblack plant)	Pollination	Increase in respiration	Action potential
Pisum sativum (Garden Pea)	Wounding	Inhibition of protein synthesis	Variation potential
Luffa sp. (Sponge gourd)	Electrical, cooling	Decreased elongation and growth of stem	Action potential

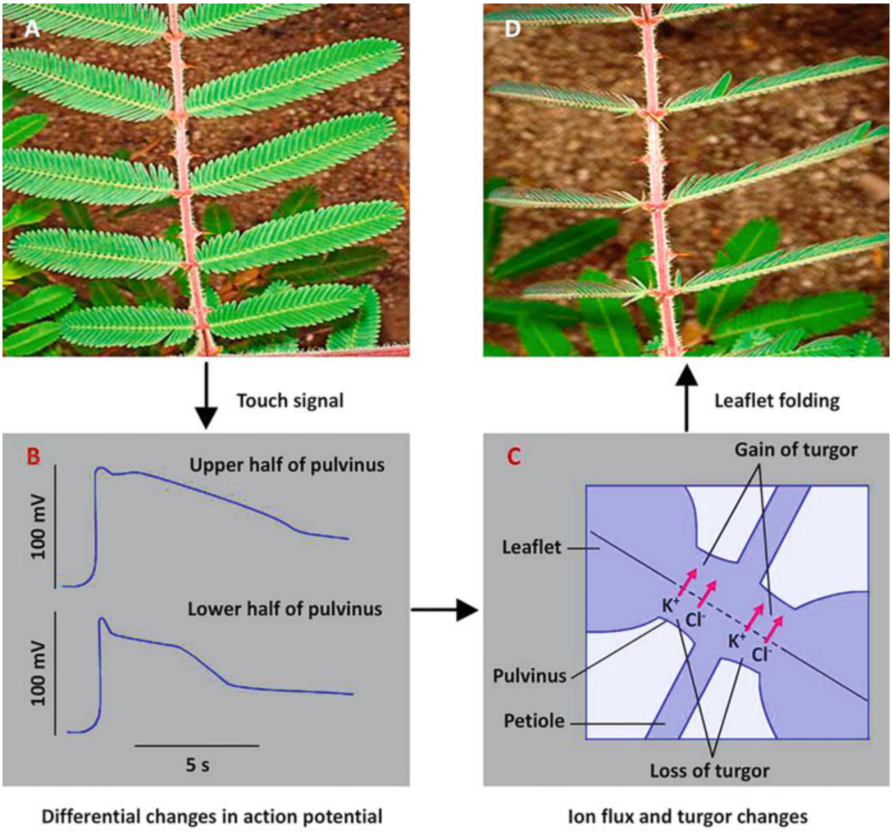


Fig. 23.6 Membrane potential as a receptor in *Mimosa pudica.* (**a**) Leaf stroking (touch) (**b**) generates an action potential (**c**) resulting in massive movement of K^+ and Cl^- in the motor cells in the pulvinus. (**d**) This causes loss of turgidity and consequent folding of leaflets

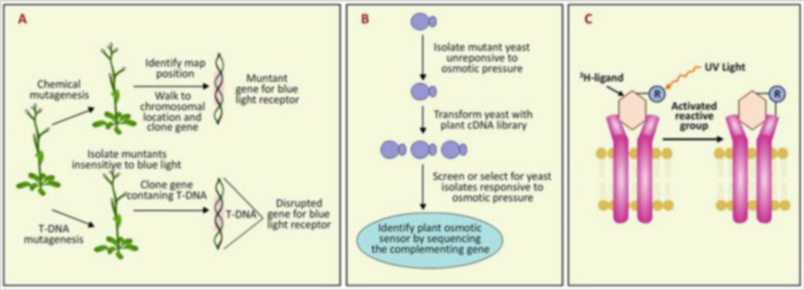
Box 23.4: Detection and Identification of Plant Receptor Proteins

As compared to enzymes and other proteins, the cellular concentrations of receptor proteins are much less. Therefore, their detection requires special techniques. Some of the approaches being used to detect plant receptor proteins are as follows:

1. **Functional complementation for gene identification:** In this approach, a plant gene restores a wild-type phenotype in a mutant strain of yeast. Complementation requires isolation of a yeast mutant which is deficient in the plant character under investigation. The yeast is transformed with a plant cDNA library, followed by isolation of a complemented yeast clone and sequencing of plant transgene. For example, osmotic pressure responses are shared by plants and yeast. Identification of osmosensing receptor is achieved by isolating yeast mutant unresponsive to osmotic pressure. Such a yeast mutant can be used to find plant cDNAs which might encode plant receptors responsible for signaling changes in osmotic pressure.
2. **Mutant analysis by molecular mapping and "chromosome walking":** Mutant populations obtained by chemical mutagenesis are used to identify desired phenotypes that are insensitive to the signal. Chromosomal regions are identified through selection and mapping. Chromosomal "walks" are used to identify the mutant sequence, which is then used to identify the wild-type sequence and the gene of interest. Genes can also be tagged with T-DNA from the bacterium (*Agrobacterium tumefaciens*) which causes crown gall disease. Transformation can be carried out in plants or seeds, and the desired phenotype is identified from the population of transformants. Known sequence of T-DNA as a tag can be used to identify the insertion site. Sequencing around the insertion site facilitates the identification of gene responsible for the phenotypic character. These methods have been used to identify receptors for blue light and ethylene.
3. **Photoaffinity labeling:** This technique uses isotopically labeled reagents that undergo bond rearrangement on exposure to UV radiation. 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. For example, ^{3}H-nitrene or ^{3}H-carbene (radioactive affinity labels) is attached to a ligand and mixed with receptor preparation to prevent binding, followed by exposure to UV radiations. Upon activation by UV radiation, the reactive nitrene or carbene (R), the labeled compound irreversibly binds to the receptor, which can then be purified and identified.

(continued)

Box 23.4 (continued)



23.3.2 Characteristic Features of Membrane Receptors

A number of plant cell membrane-localized receptors identified so far are proteins and possess following features:

1. High binding affinity for ligands.
2. Reversibility of ligand-receptor binding. This allows the system to respond to changes in ligand concentration since receptors are generally present in low abundance.
3. Saturation of ligand-receptor binding at certain concentration.
4. Selectivity of receptors for biologically active ligands.
5. Ligand-receptor binding mimics physiological activity.
6. Affinity constant (K_d) for receptor-ligand binding correlates with ligand concentration active in vivo.

23.3.3 Tissue Sensitivity for Receptor-Mediated Signaling Responses

Various tissues or cell types respond differently to the variety of signals they are exposed to. Thus, for example, fruit tissues become sensitive to ethylene after achieving a certain stage of ripening. In contrast, guard cells are totally insensitive to high concentrations of ethylene. Same signal can lead to different responses in different tissues through its ability to bind to different types of receptors. Thus, auxin can induce pericycle cells to form adventitious or lateral roots, but it promotes cell elongation in coleoptile cells. Both, different receptors and divergent downstream elements of signal transduction pathway, may be responsible for such variations in auxin (or any other hormone)-modulated responses in different cell types. It is also likely that different tissues may adapt or desensitize themselves to continuous signals and receptor concentrations during development. Thus, for example, when etiolated seedlings are exposed to red or white light, phytochrome concentration decreases rapidly through ubiquitin-mediated proteolysis of the phytochrome protein or its sequestration. As a result, the sensitivity of green tissue gets so modulated that it can still (in lowered phytochrome availability) sense light and affect development. Specific tissues also exhibit their unique dose-response relationship for ligands and receptors to induce physiological responses. For example, statocytes (the gravity-sensing cells in roots) contain much higher concentration of calmodulin than in the neighboring cells of the root meristem. Therefore, statocytes are likely to respond to much smaller increase in cytosolic calcium than other root cells do. Similarly, mechanical signals (e.g., wind, touch) can cause transient increase in cytosolic calcium concentration, calmodulin synthesis, and its accumulation in seedlings. This is how stimulation initiated by mechanical signals can render seedlings more sensitive to subsequent signals.

23.4 Signal Perception at the Plasma Membrane

Large and hydrophobic molecules, for which import channels do not exist on the plasma membrane, can only be perceived by plasma membrane receptors. Additionally, membranes also perceive physical signals, such as mechanical forces and blue light (via phototropins). Small lipophilic molecules can, at times, cross the plasma membrane and are perceived in the cytoplasm or nucleus. Thus, ethylene can cross plasma membrane and is perceived by receptors on the endoplasmic reticulum membrane. Likewise, auxins and gibberellins are perceived by soluble receptors. Phytochrome and cryptochrome receptors also have cytoplasmic and nuclear locations. Three major groups of plasma membrane receptors can be distinguished on the basis of how they interact with signaling components. These are receptor kinases, G-protein-coupled receptors, and ion channel receptors.

23.4.1 Receptor Kinases

They constitute the largest group of membrane receptors in plants and are responsible for transducing extracellular signals through phosphorylation of intracellular targets. One of the most common post-translational modifications used by cells to alter protein stability is through phosphorylation catalyzed by kinases. Kinases have the ability to alter protein stability, subcellular location, binding properties, enzyme activity, and their susceptibility to further modifications. It is also possible that a protein may be phosphorylated at more than one amino acid residue, involving more than one kind of kinases, thereby eliciting different effects. Thus, kinases and phosphatases (which dephosphorylate proteins) play crucial roles in signal transduction mechanisms. **Receptor-like kinases (RLKs)** constitute a family of over 600 proteins in *Arabidopsis* and over 1100 proteins in rice, which are known to phosphorylate target proteins in plant cells at serine and threonine residues. RLKs possess ligand-binding sites on an extracellular domain and on an intracellular kinase domain. RLKs are involved in a wide range of plant signaling processes,

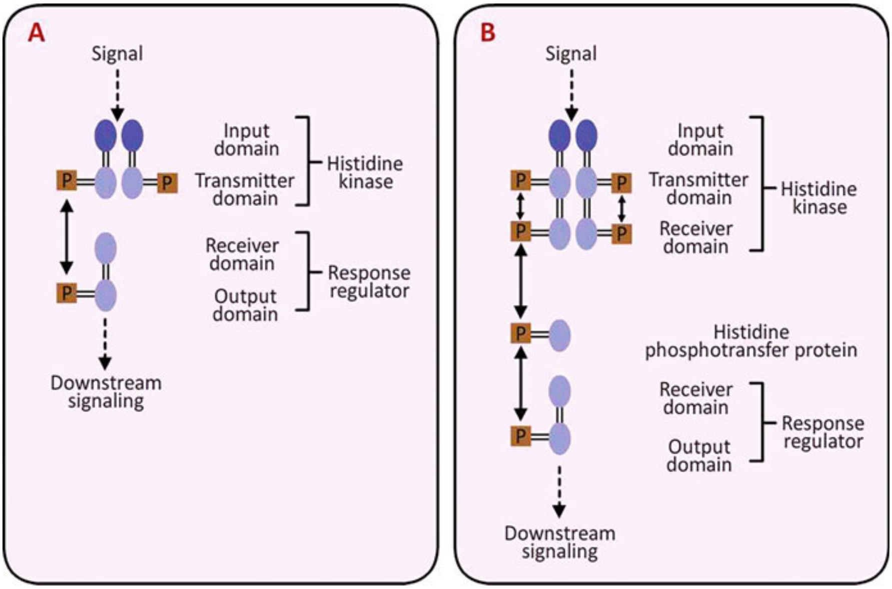


Fig. 23.7 Histidine kinase receptors. (**a**) The two-component system and (**b**) its multistep phosphorelay derivative

including hormone perception, defense, development, symbiosis, and pollen tube germination and guidance. In addition to RLKs, plants also possess **histidine kinase receptors** derived from bacterial two-component system. In addition to RLKs, plants possess **histidine kinase receptors** for ethylene and cytokinin signaling. These kinases are multistep derivatives of bacterial **two-component systems** which consist of two proteins—a **histidine kinase** and a **response regulator** (Fig. 23.7a)**.** The histidine kinase may be membrane spanning or soluble and exists as a dimer. Each kinase monomer has an input domain and a transmitter domain. The input domains of both monomers perceive signal and reciprocally autophosphorylate a histidine residue in each other's transmitter domain. The response regulator also contains a receiver domain and an output domain. Phosphoryl groups from the autophosphorylated histidine kinases's transmitter domain are transferred to an aspartate residue in the receiver domain of the response regulator, thereby activating the output domain. This triggers onward signaling events including gene transcription by activated response regulators which act as transcription factors. Prokaryotes, plants, and fungi (but not animals), in contrast with bacterial two-component system, **multistep phosphorelay** with three components: histidine kinase, histidine phosphotransfer protein, and response regulator (Fig. 23.7b). The histidine kinase often contains its own receiver domain into which phosphoryl group is transferred after autophosphorylation of its transmitter domain following signal perception. The phosphoryl group from the receiver domain histidine is transferred to an intermediate histidine phosphotransfer protein (HPt) and then to response regulator for further signaling. All phosphotransfers (both in basic and multistep systems) can occur in both the directions, ie., from histidine kinase to response regulator and vice versa. Therefore, signal transduction using these receptors depends on a balance of "forward" and "reverse" phosphotransfers.

23.4.2 G-Protein-Coupled Receptors (GPCRs)

GPCRs are membrane-associated proteins with an extracellular ligand-binding domain, a transmembrane domain (composed of seven hydrophobic helices), and an intracellular domain that interacts with inactive G-protein trimer. **G proteins** are GTP-binding proteins whose activity is regulated by GPCRs. G proteins consist of three subunits—Gα, Gβ, and Gγ.

Steps of GPCR-Mediated Signaling (Fig. 23.8):

1. Binding of an extracellular ligand to the GPCR-binding site induces release of GDP and its substitution by GTP. A single ligand bound GPCR can amplify a signal by activating multiple G proteins. Thus, GTP binding to G protein at the Gα subunit marks the first stage of signal transduction and activates G protein.
2. Activated G protein uncouples itself from the receptor (GPCR) and dissociates into Gα subunit and a Gβ/Gγ dimer.
3. Gα and Gγ possess short covalently attached lipid tails which anchor them to the inside of PM and activate downstream signaling proteins in the cytoplasm.
4. Active Gα (with GTP bound to it) is inactivated by the hydrolysis of bound GTP to GDP, which is stimulated by GTPase-activating protein (GAP).
5. Signaling is terminated by the hydrolysis of GTP to GDP by GTPase-activating proteins (GAPs). GDP-bound Gα rejoins with Gβ/Gγ dimer to form an inactive trimer which again binds to GPCR to complete the cycle. In contrast with the role played by GAPs in activating GTPase, guanine nucleotide exchange factors (GEFs) are known to activate GTPases by replacing GDP with GTP.

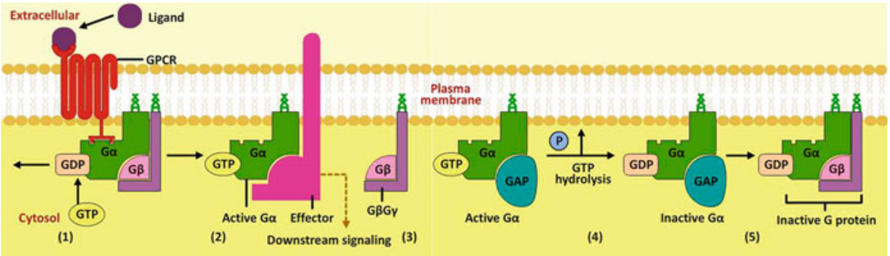


Fig. 23.8 Steps of G-protein-coupled receptor (GPCR)-mediated signaling. Gα, Gβ, and Gγ are three subunits of GTP; *GAP* GTPase-activating protein, *GDP* guanine diphosphate, *GEF* guanine nucleotide exchange factor, *GPCR* G-protein-coupled receptor, *GTP* guanine triphosphate

6. Monomeric G proteins are activated by guanine nucleotide exchange factors (GEFs) which induce conversion of GDP to GTP. The hydrolysis of GTP to GDP is stimulated by GTPase-activating proteins (GAPs). Lastly, GDP dissociation inhibitors (GDIs) prevent the spontaneous release of GDP by monomeric G proteins.

Tip growth and polar extension in plants are regulated by a family of small GTPases called ROPs (for Rho-like GTPase). ROP1 GTPases are localized on the PM at the tip of the growing pollen tube (Fig. 23.9). As with other GTPases, ROP1 activity can be switched on and off by GEFs and GAPs, respectively. PM-localized RLK is believed to activate GEF, which activates ROP1. ROP1 stimulates NADPH oxidase activity leading to ROS production. ROS, in turn, promotes Ca^{2+} influx from the extracellular space, which enhances tip growth.

23.4.3 Ion Channel-Linked Receptors

Ion channels allow inorganic ions to migrate across membranes according to their electrochemical gradients. Selectivity of ion channels is determined by the pore diameter and charges on the amino acid residues which line the channel passage way. Opening of ion channels can be regulated by their association with a ligand, change in voltage, or by mechanical force. So far, no plasma membrane-associated ion channels have been reported to function as receptor in response to ligand binding. However, PM-associated mechano-sensing calcium channels do play a role in plant responses to physical force. Plants exhibit developmental responses to touch, gravity, wind, and soil pressure, mediated by rapid and transient increase in

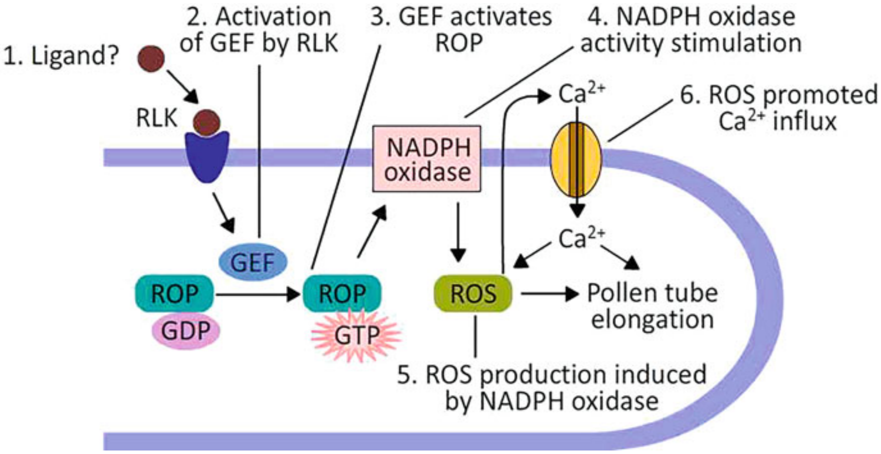


Fig. 23.9 Crosstalk between receptor-like kinases and ROP GTPases in the regulation of pollen tube growth. *GEF* guanine nucleotide exchange factor, *GTP* guanine triphosphate, *RLK* receptor-like kinase, *ROP* small GTPase, *ROS* reactive oxygen species

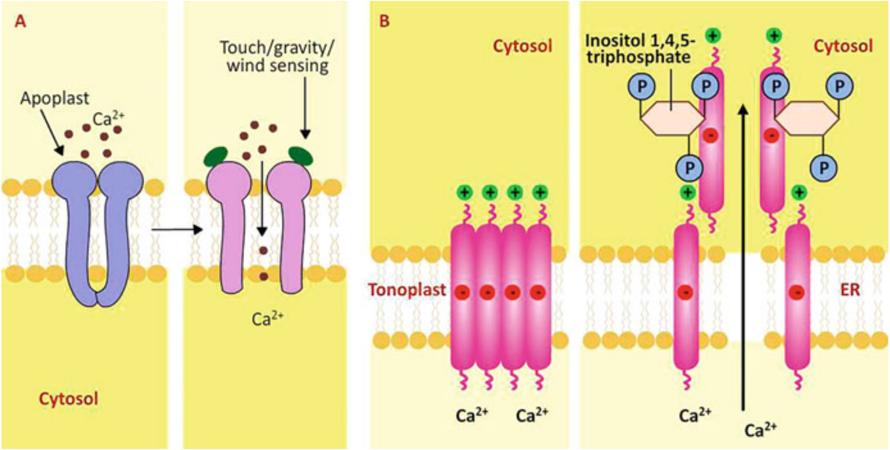


Fig. 23.10 Ion channel-linked receptors. (**a**) PM-associated Ca^{2+} channels open up as a result of mechanosensing (due to touch, wind, gravity, soil pressure, etc.). (**b**) IP_3 binding to the four-domain ion channel on the tonoplast leads to opening of Ca^{2+} channels due to conformation changes in the receptor, which are caused by stabilization of positive and negative charges

cytosolic calcium ion concentration indicating the existence of mechano-sensing ion channels. MID 1 COMPLIMENTING ACTIVITY1 (MCA1) is believed to be a PM-associated calcium channel in *Arabidopsis* which responds to mechanical stimulation in roots. Some ion channel receptors are also located on the internal membranes. The receptor for inositol 1,4,5-triphosphate (IP_3) is located on the tonoplast and the ER membranes. These receptors are composed of four subunits. IP_3 binding to the receptor leads to conformational changes resulting in opening of channel due to stabilization of positive and negative changes. This allows entry of Ca^{+2} into the cytosol from vacuole or ER lumen (Fig. 23.10).

23.5 Signal Transduction and Amplification via Second Messengers

Signal transduction, i.e., the process by which information is transferred from the site (s) of its perception by the receptors to the site(s) of response, involves a few signaling steps or an elaborate cascade of events using intermediary biomolecules called as **second messengers**. Second messengers are intracellular, diffusible small molecules and ions which are rapidly synthesized or released transiently as a concentrated pulse following signal perception by the receptors and modify the activity of target signaling proteins. Second messengers include biomolecules (ROS, phosphatidic acid, MAP kinases) and mineral ions (Ca^{+2}).

23.5.1 Significance of Second Messengers in Signal Transduction

The length of signal transduction pathway is determined to some extent by the location of receptor where signal is perceived. Perception of signals by the PM often involves subsequent signal transduction via many intermediates. It is well known that in the event of low abundance of receptors, generation of second messengers helps in further amplifying an otherwise low signal before it reaches nucleus to modulate gene expression or to any other site of response. In the absence of second messengers, the signal is likely to get diluted due to diffusion and deactivation (e.g., by dephosphorylation, degradation, or sequestration). In order to further elevate weak initial signals, cells employ amplification mechanisms such as phosphorylation cascade and second messengers. Major categories of second messengers operating in plant cells are:

1. Ca^+
2. Cytosolic or cell wall pH
3. ROS
4. Cyclic nucleotides
5. MAP kinases
6. Lipid-signaling molecules

23.5.2 Ca^{+2}: The Most Ubiquitous Second Messenger

Ca^{+2}, a divalent cation, is involved in a vast variety of signaling pathways, including pathogen infection, modulation, touch, wind, changes in gravity vector, anaerobic conditions, drought and salt stress, and responses to GA, ABA, and red and blue light. Plant cells maintain a very low resting cytosolic Ca^{+2} concentration (100–350 nM) but maintain large Ca^{+2} reserves sequestered in vacuoles and endoplasmic reticulum (1–2 mM). Plant cell wall also contains relatively high calcium concentration (0.5–1 mM). Any of the above-stated biological factors can result in spikes (rapid rise and fall) of Ca^{+2} in the cytosol (up to 1 μM), which triggers subsequent signaling action. Cytosolic Ca^{+2} increase is tightly regulated by the opening and closing of Ca^{+2} channels localized on PM, ER, and tonoplast. Plasma membrane is known to possess Ca^{+2} channels **called glutamate-like receptors (GLRs) and cyclic nucleotide-gated channels (CNGCs)**. Calcium channel opening is also regulated by changes in transmembrane electric potential, membrane tension, or binding of ligands. Intracellular spikes of cytosolic Ca^{+2} brought about by the activation of calcium channels through various activation routes result in the regulation of downstream signaling through **Ca^{+2}-sensor proteins** as the intermediates. Four major categories of Ca^{+2}-sensor proteins are calmodulin (CaM) and calmodulin-like proteins, calcium-dependent protein kinases (CDPKs), Ca^{+2}/calmodulin-dependent protein kinases (CCaMKs), and calcineurin B-like proteins (CBLs). These target proteins, upon activation by Ca^{+2}, modulate the activity of target proteins. **Target proteins** include transcription factors, various

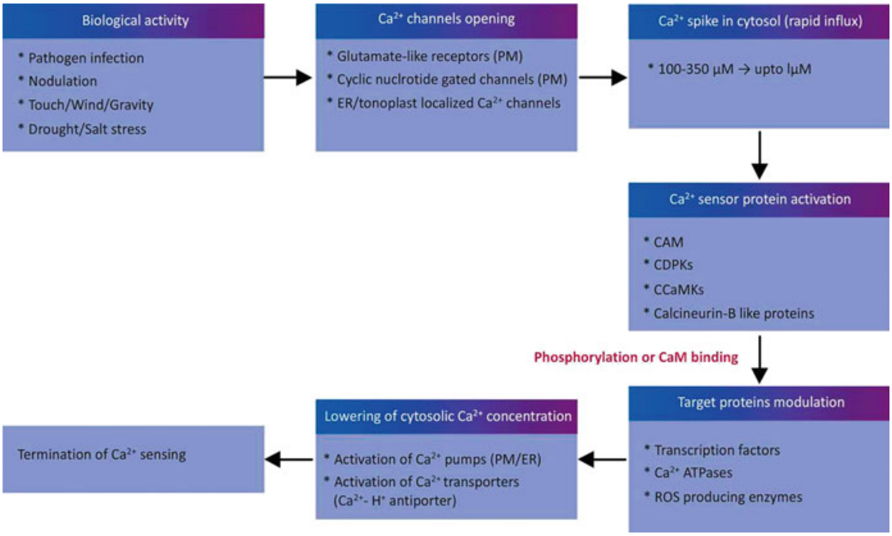


Fig. 23.11 Sequence of signal transduction events employing Ca^{2+} as a second messenger

protein kinases, Ca^{+2}-ATPases, ROS-producing enzymes, and ion channels. Soon after eliciting the response, excess Ca^{+2} is removed from the cytosol by Ca^{+2} pumps and Ca^{+2} exchangers on PM or ER/tonoplast to terminate Ca^{+2} signaling.

How does Ca^{+2} signaling link each stimulus to its appropriate response? A number of stimuli lead to $[Ca^{+2}]_{cyt}$ spikes, and a large number of Ca^{+2} and a large number of Ca^{+2}- and/or CaM-regulated kinases activate downstream pathways (Fig. 23.11). Plant cells achieve linking of these Ca^{+2} spikes with specific response through one of the following means:

1. Gradation of intracellular Ca^{+2} gradients, e.g., in growing pollen tubes and root hairs. Spatial segregation of $[Ca^{+2}]_{cyt}$ changes leads to varied subsequent biochemical events, leading to tip growth.
2. Temporal changes in $[Ca^{+2}]_{cyt.}$, e.g., oscillation of $[Ca^{+2}]_{cyt}$ in guard cells during stomatal opening.
3. Localization of different components of Ca^{+2} signal transduction machinery in different cells at a given time.

23.5.3 Modulation of Cytosolic or Cell Wall pH as a Second Messenger

One of the major roles of electrochemical proton gradient (i.e., proton motive force) across the cell membrane is to drive ATP synthesis and accelerate secondary active transport. Cytosolic pH is generally maintained at 7.5 in contrast to cell wall pH of

Table 23.3 Possible routes of pH modulation as a second messenger in plant cell signaling event

Cell wall	Cytosol
H^+-ATPase phosphorylation leading to extrusion of H^+ in the apoplast	Presence of acidic and basic amino acids on proteins
1. Expansion activation leading to cell wall loosening	1. pH-sensitized activity modulation
2. Alteration of protonation status of weakly acidic hormones (e.g., indole-3-acetic acid) leading to their altered permeability across PM	2. Effect on protein interaction with other proteins, substrates, or ligands
3. pH-sensitive gating of K^+ channels and aquaporins	

about 5.5 or less. Protons exert their signaling role both in the cell wall and cytosol. Activation of H^+-ATPase activity through its phosphorylation in the PM triggers extrusion of hydrogen ions into the apoplast where it triggers expansions, leading to breaking of hydrogen bonds between closely placed cellulose microfibrils and causing subsequent cell expansion due to turgor pressure. This is commonly observed in hypocotyls. Altered pH of the cell wall is also likely to affect protonation status of weakly acidic plant hormones, thereby affecting their ability to enter cells by diffusion. Gating of potassium channels and aquaporins has also been observed to be affected by pH. Intracellularly, presence of acidic and basic amino acids makes all proteins sensitive to pH changes in the cytosol, thereby altering their activity/function. The pKa value (dissociation constant) of these amino acids and the impact of their protonation are likely to affect the ability of the concerned proteins to interact with other proteins, substrates, or ligands (Table 23.3).

23.5.4 Reactive Oxygen Species and Reactive Nitrogen Species as Second Messengers in Environmental and Developmental Signals

Reactive oxygen species (ROS) production is an unavoidable consequence of aerobic life. ROS include both oxygen radicals, like superoxide ($O_2^{\cdot -}$), hydroxyl (${}^{\cdot}OH$), peroxyl ($ROO^{\cdot}$), etc. and non-radicals, such as hydrogen peroxide (H_2O_2), singlet oxygen (${}^1O_2$), ozone (O_3), etc. Reactive nitrogen species (RNS) include radicals, like nitric oxide ($NO\cdot$) and nitric dioxide ($NO_2^{\cdot}$), and non-radicals, such as nitrous acid (HNO_2) and dinitrogen tetroxide (N_2O_4). Apart from their roles in plant development, ROS and RNS, particularly H_2O_2 and NO, respectively, act as second messengers during plant responses to several abiotic and biotic stress factors, like salinity, drought, temperature, heavy metals, and pathogen attack. ROS signals originate from different organelles and induce transcriptional changes and cellular reprogramming, leading to either protection of the plant cell or induction of programmed cell death (PCD). The sensitive equilibrium between ROS production and scavenging at the specific intracellular location and time determines whether

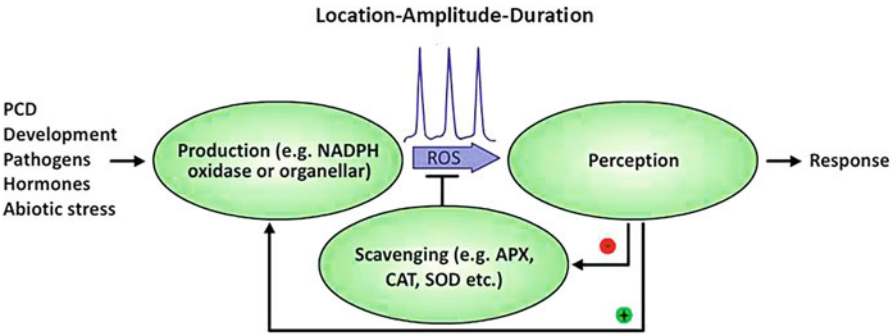
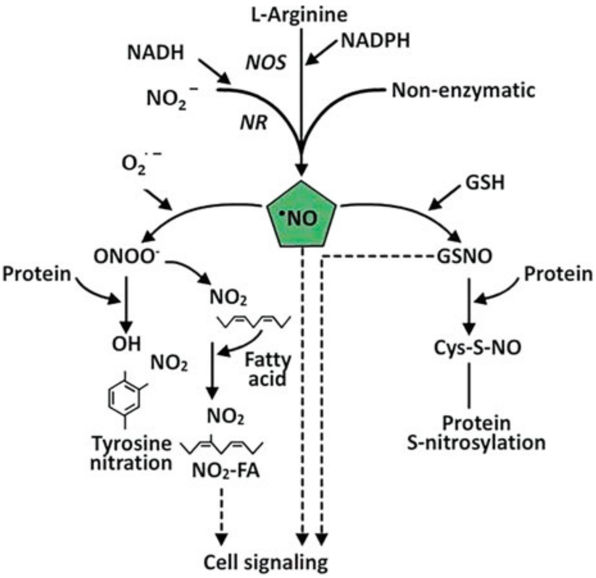


Fig. 23.12 ROS signaling in plant cells. An equilibrium between ROS production and scavenging at specific intracellular locations and timing determine the nature of its role as a damaging, protective, or signaling factor. *APX* ascorbate peroxidase, *CAT* catalase, *PCD* programmed cell death, *ROS* reactive oxygen species, *SOD* superoxide dismutase

ROS will act as damaging, protective, or signaling factor (Fig. 23.12). Cells have evolved strategies to use ROS as biological signals which activate and control various genetic stress/response programs as ROS influences the expression of a number of genes and operation of signal transduction pathways. Plants are able to regulate developmental processes and respond to environmental cues with the involvement of hormones, like auxins, cytokinins, ethylene, ABA, jasmonic (JA), and salicylic (SA) acids, in signaling together with ROS signaling. Increased levels of ROS can cause SA accumulation which, in turn, is involved in SA-induced stomatal closure. Gibberellic acid (GA) signaling is linked with ROS by stimulating the destruction of DELLA proteins which regulate transcript levels of antioxidant enzymes.

Oxidation of thiol groups by H_2O_2 may lead to inactivation of enzymes. H_2O_2 plays a concentration-dependent dual role in plants. It can act as a signal molecule in acclimatory signaling, triggering tolerance to different biotic and abiotic stress factors at low concentration, whereas it leads to PCD at high concentration (50 mM). H_2O_2 acts as a key regulator in a wide range of physiological processes, like photorespiration and photosynthesis, stomatal movement, cell cycle, and growth and development. It acts as a second messenger for signals generated by ROS due to its relatively long life and high permeability across membranes. H_2O_2 plays the role of messenger in numerous signaling pathways for regulation of many of the stress genes. In response to environmental (abiotic) stress in plants, RNS also play a significant role as signaling molecules. During defense response to pathogen attacks in plants, NO acts as a key mediator together with ROS. In general, a rapid overproduction of ROS and RNS takes place when plants are subjected to biotic and environmental stresses. PCD is an important mechanism which regulates varied aspects of growth and development and also eliminates damaged or infected cells produced in response to environmental stress and pathogen attack. Both NO and ROS play key functions during PCD. In the presence of O_2, NO can react with reduced glutathione (GSH) by an *S*-nitrosylation reaction to form *S*-

Fig. 23.13 Post-translational modifications mediated by nitric oxide in plant cells. The presence of GSNO, NO, and $ONOO^-$ leads to covalent post-translational modifications (PTMs) like S-nitrosylation and nitration of proteins and fatty acids. *GSH* reduced glutathione, *GSNO* S-nitrosoglutathione, *NOS* nitric oxide synthase, *NR* nitrate reductase, *ONOO*$^-$, peroxynitrite anion, $O_2^{\cdot-}$ superoxide anion



nitrosoglutathione (GSNO), an important mobile reservoir of bioactive NO (Fig. 23.13). The presence of GSNO has been demonstrated in different plant species. On the other hand, peroxynitrite ($ONOO^-$), a powerful oxidant/nitrating RNS species, is formed by the rapid reaction between $O_2^{\cdot-}$ and NO, and its occurrence has been reported in plant organelles, such as peroxisomes. As a result of the presence of NO and GSNO in plant tissue and generation of $ONOO^-$, important covalent post-translational modifications (PTMs), like S-nitrosylation and the nitration of proteins, can take place under natural and stress conditions. In peroxisomes, *S*-nitrosylation inhibits the activities of catalase and glycolate oxidase, and this could regulate the cellular level of key signaling molecules like H_2O_2. Generation of $ONOO^-$ can cause tyrosine nitration of plant proteins leading to nitrosative damage in plant cells, although a basal endogenous nitration could also have a regulatory function. H_2O_2 and NO have also been shown to play an important role in avoiding pathogen advancement as they activate transcriptional factors of pathogenesis-related (PR) proteins during the induction of resistance.

23.5.5 Lipid-Signaling Molecules

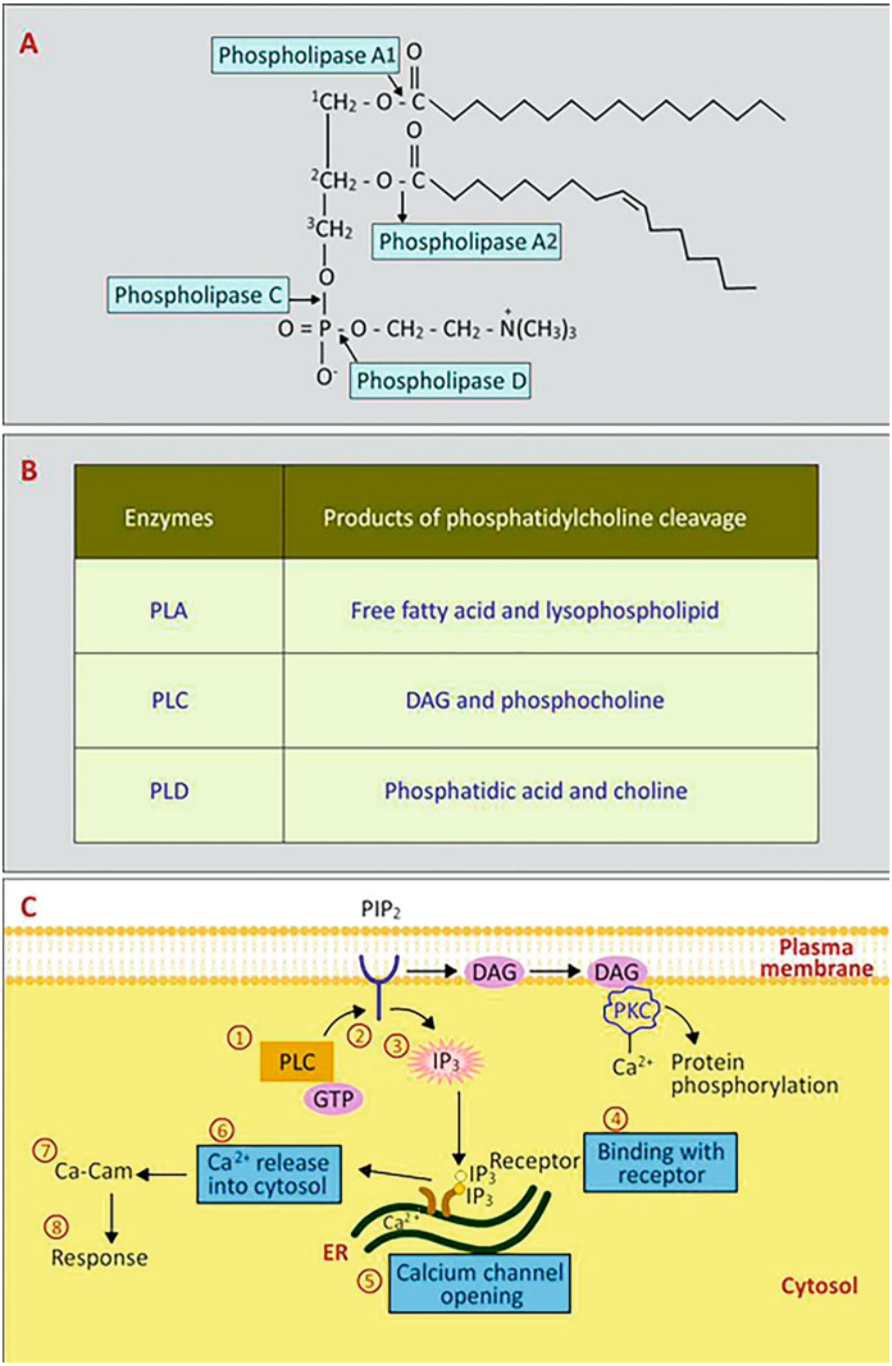
The physical properties of cell membranes such as changes on their surface and fluidic nature are modulated by their primary lipidic constituents, i.e., phosphoglycerolipids and sphingolipids. A typical phospholipid molecule consists of two fatty acid chains esterified to a glycerol backbone, a phosphate group, and a variable head group. Common head groups include choline, ethanolamine, glycerol, serine, inositol, inositol monophosphate, inositol bisphosphate, and OH group. The resulting lipids from these head groups are phosphatidylcholine (PC),

Table 23.4 Lipid-derived signaling molecules and their roles in plants

Lipid-derived signaling molecule	Derived from	By the action of	Signaling roles
Oxylipins	Glycerolipids/ phospholipids	Patatin-related acyl hydrolases	Pathogen defense (?)/abiotic stress (?)
Jasmonic acid	Phosphatidylcholine	PLA_1 (?) or PLA_2	Senescence, flower opening, anther dehiscence, pollen maturation
Phosphatidic acid	PC and other PLs	PLDs	Freezing tolerance, root hair patterning, cell differentiation, ABA signaling for stomatal closure, modulation of actin and microtubule dynamics
Diacylglycerol (DAG)	Phosphatidylinositol 4,5-bisphosphate	PLC	PKC activation; pollen tube growth; stomatal opening
Inositol triphosphate (IP_3)	PIP_2	PLC	Opening of ER/tonoplast-localized Ca^{2+} channels; interaction with cytoskeletal proteins

phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylinositolphosphate (PIP), phosphatidylinositolbisphosphate, and phosphatidic acid (PA), respectively (Table 23.4). Phospholipases (PLA_1, PLA_2, PLC, PLD) act at specific positions on these phospholipid molecules, resulting in the formation of a variety of lipid-signaling molecules which modulate a number of cellular and physiological processes. Members of **phospholipase A (PLA)** enzyme are responsible for cleaving one of the acyl ester bonds on the phospholipids, thereby releasing a fatty acid molecule and a lysophospholipid. **Lysophospholipids** are small lipid molecules consisting of a single carbon chain and a polar head group. They are more hydrophilic than their corresponding phospholipids, and they are known to regulate a number of plant processes. PLAs cleave either of the acyl bonds on the phospholipid molecule, releasing a fatty acid and a lysophospholipid. Two forms of PLAs, PLA_1 and PLA_2, are known, but only PLA_2 can be regulated by G proteins, protein kinases, and Ca^{2+} in animal cells. Phospholipase C **(PLC)** hydrolyzes the glycerophosphate bond to yield diacylglycerol (DAG) and a phosphorylated head group, such as IP_3 (inositol 1,4,5-triphosphate). Both these molecules (DAG and IP_3) are involved in the regulation of Ca^{2+} fluxes essential for a number of physiological processes. **Phospholipase D (PLD)** action on phosphatidylcholine (PC) leads to the formation of **phosphatidic acid (PA)**, which is a crucial signaling molecule to counter environmental stresses. Different PLD isoforms are expressed in different cellular location at different stages of development.

PM-associated PLC has been purified from several plants and is involved in signaling events associated with inositol phospholipid synthesis and breakdown, resulting in an increase in cytosolic calcium (Fig. 23.14). PLC is activated by its



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

Fig. 23.14 (**a**) Cleavage sites of specific phospholipases. (**b**) Products of action of phospholipases on phospholipids. (**c**) Major signal transduction events involving lipid-signaling molecules: (1) activation of PLC2, (2) activation of activated PLC on PIP_2 (DAG and IP_3 formation), (3, 4) diffusion and binding of IP_3 with the receptor on ER, (5) opening of Ca^{2+} channels in ER, (6) release of free Ca^{2+} from ER stores, (7) Ca^{2+}-CaM complex formation, and (8) response

interaction with α subunit of G protein associated with GTP. Activated PLC cleaves PIP_2, yielding the second messengers—DAG and IP_3. IP_3 diffuses into the cytoplasm and binds to its receptor on Ca^{2+} channels localized on ER or tonoplast. This results in the opening of Ca^{2+} channels, thereby releasing free calcium into the cytosol from these intracellular reserves. DAG remains membrane bound and is recycled to regenerate PI. IP_3 is also known to interact with cytoskeletal proteins to bring about changes in the cells. Likewise, PIP_2 has been shown to bind to profilin and gelosin—two important proteins involved in microfilament organization. DAG activates protein kinase C (PKC) by altering its sensitivity to its Ca^{2+} (Fig. 23.14). DAG is also involved in regulating the growth of pollen tube and opening of stomatal aperture.

PLA_2 action may be involved in the action of fungal elicitors and oxidative burst responses associated with them (1, 3). β-Glucanase is regulated by PLA_2 activation. Linolenic acid, a product of PLA_2 action, is a precursor for jasmonic acid biosynthesis via octadecanoid pathway. Phosphatidic acid (PA) generated through PLD action has been implicated in senescence, wounding, stress, and fruit ripening. PA can also act as a calcium ionophore, thereby permitting free movement of calcium ions across PM. PA regulates several protein kinases, GTP-binding proteins, vesicle trafficking, and oxidative burst response due to pathogens.

23.5.6 Mitogen-Activated Protein (MAP) Kinase Cascade

MAP kinase cascade derives its name from a series of protein kinases which phosphorylate each other in a defined sequence. They often represent points of convergence for several signaling pathways. This signal transduction mechanism processes multiple upstream signals to trigger multiple downstream signals. MAP kinases are serine/threonine kinases, and three functionally distinct families of MAP kinases operate in this series. The first kinase in this cascade is a MAP kinase kinase kinase (MAPKKK) which is activated by a receptor, kinases, or GTPases at the PM (Fig. 23.15). MAPKKK phosphorylates MAPKK which subsequently phosphorylates MAPK. MAPK is the last kinase in the sequence, and signaling through this cascade is attenuated by the appropriate phosphatases. MAPK, which serves as the "anchor" of the relay sequence, finally phosphorylates kinases, metabolic enzymes, or transcription factors to bring about downstream signal transduction, metabolic changes, or changes in gene expression. Each phosphorylated kinase can also modify the activity of many more of its own target proteins. Thus, MAP kinase cascade is able to alter the phosphorylation status (and hence activity) of thousands of proteins in response to very few ligand molecules bound to the receptor at the initial stage on the PM. *Arabidopsis* has 90 genes encoding these kinases (60 for MAPKKK, 10 for MAPKK, and 20 for MAPK). Some of the signaling cascades known to be operated through MAP kinase route include jasmonic acid and ABA signal transduction,

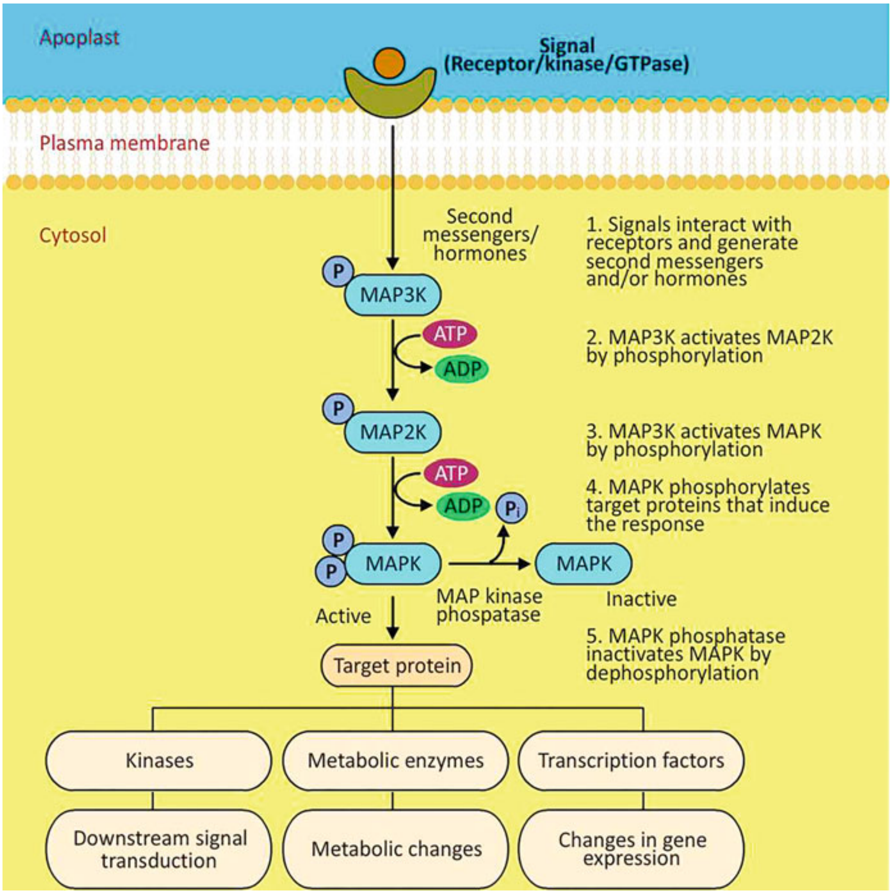

Fig. 23.15 Mitogen-activated protein (MAP) kinase-mediated signaling through a series of phosphorylation of MAP3K, MAP2K, and MAPK. This signaling route brings about multiple downstream signals

regulation of asymmetric formation of division plate during cell division, stomatal patterning, and cellular responses to abiotic stresses and pathogens.

In view of the multiple inputs and outputs, how does MAP kinase cascade maintain signal specificity? This is made possible by one of the two ways: (1) selective co-localization of kinases into a cascade forming combination to restrict kinase interactions and (2) selective repression of some cascades by phosphatases to permit only certain transduction pathways to operate.

23.5.7 Cyclic Nucleotides

Cyclic 3′,5′-adenosine monophosphate (cAMP) is known to mediate signal transduction in response to a number of animal hormones. It has also been reported to act as a second messenger in fungi and many prokaryotes. Animal cells also use cGMP as a second messenger in signal transduction. It is synthesized from GTP by the action of **guanylyl cyclase** and activates **cGMP-dependent protein kinase (PKG).** Cyclic AMP produced from the action of PM-localized adenylyl cyclase diffuses into the cytoplasm where it activates cyclic AMP-dependent protein kinase (PKA). PKA is responsible for the phosphorylation of downstream target proteins in several signal transduction pathways. In animal cells, PKA also facilitates opening of **cyclic nucleotide-gated ion channels (CNGCs)** for nonselective influx of Ca^{2+}, K^+, and Na^+. Signaling through cAMP and cGMP is terminated by the action of respective cytoplasm localized phosphodiesterases for catalyzing the conversion of cAMP and cGMP into their respective noncyclic forms (5′-AMP and 5′-GMP), respectively. Cyclic nucleotides have more restricted second messenger role in plant cells as compared to animal cells (Fig. 23.16). cAMP has been reported to affect signaling process accompanying stomatal closing, pollen tube growth, incompatibility reaction between stigma and pollen, cell cycle, and rhizobial interactions. Although no plant homologs for PKA and PKG have so far been reported, CNGCs have been implicated in GA and phytochrome signaling, pollen tube growth, cell cycle control, and stress and defense signaling. In plant cells, guanylyl cyclase

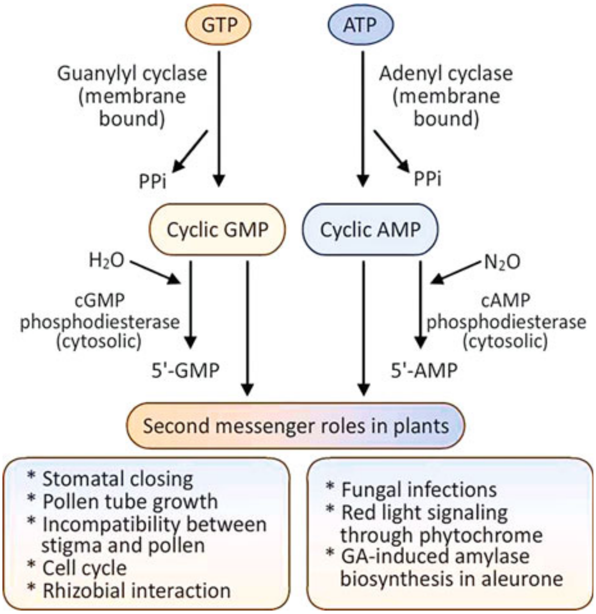

Fig. 23.16 Biosynthesis and second messenger roles of cAMP and cGMP in plant cells. Cyclic nucleotides have more restricted roles in plant cells than in animal cells

activity may be more important than adenyl cyclase in signal transduction of various processes. cGMP may participate in transducing signals of fungal infection, red light signals through phytochrome, and synthesis of amylase in aleurone layer in response to GA action.

23.6 Adaptive Mechanisms of Plant Signaling and Their Termination

1. A majority of plant hormone signal transduction pathways induce a response by **negative regulation** through inactivation of repressor proteins. This is in contrast with animal signal transduction pathways which induce responses mostly through activation of a cascade of positive regulators. Thus, for example, brassinosteroid binding to the receptor kinase BRI1 leads to inactivation of repressor protein BIN2. This results in the inactivation of transcription factors—BES1 and BZR1. Why have plant cells evolved signaling pathways based on negative regulation rather than positive regulation (based on animal cells)? Mathematical modeling has suggested that negative regulators result in faster induction of downstream response genes. Since the speed of response to environmental stress conditions is crucial for survival of sessile plants, the adoption of negative regulatory signaling is likely to confer selective advantage during evolution. Some of the major molecular mechanisms adopted by plant cells to inactivate repressor proteins include dephosphorylation, retargeting of repressors to other subcellular compartments and degradation.
2. **Plants have evolved mechanisms for switching off or attenuating signal transduction responses**: Plant hormones can be degraded or inactivated by oxidation or conjugation to sugars or amino acids. Receptors and signaling intermediates can also be inactivated by dephosphorylation. Signal amplification can further be switched off by the lowering of elevated concentrations of secondary messengers by ion transporters and cellular scavengers. Proteolytic degradation of the components of signal transduction pathways serves as an important mechanism to regulate signaling events. Feedback regulation is another mechanism for attenuation of a signal response. For example, degradation of AUX/IAA proteins thereby terminates the response. Likewise, gibberellin negatively regulates its own intracellular concentration through DELLA-mediated feedback loops. In the absence of GA, DELLA transcriptional regulators promote GA biosynthesis through enhanced expression of $GA_{20\ ox}$ and $GA_{30\ ox}$ enzymes. DELLA also inhibits genes encoding GA catabolism ($GA_{2\ ox}$), thereby causing GA degradation. As a result of these two effects of DELLA, GA concentration in the cell increases. Soon thereafter, as soon as DELLA proteins are degraded by proteosomal pathway, GA biosynthesis decreases and GA catabolism increases. Thus, GA negatively regulates its own concentration in the affected cells.

3. **Tissue specificity of signal responses**: Not all responses evoked by a signal are evident in any cell type. Any tissue exhibits some of the responses due to a signal. Thus, auxin promotes cell expression in growing aerial tissues but inhibits lateral root formation in the cells of pericycle, while leaf primordia are initiated at the shoot apical meristem. These differential auxin responses in different cells are due to auxin-dependent interaction of TIR/AFB receptors and AUX/IAA repressor proteins and variation in the response by the tissue in plants where these components are expressed, the abundance of their expression, the degree of their binding affinity and cellular auxin level.
4. **Agonistic (additive or positive) and antagonistic (inhibitory or negative) crosstalk among different signaling mechanisms**: Signal transduction pathways mostly operate as part of a complex web of signaling interactions rather than in isolation, e.g., antagonistic interaction between gibberellins and ABA during seed germination. Such interactions among various signaling routes are referred as **cross regulation**. It can be of one of the following three types:
    (a) **Primary cross regulation** where distinct signaling pathways regulate a shared transduction pathway. Both pathways have the same effect on the response leading to positive interaction. When one of the pathways inhibits the effect of the other, it leads to negative primary interaction. For example primary cross regulation is evident when both cytokinin and ABA induce transcription of ABI4 to further regulate transcription of a number of biosynthetic and response genes (Fig. 23.17).
    (b) **Secondary cross regulation** where output of one signaling route regulates the abundance or perception of another signal. It is positive interaction when one pathway enhances the input levels or perception of the other pathway and

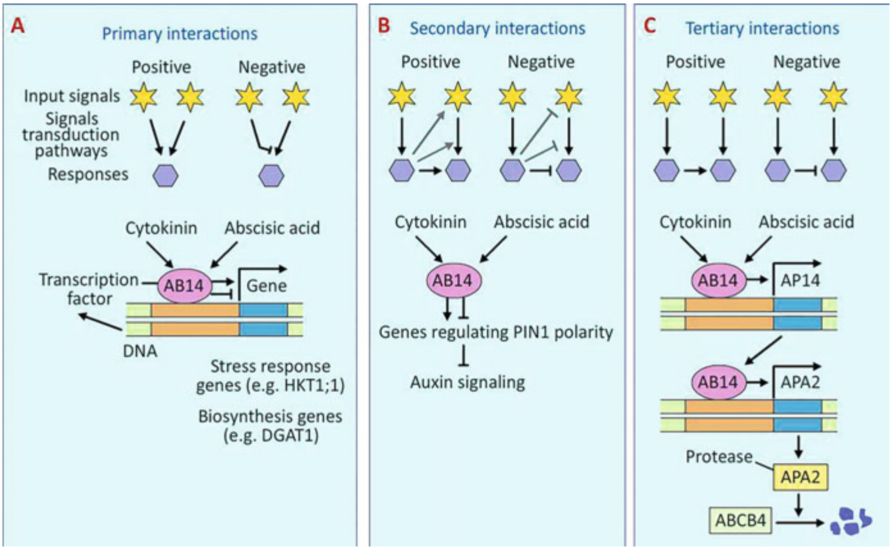

Fig. 23.17 Agonistic and antagonistic crosstalk among different signaling mechanisms

is negative when the second pathway is repressed by the first. For example, induction of transcription factor ABI4 acts on auxin signaling by lowering auxin flow and polar localization and abundance of PIN 1 in root vasculature, which is responsible for lateral root initiation.

(c) **Tertiary cross regulation** where outputs of two pathways influence each other positively or negatively. ABI4 positively regulates APA_2 protease expression which induces degradation of ABCB4 auxin transporter that regulates auxin flow in root epidermal cells during lateral root elongation. ABCB4 abundance on PM is reduced after ABA or CK treatment in wild plants (Fig. 23.17).

An understanding of the complexities of these cross regulations is possible through mathematical and computational models to simulate these biological networks, an approach often referred as **system biology**.

Summary

- Plant growth and development are modulated by a variety of environmental (external) and physiological (internal) signals. A number of signaling events in plants involve signal reception and response in the same cell (**cell autonomous responses**). Many other responses involve signal perception in one cell and response in distal cells, tissues, or organs (**non-cell autonomous responses**). Same signal can lead to different responses in different tissues through its ability to bind to different families of receptors. Different tissues may adapt or desensitize themselves to continuous signals and receptor concentrations during development. Signal transduction pathways mostly operate as part of a complex web of signaling interactions rather than in isolation.
- Any environmental or intracellular input, which initiates one or more responses in the cell/plant, is referred as a **signal**. Plant cells are sensitized to the signals (**perception**) by employing specialized sensor proteins, termed **receptors**. Receptors may be located on the plasma membrane, cytoplasm, endomembrane system, or in the nucleus. Receptors located on the plasma membrane can perceive both physical and chemical signals. Sensing of signal by the receptor is followed by the transfer of the information from the signal from one biochemical form to another (**transduction**) so as to amplify the impact of signal leading to a cellular response.
- Plants have also developed pathways for electrical signaling to rapidly respond to external stimuli. In contrast to chemical signals, electrical signals rapidly transmit information over long distances. Touch-sensitized drooping of leaflets of *Mimosa pudica* is a classic example where membrane potential serves as a receptor.

- Three major groups of plasma membrane receptors can be distinguished on the basis of how they interact with signaling components. These are receptor kinases, G-protein-coupled receptors (GPCRs), and ion channel receptors. **Receptor kinases** are responsible for transducing extracellular signals through phosphorylation of intracellular targets. Plants also possess **histidine kinase receptors** derived from bacterial two-component system. Signal transduction using these receptors depends on a balance of “forward” and “reverse” phosphotransfers.
- **G-protein-coupled receptors (GPCRs)** are membrane-associated proteins with an extracellular ligand-binding domain, a transmembrane domain (composed of seven hydrophobic helices), and an intracellular domain that interact with inactive G-protein trimer. **G proteins** are GTP-binding proteins whose activity is regulated by GPCRs. G proteins consist of three subunits—Gα, Gβ, and Gγ. Tip growth and polar extension in plants are regulated by a family of small GTPases called ROPs (for Rho-like GTPase). ROP1 GTPases are localized on the PM at the tip of the growing pollen tube.
- So far, no plasma membrane-associated ion channels have been reported to function as receptor in response to ligand binding. However, PM-associated mechanosensing calcium channels do play a role in plant responses to physical force.
- **Second messengers** are intracellular, diffusible small molecules and ions which are rapidly synthesized or released transiently as a concentrated pulse following signal perception by the receptors and modifying the activity of target signaling proteins. Ca^{+2} is involved in a vast variety of signaling pathways. Cytosolic Ca^{+2} increase is tightly regulated by the opening and closing of Ca^{+2} channels localized on PM, ER, and tonoplast. Plasma membrane is known to possess Ca^{+2} channels **called glutamate-like receptors (GLRs) and cyclic nucleotide-gated channels (CNGCs)**. Intracellular spikes of cytosolic Ca^{+2} brought about by the activation of calcium channels through various activation routes result in the regulation of downstream signaling through **Ca^{+2}-sensor proteins** as the intermediates. Four major categories of Ca^{+2}-sensor proteins are calmodulin (CaM) and calmodulin-like proteins, calcium-dependent protein kinases (CDPKs), Ca^{+2}/calmodulin-dependent protein kinases (CCaMKs), and calcineurin B-like proteins (CBLs). These target proteins, upon activation by Ca^{+2}, modulate the activity of target proteins. **Target proteins** include transcription factors, various protein kinases, Ca^{+2}-ATPases, ROS-producing enzymes, and ion channels.
- Protons also exert their signaling role both in the cell wall and cytosol. Altered pH of the cell wall is also likely to affect protonation status of weakly acidic plant hormones, thereby affecting their ability to enter cells by diffusion. Gating of potassium channels and aquaporins has also been observed to be affected by pH. ROS signals originate in different organelles and induce transcriptional changes and cellular reprogramming, leading to either protection of the plant cell or induction of programmed cell death (PCD). H_2O_2 plays the role of messenger in numerous signaling pathways for regulation of many of the stress genes. RNS also play a significant role as signaling molecules. As a result of the presence of NO and GSNO in plant tissue and generation of $ONOO^-$, important covalent post-translational modifications (PTMs), like *S*-nitrosylation and the

nitration of proteins, can take place in plants under natural and stress conditions. In peroxisomes, *S*-nitrosylation inhibits the activities of catalase and glycolate oxidase, and this could regulate the cellular level of key signaling molecules, like H_2O_2. H_2O_2 and NO have also been shown to play an important role in avoiding pathogen advancement as they activate transcriptional factors of pathogenesis-related (PR) proteins during the induction of resistance.

- PM-associated PLC is involved in signaling events associated with inositol phospholipid synthesis and breakdown, resulting in an increase in cytosolic calcium. PLC is activated by its interaction with α subunit of G protein associated with GTP. Activated PLC cleaves PIP_2, yielding the second messengers—DAG and IP_3. IP_3 diffuses into the cytoplasm and binds to its receptor on Ca^{2+} channels localized on ER or tonoplast. This results in the opening of Ca^{2+} channels, thereby releasing free calcium into the cytosol from these intracellular reserves. IP_3 is also known to interact with cytoskeletal proteins to bring about changes in the cells. PLA_2 action may be involved in the action of fungal elicitors and oxidative burst responses associated with them. Phosphatidic acid (PA) generated through PLD action. PA can also act as a calcium ionophore, thereby permitting free movement of calcium ions across PM. PA regulates several protein kinases, GTP-binding proteins, vesicle trafficking, and oxidative burst response due to pathogens.
- MAP kinases are serine/threonine kinases, and three functionally distinct families of MAP kinases operate in this series. The first kinase in this cascade is a MAP kinase kinase kinase (MAPKKK) which is activated by a receptor, kinases, or GTPases at the PM. MAPKKK phosphorylates MAPKK which subsequently phosphorylates MAPK. MAPK is the last kinase in the sequence, and signaling through this cascade is attenuated by the appropriate phosphatases. MAPK, which serves as the “anchor” of the relay sequence, finally phosphorylates kinases, metabolic enzymes, or transcription factors to bring about downstream signal transduction, metabolic changes, or changes in gene expression.
- Cyclic nucleotides have more restricted second messenger role in plant cells as compared to animal cells. In plant cells, guanylyl cyclase activity may be more important than adenyl cyclase in signal transduction of various processes. cGMP may participate in transducing signals of fungal infection, red light signals through phytochrome, and synthesis of amylase in aleurone layer in response to GA action.
- A majority of plant hormone signal transduction pathways induce a response by negative regulation through inactivation of repressor proteins. This is in contrast with animal signal transduction pathways which induces responses mostly through activation of a cascade of positive regulators. Thus, for example, brassinosteroid binding to the receptor kinase BRI1 leads to inactivation of repressor protein BIN2. Since the speed of response to environmental stress conditions is crucial for survival of sessile plants, the adoption of negative regulatory signaling is likely to confer selective advantage during evolution. Proteolytic degradation of the components of signal transduction pathways serves as an important mechanism to regulate signaling events. Feedback regulation is another mechanism for attenuation of a signal response.

Multiple-Choice Questions

1. Which of the following is NOT a non-cell autonomous response?
   - (a) Radial patterning of *Arabidopsis* primary root regulated by transcription regulator-SHR
   - (b) Auxin gradient in the root by rapid redistribution of PIN3 (auxin efflux protein)
   - (c) Opening of stomata in response to blue light
   - (d) Transition from vegetative to reproductive phase (floral induction)
2. Signal perception via symplast regulates the movement of which of the molecules listed below?
   - (a) Lipophilic molecules
   - (b) Chemicals like signal peptides
   - (c) Mechanical forces like touch and blue light
   - (d) RNAs and transcription factors
3. In GPCRs, the Gα subunit of G protein is active when bound with _____. Inactivation of Gα subunit is mediated via the action of _________.
   - (a) GTP, GTPase-activating protein (GAP)
   - (b) GDP, Guanine nucleotide exchange factors (GEFs)
   - (c) GDP, GTPase-activating protein (GAP)
   - (d) GTP, Guanine nucleotide exchange factors (GEFs)
4. Tip growth and polar extension in plants is regulated by:
   - (a) GSNO
   - (b) Phospholipase A (PLA)
   - (c) Rho-like GTPase (ROP1)
   - (d) Phospholipase D (PLD)
5. In peroxisomes, S-nitrosylation has the ability to inhibit the activity of:
   - (a) Superoxide dismutase (SOD)
   - (b) Catalase
   - (c) Glutathione peroxidase
   - (d) b and c
6. Small lipid molecules consisting of a single carbon chain and a polar head group are known as:
   - (a) Sphingolipids
   - (b) Phosphoglycerolipids
   - (c) Lysophospholipids
   - (d) None of these
7. Calcium channels—glutamate-like receptors (GLRs) and cyclic nucleotide-gated channels (CNGCs)—are located in:
   - (a) Tonoplast
   - (b) Plasma membrane
   - (c) Endoplasmic reticulum
   - (d) All of these

8. Activated _____ cleaves PIP 2 to yield secondary messengers- ______ and _______.
   (a) PLD, IP 5, and DAG
   (b) PLC, IP 3, and DAG
   (c) PLC, IP 2, and DAHA
   (d) PLD, IP 3, and DAG

Answers

1. c 2. d 3. a 4. c 5. b 6. c 7. b 8. b

Suggested Further Readings

Leyser O, Ray S (2015) Signal transduction. In: Buchanan BB, Gruissem W, Jones RL (eds) Biochemistry and molecular biology of plants. Wiley-Blackwell, Chichester, pp 834–871

Taiz L, Zeiger E (2010) Plant physiology, 5th edn. Sinauer Associates Inc, Massachusetts, pp 407–445