19

Multiple signals regulate the growth and development of plant organs and enable their adaptation to environmental conditions

In complex multicellular organisms such as higher plants and animals, metabolism, growth, and development of the various organs are coordinated by the emission of signal compounds. In animals these signals can be hormones, which are secreted by glandular cells. Hormones are classified in paracrine hormones, which function as signals to neighboring cells, and endocrine hormones, which are emitted to distant cells (e.g., via the blood circulation). Also in plants, signal compounds are released from certain organs, often signaling to neighboring cells, but also to distant cells via the xylem or the phloem. All these plant signal compounds are termed **phytohormones**. Some of the phytohormones (e.g., brassinosteroids) resemble animal hormones in their structure, whereas others are structurally completely different. Like animal hormones, phytohormones also have many different signal functions. They control the adjustment of plant metabolism to environmental conditions, such as water supply, temperature, and day length, and regulate plant development**.** Light sensors including **phytochromes**, which recognize red and far-red light, and **cryptochromes** and **phototropin** monitoring blue light, control the growth and the differentiation of plants depending on the intensity and quality of light.

The signal transduction chain between the binding of a certain hormone to the corresponding receptor and its effect on specific cellular targets, such as the transcription of genes or the activity of enzymes, is now known for many animal hormones. In contrast, signal transduction chains have not been fully resolved for any of the phytohormones or light sensors. However,

partial results indicate that certain components of the signal transduction chain in plants may be similar to those in animals. The phytohormone receptors and light sensors apparently act as a **multicomponent system**, in which the signal transduction chains are interwoven to a **network**.

19.1 Signal transduction chains known from animal metabolism also function in plants

G-proteins act as molecular switches

A family of proteins, which by binding of GTP or GDP can alternate between two conformational states, is widely distributed in the animal and plant kingdoms. These proteins are **GTP-binding proteins**, or simply **G-proteins**. The heterotrimeric G-proteins, discussed in the following, are bound at the inner side of the plasma membrane interacting with integral membrane receptor proteins consisting of seven transmembrane helices (Fig. 19.1). The receptors have a binding site for the signal molecule at the outside and a binding site for G-proteins at the cytoplasmic site of the plasma membrane, and are therefore well suited to pass external signals into the cell. **Heterotrimeric-G-proteins** are composed of three different subunits: $\mathbf{G_\alpha}$ (molecular mass 45–55 kDa), $\mathbf{G_\beta}$ (molecular mass 35 kDa), and $\mathbf{G_\gamma}$ (molecular mass 8 kDa). In *Arabidopsis thaliana* the α- and β-subunits are each encoded by one gene, whereas the γ-subunit is encoded by two genes. Subunit G_α has a binding site that can be occupied by either GDP or GTP. In animals, binding of the heterotrimer to a receptor (e.g., an adrenaline receptor occupied by adrenaline) enables the exchange of the GDP for GTP at the G_α subunit.

The binding of GTP results in a **conformational change** of the G_α subunit, which subsequently dissociates from the trimer. The liberated G_α-GTP unit functions as an activator of various enzymes that synthesize components of the signal transduction chain. For instance, G_α-GTP stimulates a **GMP-cyclase** that forms the signal compound guanosine-3′-5′-monophosphate (**cGMP**) from GTP (Fig. 19.2), as has been found in plants and animals. G_α-GTP also stimulates **phospholipase C** (see Fig. 19.4). The function of this reaction in the liberation of Ca^{2+} as a signal component will be discussed in the following section. In fungi and animals, G_α-GTP also stimulates the synthesis of the signal compound adenosine-3′-5′-monophosphate (**cAMP**) from ATP via an activation of AMP-cyclase. It has so far not been definitely proved whether cAMP plays a role also in plant metabolism.

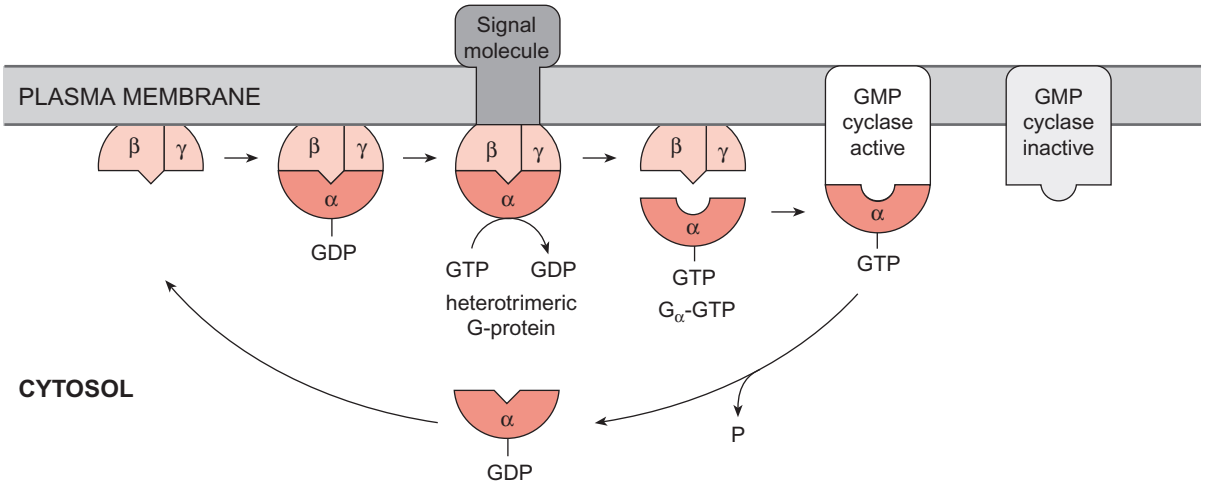


Figure 19.1 Schematic presentation of G-protein action.

G_α-GTP has a half-life of only a few minutes. Bound GTP is hydrolyzed to GDP by an intrinsic GTPase activity, and the resulting conformational change causes the G_α subunit to lose its activator function. It binds again to the dimer to form a trimer and a new cycle can begin. The short life of G_α-GTP makes the signal transduction very efficient.

Small G-proteins have diverse regulatory functions

All eukaryotes also contain **small G-proteins**, which have only one subunit and are related to the α-subunit of heterotrimeric G-proteins discussed in the preceding. All small G-proteins belong to a superfamily termed the **Ras superfamily**. These small G-proteins, located in the cytosol, have binding domains for GDP/GTP and an effector domain. Binding of a GDP renders the protein inactive and that of GTP active. When stimulated by a signal, the small G-protein interacts with an **exchange factor**, which converts the GDP-bound inactive protein to a GTP-active conformation by GTP/GDP replacement. Through its effector domain the active GTP-conformation interacts with other proteins in analogy to the G_α-GTP of the heterotrimeric G-protein. It has been predicted from genomic analyses that the *Arabidopsis* genome encodes more than 90 small G-proteins. Small G-proteins have various regulatory functions, such as the regulation of defense reactions, ABA responses, vesicle transport, cell polarity, and the growth of pollen tubes and root hairs. Present knowledge about the role of small G-proteins in plants is still at an early stage.

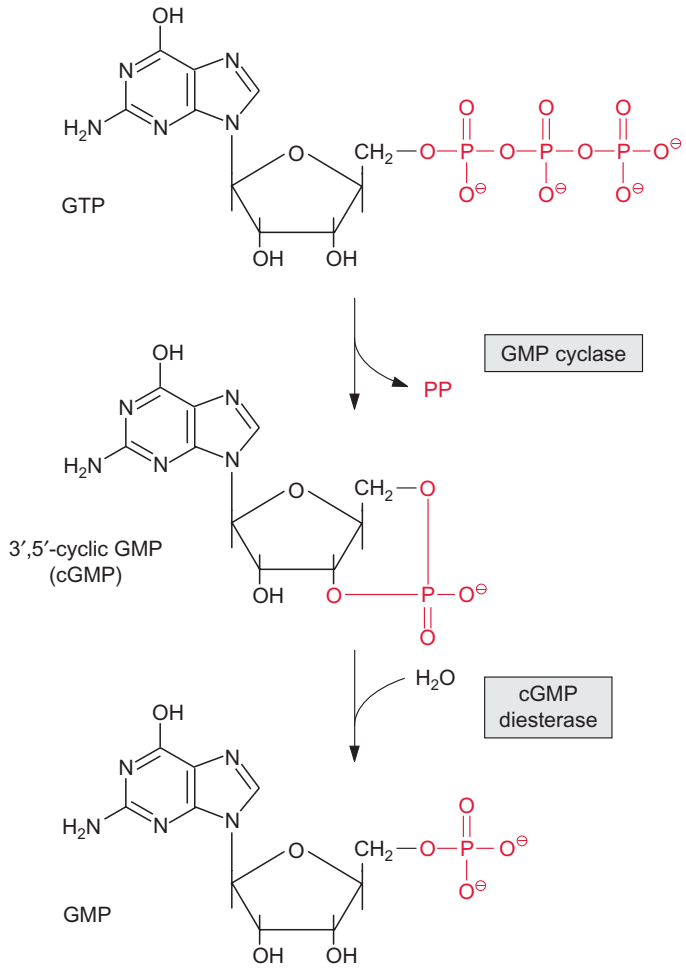


Figure 19.2 cGMP is synthesized by GMP-cyclase from GTP and is degraded to GMP by a diesterase.

Ca^{2+} is a component of signal transduction chains

In animal cells as well as in plant cells, the cytosolic concentration of free Ca^{2+} is normally lower than 10^{-7}mol/L. These very low Ca^{2+} concentrations are maintained by ATP-dependent pumps (Ca^{2+}-P-ATPases, section 8.2), which accumulate Ca^{++} in the lumen of the endoplasmatic reticulum and the vacuole (in plants) or transport Ca^{2+} via the plasma membrane to the extracellular compartment (Fig. 19.3). Alternatively, Ca^{2+} can be taken up into mitochondria by H^+/Ca^{2+} antiporters. Signals (e.g., salt, ABA, dryness or coldness) induce Ca^{2+} channels in the endomembranes of intracellular stores to open for a short time, resulting in a rapid increase in the cytosolic concentration of free Ca^{2+}. In almost all cells free Ca^{2+} stimulates

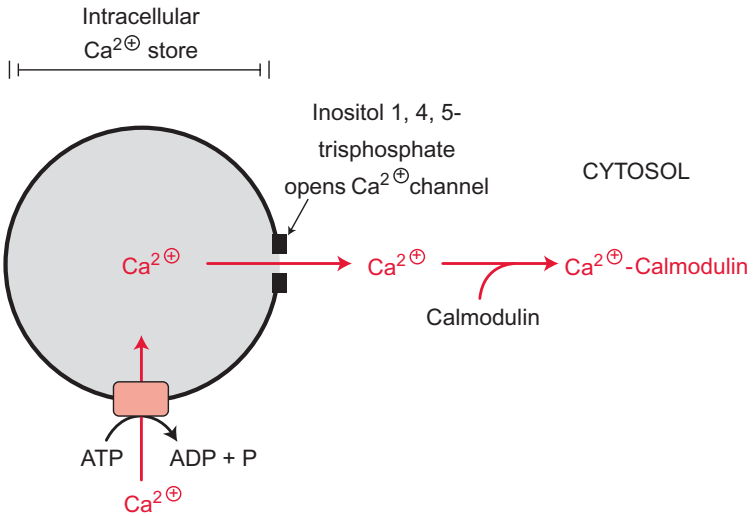


Figure 19.3 The endoplasmatic reticulum of animals and plants and the plant vacuole (designated here as intracellular Ca^{2+} store) contain in their membrane a Ca^{2+}-P-ATPase (section 8.2), which pumps Ca^{2+} into the lumen or into the vacuole. Ca^{2+} can be released into the cytosol by an IP_3-dependent Ca^{2+} channel.

regulatory enzymes such as protein kinases that are often components of signal cascades regulating a multitude of cellular processes (see following section).

The phosphoinositol pathway controls the opening of Ca^{2+} channels

Ca^{2+} channels can be controlled by the phosphoinositol signal transduction cascade (Fig. 19.4), which has initially been resolved in animal metabolism, but has also been shown to exist in plants. **Phosphatidyl inositol** is present, although in relatively low amounts, as a constituent of cell membranes. In animal cells, the two fatty acids of phosphatidyl inositol are usually stearic acid and arachidonic acid. The inositol residue is phosphorylated at the hydroxyl groups in 4′ and 5′ position by a kinase. **Phospholipase C**, stimulated by a G-protein, cleaves the lipid to **inositol-1,4,5-trisphosphate** (**IP_3**) and **diacylglycerol** (DAG). IP_3 causes a rise in the cytosolic Ca^{2+} concentration, whereas in animals diacylglycerol stimulates a Ca^{2+}-dependent protein kinase. In plants, diacylglycerol as such does not seem to play a role in the metabolism. However, it has an indirect effect, since **phosphatidic acid** (Fig. 15.5) deriving from the phosphorylation of diacylglycerol affects protein kinases and ion channels.

Patch-clamp studies (see section 1.10) demonstrated that in plant vacuoles and other Ca^{2+} stores, such as the endoplasmatic reticulum, IP_3 causes Ca^{2+} channels to open. The rapid influx of Ca^{2+} into the cytosol is limited

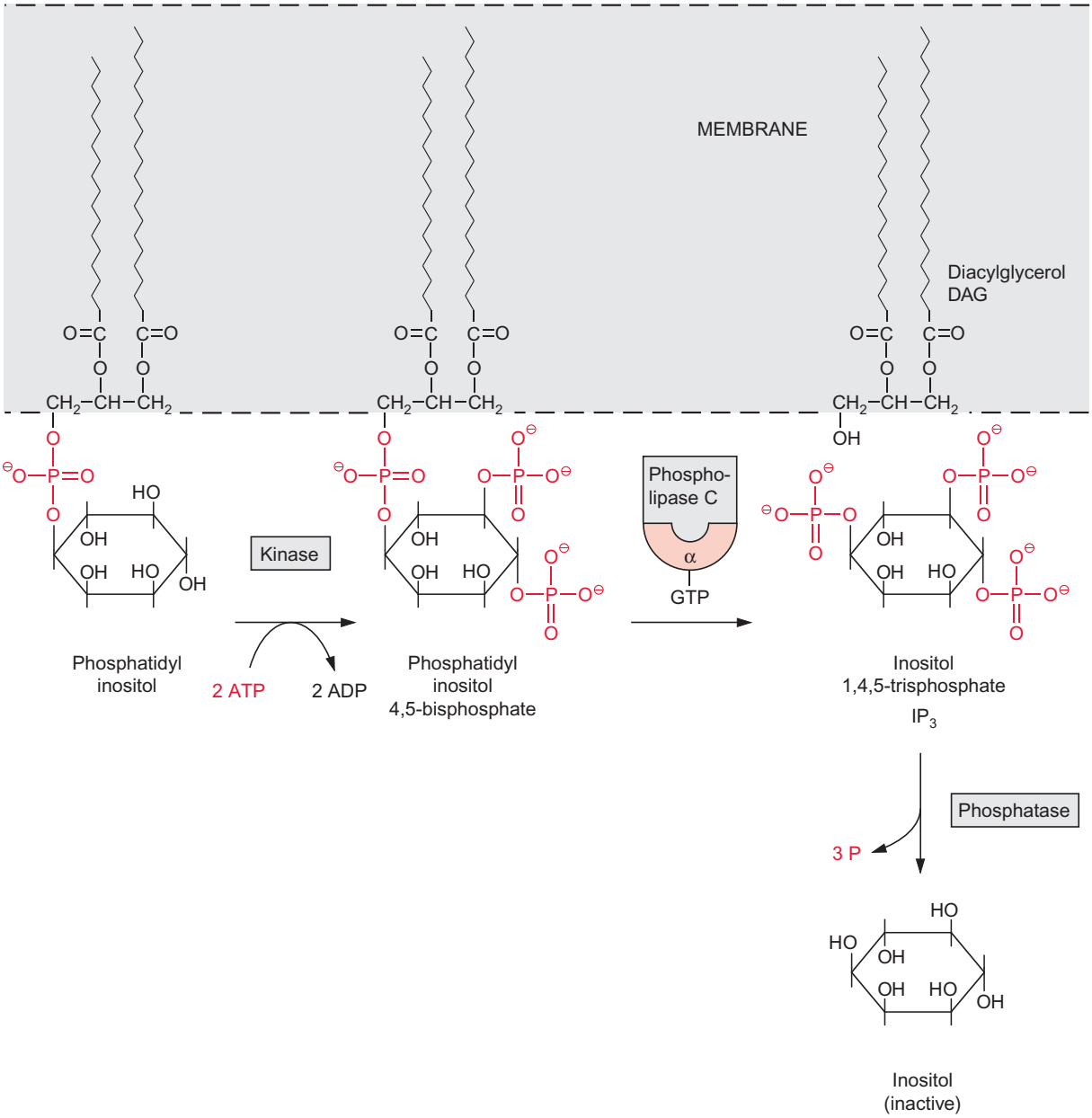


Figure 19.4 Inositol-1,4,5-trisphosphate (IP_3) is part of a signal transduction chain. Two hydroxyl groups of the inositol bound to a membrane anchored phospholipid are phosphorylated by a kinase and IP_3 is liberated by a G-protein ($G_\alpha GTP$)-dependent phospholipase C. The signal component IP_3 formed in this way can be degraded by phosphatases.

by the very short life of IP_3 (often less than 1 s). The rapid elimination of IP_3 proceeds either via additional phosphorylation of IP_3 or the hydrolytic liberation of the phosphate groups by a phosphatase. The short lifetime of IP_3 enables a very efficient signal transduction.

In plants the phosphoinositol cascade has an important role in transmitting signals from the environment to cellular functions (e.g., in adjusting the stomata opening to the water supply). A specific kinase has been identified in plants, which catalyzes the phosphorylation of phosphatidyl inositol to **phosphatidyl inositol-3-phosphate**. This modified membrane lipid functions as a signal for vesicle transfer (Fig. 1.16) (e.g., in the transfer of hydrolytic enzymes from the ER to the vacuole).

Calmodulin mediates the signal function of Ca^{2+} ions

Ca^{2+} often does not act directly as a signal component but by binding to calmodulin. **Calmodulin** is a soluble protein (molecular mass 17 kDa) that occurs in animals as well as in plants. It is a highly conserved protein; the identity of the amino acid sequences between the calmodulin from wheat and cattle is as high as 91%. Calmodulin is present mainly in the cytosol. It consists of a flexible helix connecting the two loops of both ends. Each loop possesses a binding site for a Ca^{2+} ion and contains glutamate (E) and phenylalanine (F). For this reason these loops are designated **EF hands** (Fig. 19.5). The binding of Ca^{2+} to all four EF hands results in a conformational change of calmodulin by which its hydrophobic domain is exposed. This domain interacts with certain protein kinases **(calmodulin-binding kinases** (**CBK**)), which are subsequently activated. The activated protein kinase-CBK II first catalyzes its own phosphorylation (autophosphorylation), and then reaches its full activity, and even retains it after the dissociation of calmodulin, until the phosphate residue is released by hydrolysis.

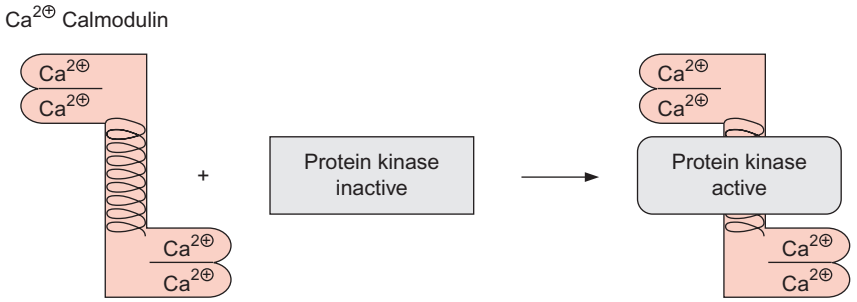


Figure 19.5 The protein calmodulin contains two Ca^{2+} binding domains, which are connected by a flexible α-helix. Ca^{2+}-calmodulin activates certain protein kinases, e.g., CBK.

Ca^{2+}-calmodulin also binds to other proteins, thus changing their activity, and is therefore an important component of signal transduction chains.

Moreover, plants encode a family of protein kinases, which possess Ca^{2+}-binding EF hands as essential domains of the protein. They are termed Ca^{2+}-dependent protein kinases (**CDPK**). By now more than 30 genes of the CDPK-family have been detected in *Arabidopsis*, although the function of part of them is still not known. CDPK kinases are involved in the phosphorylation of sucrose phosphate synthase (Fig. 9.18) and nitrate reductase (Fig. 10.9), pathogen defense reaction, and the response to various abiotic stresses.

There are also other proteins with calmodulin domains, the so-called **calmodulin-related proteins** (**CRK**), but their functions are to a large extent not known.

Phosphorylated proteins are components of signal transduction chains

Protein kinases, several of which have been discussed previously, and **protein phosphatases** are important elements in the regulation of intracellular processes. Phosphorylation and dephosphorylation change proteins between two activity states. Similarly many protein kinases are switched on or off by phosphorylation; therefore protein kinases represent a **network of on-off switches in the cell**, comparable to those of computer chips. These switches control differentiation, metabolism, defense against pests, and many other cell processes. It is estimated that in a eukaryotic cell 1% to 3% of the functional genes encode protein kinases. Initially protein kinases were investigated primarily in yeast and animals, but in the meantime several hundred genes encoding protein kinases have been identified in plants, although the physiological functions of only some of them are known. The elucidation of the interacting cellular components of protein kinases is at present a dynamic field in plant biochemistry.

Most protein kinases in eukaryotes, such as fungi, animals, or plants, encompass 12 structurally conserved regions. Since all these protein kinases are homologous and thus descend from a common ancestor, they are grouped in a **superfamily of eukaryotic protein kinases** (Table 19.1). They phosphorylate mainly the -OH group of **serine** and/or **threonin**e and in some cases also of **tyrosine**. Protein kinases phosphorylating histidine (e.g., receptors for ethylene and cytokinin) and aspartate residues are not members of this family (see section 19.7). The protein kinases, which are regulated by cGMP, are named **protein kinases G**. The existence in plants of **protein kinases A**, regulated by cAMP, is still a matter of dispute. The

Table 19.1: Some members of the eukaryotic protein kinase super family

	Modulator
Protein kinase-A	cAMP
Protein kinase-G	cGMP
Ca^{2+}-dependent protein kinase (CDPK)	Ca^{2+}
Calmodulin-binding kinase (CBK)	Ca^{2+}-Calmodulin
Receptor-like protein kinase (RLK)	e.g., Phytohormones
Cyclin-dependent protein kinase (CDK)	Cyclin
Mitogen-activated protein kinase (MAPK)	Mitogen
MAPK-activated protein kinase (MAPKK)	MAPK
MAPKK-activated protein kinase (MAPKKK)	MAPKK

protein kinases regulated by Ca^{2+}-calmodulin (**CBK**) were already mentioned, as well as the Ca^{2+}-dependent protein kinases (**CDPK**). Further members of the superfamily are the **receptor-like protein kinases (RLK**). These protein kinases are generally located in plasma membranes. They contain an extra cytoplasmatic domain with a receptor function (e.g., for a phytohormone). The occupation of this receptor by a signal molecule results in the activation of a protein kinase at the cytoplasmatic side of the membrane, and subsequent reaction with cellular proteins. Genome sequencing revealed that the *Arabidopsis* genome contains more than 400 genes encoding RLKs.

The superfamily of eukaryotic protein kinases also encompasses the **cyclin-dependent-protein kinases** (**CDK**) (Table 19.1). **Cyclin** is a protein that is present in all eukaryotic cells, as it has an essential function in the cell cycle. CD kinases activate a number of proteins that are involved in mitosis. Additional members of the superfamily are the **mitogen-activated-protein kinases** (**MAPK**). **Mitogen** is a collective term for a variety of compounds, many of them of unknown structure, which stimulate mitosis, and thus the cell cycle, but also other reactions. G-proteins and phytohormones may act as mitogens. MAPKs play an important role in **protein kinase cascades**, where protein kinases are regulated through phosphorylation by other protein kinases. In such a cascade, a G-protein, for example, activates an MAP-kinase-kinase-kinase (**MAPKKK**), which activates by phosphorylation an MAP-kinase-kinase (**MAPKK**), which activates an MAP-kinase (**MAPK**). The MAP-kinase in turn phosphorylates various cellular components. In a

plant several of these signal cascades with different target proteins operate in parallel. Some of the cascade components overlap. The signal cascades regulate the expression of different genes by phosphorylation of a series of transcription factors (section 20.2). The **MAP-kinase-cascade** thus has an important regulatory function in the process of cell development and differentiation. Moreover, the MAP-kinase system is also involved in the signal cascades of **pathogen defense systems**, which are triggered by elicitors (section 16.1), and in the response to abiotic stress (e.g., heavy metals, salt, dryness, coldness, wounding). Genome sequencing revealed that 20 MAPKs, 10 MAPKKs, and 60 MAPKKKs exist in *Arabidopsis*.

Recently, protein kinases have been identified that phosphorylate **histidine** and **aspartate** residues of proteins and which do not belong to the superfamily mentioned previously. As will be discussed in section 19.7, histidine protein kinases are involved in the function of the receptors for ethylene and cytokinin.

Also, **protein phosphatases** exist in eukaryotes as a superfamily, with **serine-threonine-phosphatases** and **tyrosine-phosphatases** as different groups. Many of these phosphatases are regulated similarly to protein kinases (e.g., by binding of Ca^{2+} plus calmodulin or by phosphorylation). In this way the protein phosphatases also play an active role in signal transduction cascades. Research in this field is still at the beginning.

19.2 Phytohormones contain a variety of very different compounds

Phytohormones (Fig. 19.6) have very diverse structures and functions. Only a few examples of these functions will be summarized here. Indole acetic acid, an **auxin** derived from indole, stimulates cell elongation**. Gibberellins**, derivatives of gibberellane, induce elongation growth of internodes. Zeatin, a **cytokinin**, is a prenylated adenine and stimulates cell division. **Abscisic acid**, which is formed from carotenoids, regulates the water balance. **Ethylene** and **jasmonic acid** (the latter being a derivative of fatty acids, section 15.7) enhance senescence; **methyl jasmonate** plays a role in pathogene defense. **Brassinosteroids** have a key function in the regulation of cell development. **Peptide hormones** regulate plant development, and, in addition to **salicylic acid** and jasmonic acid, play a role in pathogen defense. In many cases, phytohormone function is caused by a pair of antagonistic phytohormones. Thus abscisic acid induces seed dormancy, and gibberellic acid terminates it.

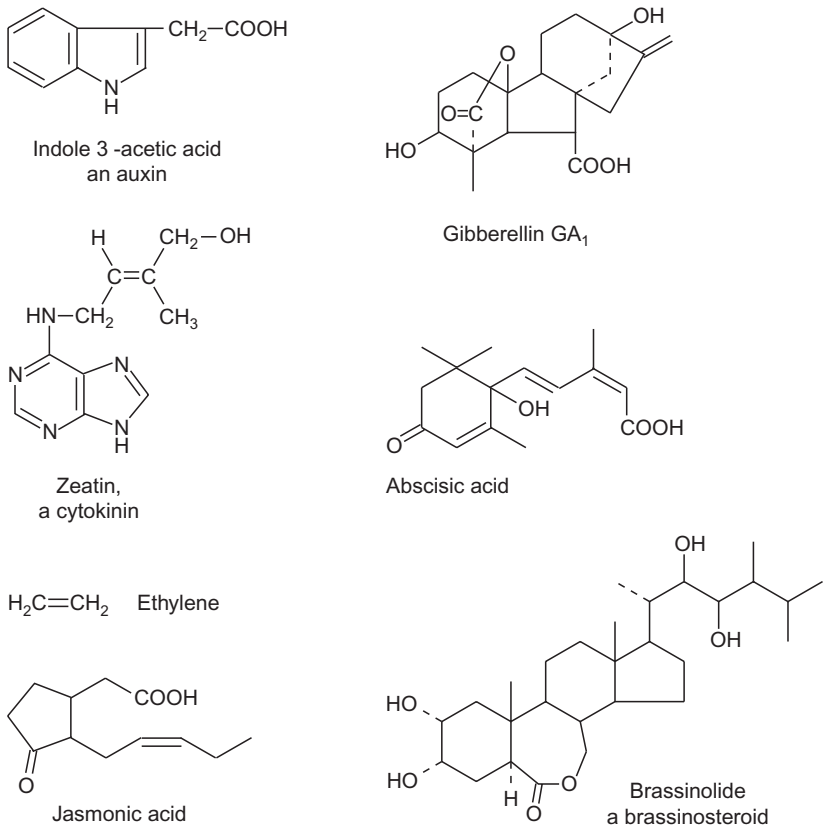


Figure 19.6 Chemical structures of some important phytohormones.

19.3 Auxin stimulates shoot elongation growth

Charles Darwin and his son Francis had already observed in 1880 that growing plant seedlings bend towards sunlight. They found that illumination of the tip initiated the bending of seedlings of canary grass (*Phalaris canariensis*). Since the growth zone is only a few millimeters from the tip, they assumed that a signal is transmitted from the tip to the growth zone. In 1926 the Dutch researcher Frits Went isolated from the tip of oat seedlings a growth-stimulating compound, which he named **auxin** and which was later identified as **indoleacetic acid** (**IAA**). Besides IAA, some other compounds are known with auxin properties (e.g., phenylacetic acid) (Fig. 19.8). The synthesis of IAA occurs not only in the shoot but also in the root. Different biosynthesis pathways are operating in different plants. Figure 19.7 shows two of these pathways.

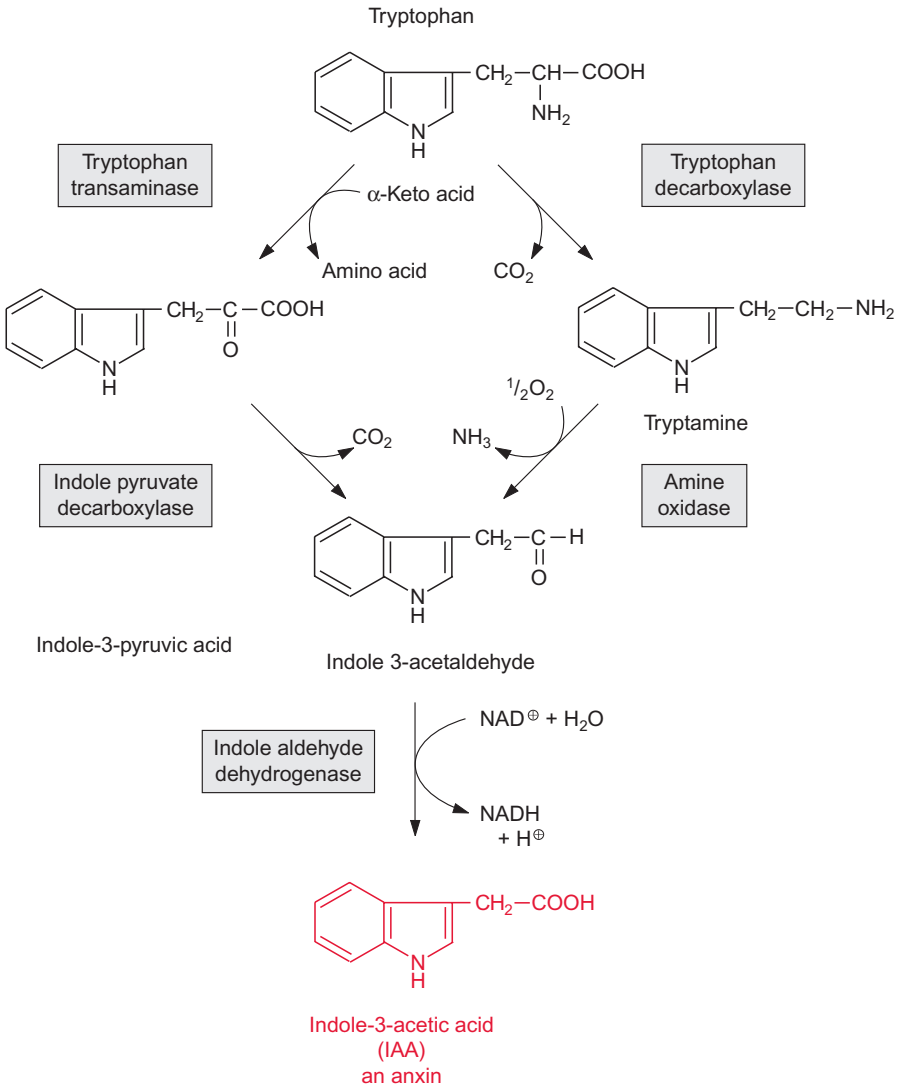


Figure 19.7 Presentation of two biosynthetic pathways for the synthesis of indole-3-acetic acid from tryptophan.

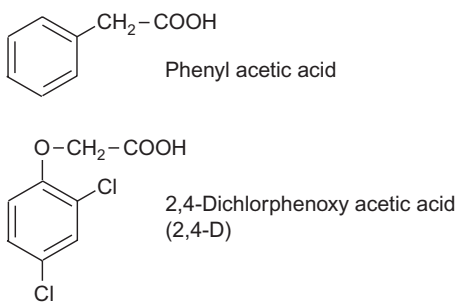


Figure 19.8 Phenylacetic acid, a compound with auxin properties, and 2,4D, a structural analogue of auxin, applied as a herbicide.

The synthetic auxin **2,4-dichlorophenoxyacetic acid** (**2,4-D**, Rohm & Haas) is used as a **herbicide**. It kills plants by acting as an especially powerful auxin, resulting in disordered morphogenesis and an increased synthesis of ethylene, which leads to a premature senescence of leaves. In the Vietnam War it was used as **Agent Orange** to defoliate forests. 2,4-D is a selective herbicide that destroys dicot plants. Monocots are insensitive to it, because they eliminate the herbicide by degradation. For this reason, 2,4-D is used for combating weeds in cereal crops.

Auxin functions in many ways. It influences embryogenesis, all types of organogenesis, maintenance of the root meristem, differentiation of vascular tissue, elongation growth of hypocotyls and roots, curvature of the coleoptiles, apical dominance, fruit ripening and the effects of the environment on plant growth. For a long time it was not clear how auxin was able to affect all these different processes. The key to this is the **polar transport** of auxin between different cells, resulting in its **asymmetric accumulation** in tissues and cells. Auxin is primarily synthesized at the tip of the shoot. From there it is transported from cell to cell by specific **influx** and **efflux carriers** of the plasma membrane. The protonated form of IAA (IAAH) is transported by a proton driven influx carrier (AUX1) into the cell where it is deprotonated and trapped. The efflux of IAA proceeds via another specific carrier (PIN). The polar transport is caused by an asymmetric distribution of these carriers. The membrane-bound efflux carrier proteins are transferred in a reversible fashion between membrane regions by vesicle transport via the Golgi apparatus. In this way the efflux carriers can be moved rapidly from one area of the plasma membrane to another to facilitate a polar transport. During the curvature of the coleoptiles, IAA is transported laterally to one side. The resulting differential stimulation of cell elongation at only one side of the shoot leads to the bending. IAA is also transported via the phloem from the leaves to distant parts of the plant.

The effect of IAA on cell growth in the shoot can be shown experimentally to occur within a few minutes after adding IAA. The hyperpolarization of the cell and an increase of phospholipase activity result in the opening of Ca^{2+} channels (section 19.1). The subsequent activation of **H^+-P-ATPases** (section 8.2) leads to the acidification of the cell wall region and subsequently to a loosening of the normally rigid cell wall. Shortly thereafter (15–30 min), the synthesis of proteins and xyloglucans begins, both as part of the epidermal cell wall synthesis and the elongation growth.

The **auxin receptor** (TIR) has been identified recently. The binding of auxin to this receptor recruits **AUX/IAA transcription factors** (section 20.2), which at low auxin concentrations together with other transcription factors (ARF) repress the expression of certain genes. Increased auxin concentrations result in a derepression of these genes and the formation

of auxin-inducible proteins. AUX/IAA transcription factors have a very short lifetime and are degraded in the proteasome after conjugation with **ubiquitin** (section 21.4). For this reason the AUX/IAA proteins are very well suited to function as on/off switches. A plant contains many genes of these TIR and AUX/IAA proteins, which explains the large repertoire of diverse auxin effects in different tissues.

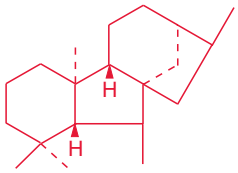
In different tissues and organs IAA has different cellular impacts. IAA stimulates cell division in the cambium, enhances **apical dominance** by suppression of lateral bud growth, and controls embryo development. Moreover, IAA prevents the formation of an abscission layer for leaves and fruits and is thus an antagonist to ethylene (section 19.7). On the other hand, increased IAA concentrations can induce the synthesis of ethylene.

Moreover, auxin induces the **formation of fruits**. Normally, seeds produce IAA only after fertilization. Transformants of eggplants that express a bacterial enzyme of IAA synthesis in the unfertilized seed were generated by genetic engineering. This IAA prevents the formation of seeds, resulting in seedless eggplants of normal consistency which are four times larger than usual. This is an impressive example of the importance of auxin for fruit growth and shows the possibilities of generating genetically altered vegetables.

19.4 Gibberellins regulate stem elongation

The discovery of gibberellins is related to a plant disease. The infection of rice by the fungus *Gibberella fujikuroi* results in the formation of extremely tall plants that fall over and bear no seeds. In Japan this disease was called "foolish seedling disease." In 1926 Eiichi Kurozawa and collaborators (Japan) isolated a compound from this fungus that induces unnatural growth. It was named **gibberellin**. These results were known in the West only after World War II. Structural analysis revealed that gibberellin is a mixture of various compounds with similar structures, which also occur in plants and act there as phytohormones.

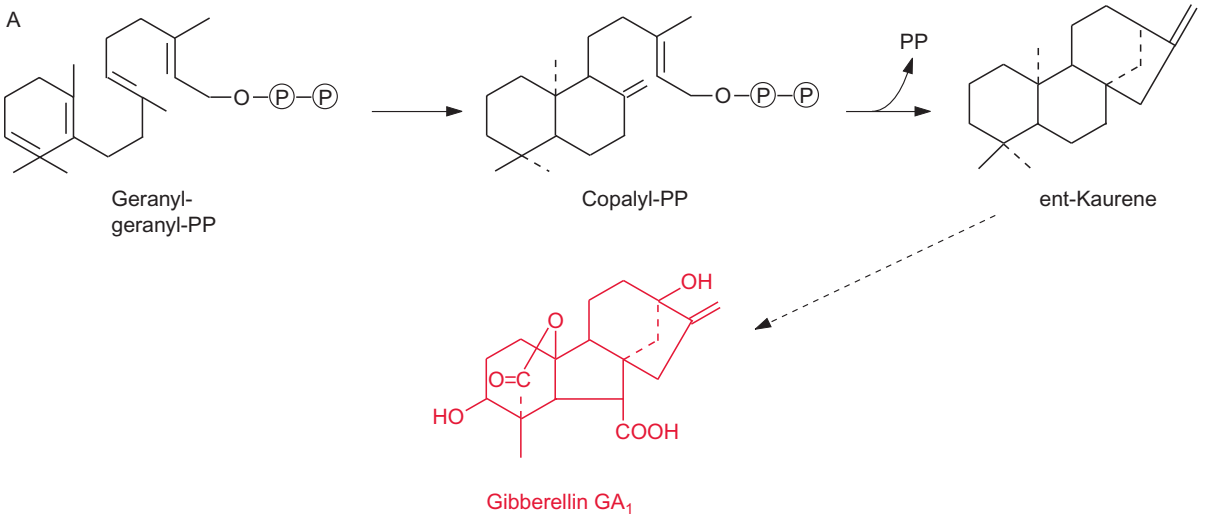
Gibberellins are derived from the hydrocarbon ***ent*-gibberellane** (Fig. 19.9). More than 100 gibberellin derivatives are now known in plants, which are numbered in the order of their identification. Therefore the numbering gives no information about structural relationships or functions. Many of these gibberellins are intermediates or by-products of the biosynthetic pathway. Only a few of them have been shown to act as phytohormones. Whether other gibberellins have a physiological function is not



ent-Gibberellane

Figure 19.9 Hydrocarbon from which gibberellins are derived.

A



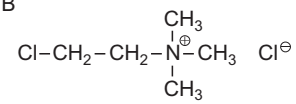
Geranyl-geranyl-PP

Copalyl-PP

ent-Kaurene

Gibberellin GA_1

B



2-Chlorethyltrimethylammonium-chloride (Cycocel, BASF)

Figure 19.10 A. Synthesis of gibberellin GA_1. B. Cycocel (BASF), a retardant that decreases the growth of stalks in wheat and other cereals, inhibits kaurene synthesis and thus also the synthesis of gibberellins.

known. The most important gibberellins are GA_1 (Fig. 19.10A) and GA_4 (not shown). Gibberellins derive from **isoprenoids** (Chapter 17). The synthesis proceeding in 13 steps takes place in three compartments. At first geranylgeranyl pyrophosphate is converted in the **plastids** to *ent*-kaurene. Subsequently *ent*-kaurene is transformed to GA_{12}-aldehyde by a cyt-P450 monooxygenase located in the membrane of the endoplasmatic reticulum

(**ER**) (section 18.2). Finally gibberellin (GA_{12}) is synthesized in the **cytosol**. This reaction involves the catalysis by oxoglutarate-dependent dioxygenases (GA oxidases).

Similar to IAA, gibberellins **stimulate shoot elongation**, especially in the internodes of the stems. A pronounced gibberellin effect is that it induces rosette plants (e.g., spinach or lettuce) to initiate and regulate the formation of flowers and flowering. Additionally, gibberellins have a number of other functions such as the preformation of fruits and the stimulation of their growth. Gibberellins terminate **seed dormancy**, probably by softening the seed coat, and facilitate seed germination by the expression of genes for the necessary enzymes (e.g., amylases).

The use of gibberellins is of economic importance for the production of **long**, **seedless grapes**. In these grapes, GA_1 causes not only extension of the cells, but also parthenocarpy (the generation of the fruit as a result of parthogenesis). Moreover, in the malting of barley for beer brewing, gibberellin is added to induce the formation of α-amylase in the barley grains. The gibberellin GA_3, produced by the fungus *Gibberella fujikuroi*, is generally used for these purposes. Inhibitors of gibberellin biosynthesis are commercially used as **retardants** (growth inhibitors). A number of substances that inhibit the synthesis of the gibberellin precursor *ent*-kaurene, such as **chloroethyltrimethyl ammonia chloride** (trade name Cycocel, BASF) (Fig. 19.10B) are sprayed on cereal fields to decrease the **growth of the stalks**. This enhances the strength of the cereal stalks and at the same time increases the proportion of total biomass in seeds. Slowly degradable gibberellin synthesis inhibitors are used in horticulture to keep indoor plants small.

Gibberellins influence gene expression. A soluble protein (GID1), which resembles a hormone-sensitive lipase, has been identified as a GA receptor. *Arabidopsis* contains three genes for GID1 proteins. When GA binds to this receptor, the complex then transmits the information to so-called cytosolic **DELLA proteins**. The latter usually repress plant growth. In this way the binding of GA to the receptor ultimately leads to an increase of gene expression, resulting in an increase of elongation growth. Thus, GA causes **the relief of a restraint**.

Mutants, in which the synthesis of GA or the function of GA on growth was impaired, turned out to be important for agriculture. The dramatic increase in the yield of cereal crops achieved after 1950, often named the "**green revolution**," is in part due to the introduction of **dwarf wheat lines**. At that time, attempts to increase the crop yield of traditional wheat varieties by an increased application of nitrogen fertilizer failed, since it produced more straw biomass instead of enhancing the grain yield. This was averted by breeding wheat varieties with **reduced stalk growth**, where the portion of grains in the total biomass (harvest index) was increased

considerably. It turned out that the decreased stalk growth in these varieties was due to the mutation of genes encoding transcription factors of the gibberellin signal transduction chain. In wheat, the mutated genes have been termed **Rht** (reduced height).

19.5 Cytokinins stimulate cell division

Cytokinins are prenylated derivatives of adenine. In **zeatin**, which is the most common cytokinin, the amino group of adenine is linked with the hydroxylated isoprene residue in the *trans*-position (Fig. 19.11). In other cytokinins benzyl derivatives, sugars or sugar phosphates are attached to the adenine. Cytokinins enhance plant growth by stimulating **cell division** and increase the sprouting of lateral buds. As cytokinins override apical dominance, they are antagonists of the auxin IAA. Cytokinins **retard senescence** and thus counteract the phytohormone ethylene (section 19.7). The larvae of some butterflies (e.g., *Stigmella,* which invade beech trees) use this principle for their nutrition. They excrete cytokinin with their saliva and thus prevent senescence of the leaves on which they are feeding. As a result, **green islands** of intact leaf material remain in yellowing autumn leaves, which provide, beyond the actual vegetation period, the caterpillars with the forage they need to form pupae.

Mature (i.e., differentiated) plant cells normally stop dividing. By adding cytokinin and auxin, differentiated cells can be induced to initiate cell division again. When a leaf piece is placed on a solid culture medium containing auxin and cytokinin, leaf cells start unlimited growth, resulting in the formation of a callus that can be propagated in tissue culture. Upon the application of a certain cytokinin/auxin ratio, a new shoot can be regenerated from single cells of this callus. The use of tissue culture for the generation of transgenic plants will be described in section 22.3.

In nature some plant associated bacteria and fungi produce auxin and cytokinin to induce unlimited cell division, which results in tumor growth of the plant. The formation of the **crown gall** induced by ***Agrobacterium tumefaciens*** (see section 22.2) is caused by a stimulation of the production of cytokinin and auxin. The bacterium does not produce these phytohormones itself, but transfers the genes for the biosynthesis of cytokinin and auxin from its T_i plasmid to the plant genome.

Zeatin is formed from AMP and dimethyallylpyrophosphate (Fig. 19.11). The isoprene residue is transferred by **cytokinin synthase** (a prenyl transferase, see section 17.2) to the amino group of the AMP and is then hydroxylated. Cytokinin synthesis takes place primarily in the meristematic

tissues. Transgenic tobacco plants in which the activity of cytokinin synthase in the leaves is increased have a much longer lifespan than normal plants, since their senescence is suppressed by the enhanced production of cytokinin.

Cytokinin receptors, like ethylene receptors, are **dimeric histidine kinases**. They are located in the plasma membrane, where the receptor site is directed to the extracellular compartment and the kinase is directed to the cytoplasm. The kinase moiety of the dimer comprises two histidine residues and two aspartyl residues. Upon binding of cytokinin, the two histidine kinases phosphorylate their histidine residues reciprocally (**autophosphorylation**). Subsequently, the phosphate groups are transferred to histidine residues or aspartyl residues of **transmitter proteins** (signal components). The transmitter proteins are channeled into the nucleus, where they function as transcription factors and thus regulate the expression of many genes.

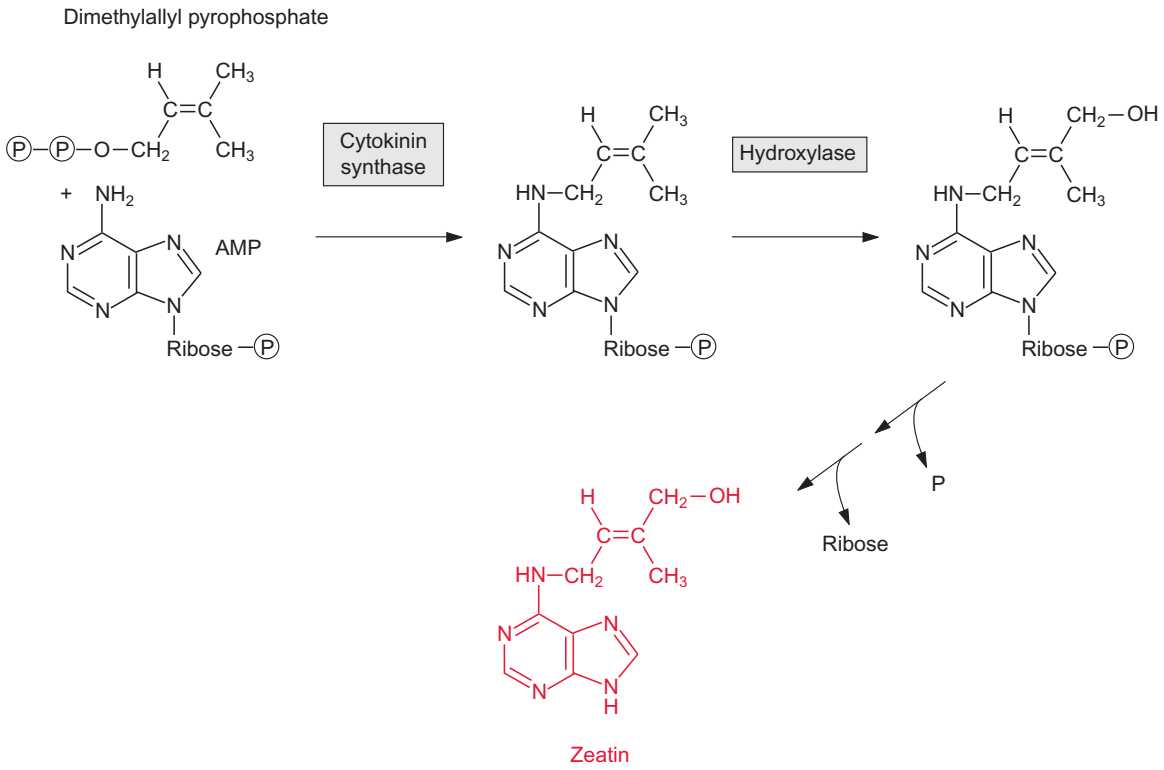


Figure 19.11 Synthesis of zeatin, a cytokinin.

19.6 Abscisic acid controls the water balance of the plant

When searching for what causes the abscission of leaves and fruits, **abscisic acid** (**ABA**) (Fig. 19.12) was found to be an inducing factor and was named accordingly. Later it turned out that the formation of the abscission layer for leaves and fruits is induced primarily by ethylene (section 19.7). An important function of the phytohormone ABA, however, is the induction of **dormancy** (endogenic rest) of seeds and buds. Moreover, ABA has a major function in maintaining the **water balance of plants**, since it induces with nitric oxide (**NO**) the closure of the stomata during water shortage (section 8.2). In addition, ABA prevents germination before the seeds are mature (vivipary). ABA deficient tomato mutants have wilting leaves and fruits, due to the disturbance of the water balance. In these wilting mutants, the immature seeds germinate within the tomato fruits while they are still attached to the mother plant.

ABA is a product of isoprenoid metabolism. The synthesis of ABA proceeds in two different ways via oxidation of **violaxanthin** (Fig. 19.12, see also Fig. 3.41). ABA synthesis occurs in leaves and also in roots, where water shortage would have a direct impact. ABA can be transported by the transpiration stream via the xylem vessels from the roots to the leaves, where it induces closure of the stomata (section 8.2).

In leaves, beside stoma closure ABA also causes rapid alterations in metabolism by influencing gene expression. Two ABA receptors have been identified, a soluble receptor named **FCA** (flowering time control) and a

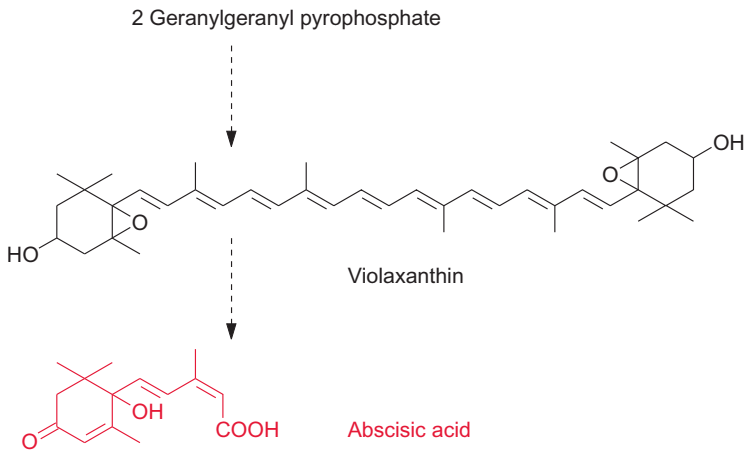


Figure 19.12 Abscisic acid is synthesized in several steps by the oxidative degradation of violaxanthin.

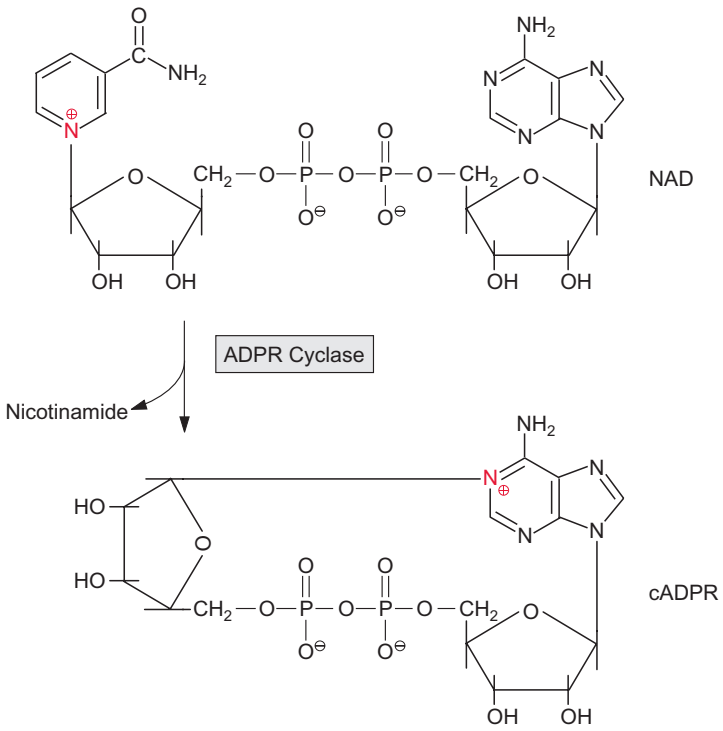


Figure 19.13 Cyclic ADP-ribose, a signaling component, causes Ca^{2+} ions to be released into the cytosol. This compound is ubiquitous in the plant and animal kingdoms. The synthesis of cyclic ADP-ribose from NAD^+ by an ADPR-cyclase: the ribose moiety (left side of the figure) is transferred from the positively charged N atom of the pyridine ring (nicotinamide) to the likewise positively charged N atom in the adenine ring.

membrane bound receptor **GRC2** (G-protein coupled receptor). The formation of the soluble FCA-ABA complex causes a delay in flowering. The ABA-GRC2 complex reacts with a G-protein (section 19.1). The release of a Gα-subunit regulates the K^+-dependent stoma closure and also embryo development. There is, however, yet another signal chain for ABA action involving the release of Ca^{2+} ions via the phosphoinositol pathway with the participation of phospholipase C (Fig. 19.13) and the signal component cyclic ADP ribose (cADPR, Fig. 19.13).

19.7 Ethylene makes fruit ripen

Ethylene is involved in the induction of **senescence**. During senescence, the degradation of leaf material is initiated. Proteins are degraded to amino acids, which, together with certain ions (e.g., Mg^{2+}), are withdrawn from the senescing leaves via the phloem for reutilization. In perennial plants, these substances are stored in the stem or in the roots, and in annual plants

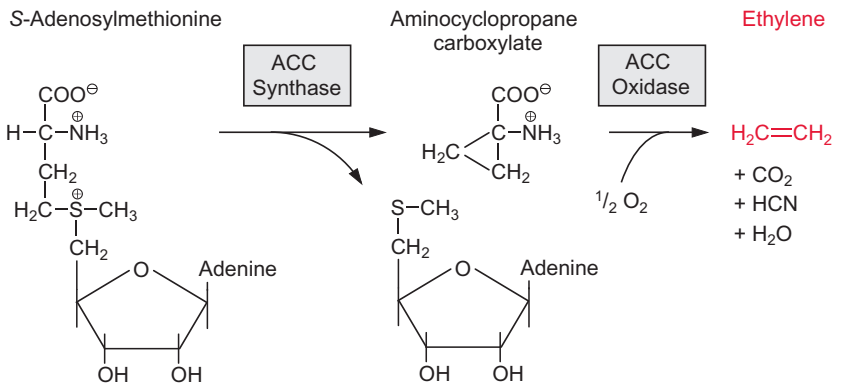


Figure 19.14 Synthesis of ethylene.

they are utilized to enhance the formation of seeds. Ethylene induces **defense reactions** after infection by fungi or when plants are wounded by feeding animals. As an example, the induction of the synthesis of tannins by ethylene in acacia as a response to feeding antelopes has been discussed in section 18.7.

In addition to stimulating the **abscission of fruit**, ethylene has a general function in **fruit ripening**. The ripening of fruit is to be regarded as a special form of senescence. The effect of gaseous ethylene can be demonstrated by placing a ripe apple and a green tomato together in a plastic bag; ethylene produced by the apple accelerates the ripening of the tomato. Bananas are harvested green and transported halfway around the world under conditions that suppress ethylene synthesis (low temperature, CO_2 atmosphere). Before being sold, these bananas are ripened by gassing them with ethylene. Also, tomatoes are often ripened only prior to sale by exposure to ethylene.

***S*-adenosylmethionine** (Fig. 12.10) is a biological methyl group donor as well as the precursor for the synthesis of ethylene (Fig. 19.14). The positive charge of the sulfur atom in *S*-adenosylmethionine enables its cleavage to form a cyclopropane, in a reaction catalyzed by **aminocyclopropane carboxylate synthase**, abbreviated **ACC synthase**. This enzyme limits the rate of ethylene biosynthesis. The fact that *Arabidopsis* contains eight genes for this enzyme illustrates its importance. The amount and stability (half life time 20 min–2 h) of ACC synthase is regulated by MAPK and CDPK phosphorylation (section 19.1). Subsequently, **ACC oxidase** catalyzes the oxidation of the cyclopropane to ethylene with the release of CO_2, HCN, and water. HCN is immediately detoxified by conversion to β-cyanoalanine (reaction not shown).

Genetic engineering has been employed to suppress ethylene synthesis in tomato fruits in two different ways. One possibility is to decrease the activities

of ACC synthase and ACC oxidase by antisense technique (section 22.5). Another alternative is the introduction of a bacterial gene into the plants, which encodes an ACC deaminase. This enzyme degrades the ACC in the tomato fruits so rapidly that consequently the ethylene levels are significantly reduced. The aim of this genetic engineering is to produce tomatoes that delay the ripening process during transport. It may be noted that transgenic tomato plants have also been generated, in which the durability of the harvested fruits is prolonged by an antisense repression of the polygalacturonidase, which is an enzyme that plays a role in lysing the cell wall.

The effect of ethylene is caused by an alteration of gene expression. Since ethylene, like other phytohormones, exerts its effect at very low concentrations (~10^{-9}mol/L), the **ethylene receptor** is expected to have a very high affinity. Like the cytokinin receptor, it consists of a dimer of **histidine receptor kinases**, each containing a histidine residue, which, after autophosphorylation, transfers the phosphate group to histidine or aspartyl residues of target proteins. By the binding of ethylene to the receptor dimer, in which a copper cofactor is involved, the kinase is inactivated and autophosphorylation is prevented. Depending on the phosphorylation state of the ethylene receptor, a signal is transmitted via protein kinase cascades, in which MAPKK and MAPK (section 19.1) participate. This activates signal cascade transcription factors which control the expression of certain genes. It may be noted that histidine kinases occur in plants, yeast, and bacteria, but not in animals.

The signal cascades of the action of ethylene, auxin, cytokinins, brassinosteroids, gibberellins, abscisic acid, and of abiotic stress are interwoven to a network.

19.8 Plants also contain steroid and peptide hormones

Brassinosteroids control plant development

For a long time many steroid hormones with a multitude of effects have been known in animals, but only recently was it discovered that steroid hormones also occur in plants. So-called **brassinosteroids** have essential functions as phytohormones**. Brassinolide** (Fig. 19.15) is the most well-known member of this phytohormone class. In the meantime more than 40 other polyhydroxylated steroids have been identified in plants. Brassinosteroids are synthesized via the isoprenoid biosynthesis pathway with the membrane lipid campesterol (Fig. 15.3) as intermediate. Brassinosteroids are contained

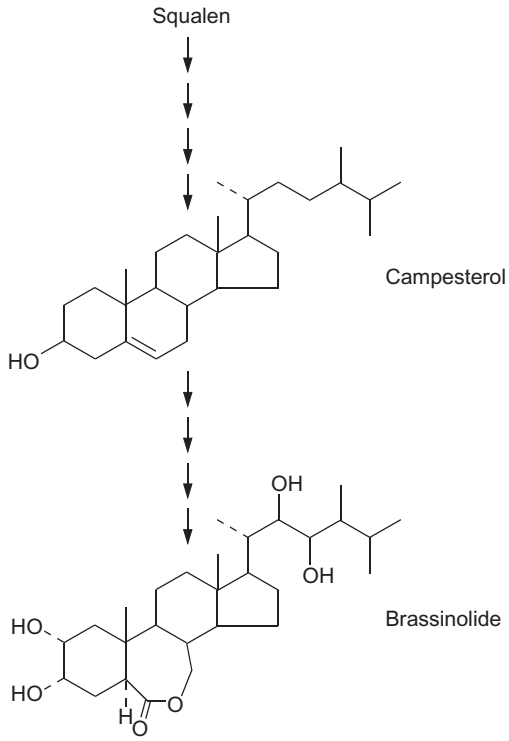


Figure 19.15 Brassinolide is synthesized from the membrane lipid campesterol via a series of synthesis steps involving cyt P_{450}-dependent hydroxylases, a reductase, and others. The synthesis pathway is very similar to the corresponding steroid synthesis pathways in animals.

in all plant organs and regulate the plant development in multiple ways. They stimulate the growth of the shoot, the unfolding of leaves, the differentiation of the xylem, retardation of root growth and the formation of anthocyans.

Brassinosteroids were first isolated from pollen. It was known that pollen contains a growth factor. In 1979 scientists from the US Department of Agriculture isolated from 40 kg of rape pollen collected by bees, 4 mg of a substance that they identified as brassinolide. Later, using very sensitive analysis techniques, it was shown that plants in general contained brassinolide and other brassinosteroids. The function of brassinosteroids as essential phytohormones was clearly established from the study of *Arabidopsis* mutants with developmental defects, such as **dwarf growth**, **reduced apical dominance**, and **lowered fertility**. The search for the defective gene responsible revealed that such mutations affected enzymes of the brassinolide synthesis pathway, which turned out to have very great similarities with

the synthesis pathway of animal steroid hormones. These developmental defects could not be prevented by the addition of "classic" phytohormones, but only by an injection of a nanomolar amount of brassinolide, as contained in plants. These results clearly demonstrated the essential function of brassinosteroids for the growth and development of plants.

In **animal cells**, steroid hormones bind to defined **steroid receptors**, which are present in the cytoplasm. Once activated, the receptor complex is transferred to the nucleus to promote or repress the expression of certain genes. It seems that plant steroids do not function in this way, as plants lack homologues of animal steroid receptors. In plants the steroid hormones associate with receptors bound to the plasma membrane. These receptors belong to the class of leucin rich repeat receptor-like kinases (**LRR-RLK**, section 19.1). Via a signal cascade, not fully resolved and involving several protein kinases and transcription factors, genes are activated which govern, e.g., cell modification, the synthesis of the cytoskeleton and the synthesis and transport of other phytohormones.

Polypeptides function as phytohormones

It is well known that small peptides, such as insulin and glucagon, have an important function in the intercellular communication in animals. Peptide hormones also play a role in plants. There is increasing evidence that plants contain secretory and nonsecretory peptides involved in the regulation of various aspects of plant growth (e.g., growth of callus and roots, organization of the meristem, nodule formation, self-incompatibility and defense reactions).

Systemin induces defense against herbivore attack

Many plants respond to insect attacks by accumulating **proteinase inhibitors**, which are toxic to insects because they impair their digestion of proteins. It was shown in tomato plants that the polypeptide **systemin**, consisting of 18 amino acids, is involved in defense reactions. In response to insect attack, a systemin precursor protein, consisting of 200 amino acids, is synthesized, and then processed by endoproteases to release the active polypeptide. Systemin is perceived by a membrane-bound receptor of very high affinity. A systemin concentration of as low as 10^{-10}mol/L is sufficient for a half saturation of the receptor. The receptor was identified as a receptor-like kinase (**RLK**) (section 19.1), similar to the LRR-RLK that binds brassinoid steroids. The binding of systemin induces a signal transduction chain involving Ca^{2+} calmodulin accumulation, the inactivation of an H^+-P-ATPase, the activation of MAPK protein kinases and of a phospholipase

(section 19.1). The latter catalyzes the release of linolenic acid from membrane lipids, which is a precursor for the synthesis of **jasmonic acid** (section 15.7). **Jasmonic acid** is a key signal in the transcription activation of defense-related genes (e.g., for the synthesis of proteinase inhibitor) (section 19.9). It may be noted that the effect of systemin is species specific. Systemin from tomato also affects potato and pepper, but it has no effect on the closely related tobacco or on other plants. Tobacco produces a systemin-like polypeptide, with a structure similar to the tomato systemin, and has analogous effects. It remains to be elucidated whether systemin-like polypeptide hormones may be involved in defense reactions of other plants.

Phytosulfokines regulate cell proliferation

A factor enhancing the proliferation of the cells was found in media of cell suspension cultures. This factor was isolated and identified as a pentapeptide, named **phytosulfokine** (PSK) containing two tyrosine residues, of which the hydroxyl group is esterified with sulfate:

PSK$_{\alpha}$: Tyr (SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln

The *Arabidopsis* genome has five genes encoding phytosulfokine precursors consisting of about 80 amino acids and an N-terminal secretion signal. Phytosulfokines with identical structures occur in many plants and have, in addition to auxin and cytokinins, an important regulatory effect on the **dedifferentiation** of cells. Plant cells can retain the ability of totipotency, which means that cells can dedifferentiate so that they can reenter the cell cycle to form all the organs of a new plant. A receptor-like protein kinase (**RLK**) has been identified as a receptor for phytosulfokines and is probably connected via signal cascades to transcription factors regulating genes of dedifferentiation and proliferation.

A small protein causes the alkalization of cell culture medium

In the course of the isolation of systemin another peptide of 49 amino acids (deriving from a precursor of 115 amino acids) was identified that caused a rapid alkalization of the media of tobacco cell suspension cultures. This peptide was called **RALF** (rapid alkalization factor). The application of RALF in nanomolar concentrations resulted in a rapid activation of MAPK, the termination of root growth and the enlargement of meristematic cells. Homologues of RALF are found in many plant species. In

Arabidopsis, nine different RALF genes have been identified that are expressed in different organs of the plant. The ubiquity of RALF polypeptides suggests that they play a general role in plants, which remains to be elucidated.

Small cysteine-rich proteins regulate self-incompatibility

Self-incompatibility is a mechanism ensuring that the pollen does not self-fertilize or fertilizes the same or closely related plants. Various plants have different methods for excluding self-fertilization. In *Brassica* species, for example, **S-locus proteins** are involved, including SL glycoproteins (**SLG**), SL receptor kinases (**SRK**) and small proteins (**SCR**) of 74–81 amino acids. The application of only 50×10^{-12}mol of a recombinant SCR protein to a stigma resulted in the inhibition of the hydration of the pollen, which prevents it from being fertilized. It was also shown that SCR proteins interact with an RLK receptor kinase SLK, resulting in an autophosphorylation. Other elements of the signal cascade remain to be elucidated. Characteristic for the small SCR proteins are four disulfide linkages between cysteine residues C1 and C8, C2 and C5, C3 and C6, and C4 and C7.

19.9 Defense reactions are triggered by the interplay of several signals

Plants defend themselves against pathogenic bacteria and fungi by producing **phytoalexins** (section 16.1), and in some cases also by programmed cell death (**hypersensitive reaction**), in order to control an infection. Animals feeding on plants may stimulate the production of defense compounds, which make the plant poisonous or indigestible.

These various defense reactions are initiated by the interplay of several signal components in a network. After an attack by pathogens or as a response to abiotic stress, signal cascades, including the phosphoinositol cascade (section 19.1), are induced, which lead to an increase of the **Ca^{2+}**concentration in the cytosol, which activates the Ca^{2+}-dependent protein kinases (**CDK**). This in turn activates **protein kinase cascades**, which modulate gene expression via transcription factors (section 19.1). Moreover, in an early response, superoxide ($\bullet O_2^-$) and/or H_2O_2 (**r**eactive **o**xygen **s**pecies (**ROS**)) are synthesized by an NADPH oxidase located in the plasma membrane. The ROS represent chemical weapons for a direct attack on the pathogens, but they are also signal components for inducing signal cascades to initiate the production

of other defense compounds. H_2O_2 is involved in the lignification process (section 18.3) and thus plays a role in the solidification of the cell wall, another defense strategy against pathogens.

The formation of nitric oxide (**•NO**), a radical, is a further early response to pathogen attack. It is known as a signal component in animals and plants, and is released by the oxidation of arginine, which is catalyzed by nitric oxide synthase.

$$\text{Arginine} + O_2 + \text{NADPH} \xrightarrow{\textit{Nitric oxide synthase}} \text{Citrulline} + \text{NADP}^+ + \text{NO}$$

Alternatively, NO can be formed from nitrite in a side-reaction of the nitrate reductase (section 10.1).

$$NO_2^- + \text{NAD(P)H} + H^+ \xrightarrow{\textit{Nitrate reductase}} \text{NO} + H_2O + \text{NAD(P)}^+$$

NO is an important messenger in hormonal responses in plants and is involved in the defense against biotic and abiotic stress. NO is an important signal component that regulates, via cGMP and ADP ribose, Ca^{2+} channels to increase the cytoplasmic Ca^{2+} concentration from intracellular stores, and in this way activates signal cascades. In connection with MAPK cascades, it promotes the synthesis of phytoalexins and is involved in the initiation of programmed cell death. In addition to abscisic acid, it induces the opening of stomata (section 8.2).

A precise control of the NO concentration in the cell is needed for it to function as a signal component. The regulation of its synthesis is to a large extent still unknown. A glutathione-dependent formaldehyde dehydrogenase (FALDH) is involved in the control of the cellular NO concentration, by which NO is reversibly bound to glutathione. NO is eliminated by oxidation to nitrate.

Salicylic acid and jasmonic acid are signal molecules in pathogen defense

The biosynthesis of **salicylic acid** (**SA**) is described in section 18.2 and that of **jasmonic acid** (**JA**) in section 15.7. Jasmonic acid and salicylic acid are both involved in signal cascades induced during pathogen attack. SA plays a crucial role in defense responses against biotrophic pathogens (which keep the cell alive), and hemi-biotrophic pathogens (which initially keep the cell alive but kill them at a later stage). Mutants of transgenic tobacco plants,

where the synthesis of salicylic acid had been intercepted, proved to be very vulnerable to infections by biotrophic and hemi-biotrophic pathogens. Enzymes induced by salicylic acid include β1.3 glucanase, which digests the cell wall of fungi, and lipoxygenase, a crucial enzyme in the pathway of the synthesis of jasmonic acid (Figs. 15.29 and 15.30). JA and ethylene are involved in the defense against herbivorous insect and necrotrophic pathogens (which kill cells).

A number of components of the SA signaling cascade which regulate gene expression via transcription factors have by now been identified. Also for JA main signaling components are known, which interact with transcription factors. Both the SA and JA defense pathways contain different components, between which there is, however, positive and negative cross talk. Plant hormone signaling pathways are not isolated pathways but are interconnected with complex regulatory networks involving various defense signaling pathways and developmental processes. A better understanding of phytohormone-mediated plant defense responses is important in designing effective strategies for engineering crops for disease and pest resistance.

JAs are involved in diverse processes such as seed germination, root growth, tuber formation, tendril coiling, fruit ripening, leaf senescence and stomatal opening. Jasmonic acid regulates the **development of pollen** in some plants. *Arabidopsis* mutants, which are unable to synthesize jasmonic acid, cannot produce functioning pollen and therefore are **male-sterile**. The formation of **jasmonic acid** (e.g., induced by systemin) is regulated by a signal cascade involving **Ca^{2+} ions** and **MAP kinases**. Also in the perception of jasmonic acid a MAP kinase cascade regulating transcription factors appears to be involved. Jasmonic acid, its methylester and its precursor 12-oxo-phytodienoic acid (**OPDA**) play a central role in defense reactions. As a response to fungal infection, jasmonic acid induces the synthesis of **phenylammonium lyase** (**PAL**) (Fig. 18.2), the entrance enzyme of phenyl propanoid biosynthesis and **chalcone synthase** (**CHS**) (Fig.18.11), the key enzyme of flavonoid biosynthesis (see Chapter 18). As a response to wounding by herbivores, jasmonic acid causes plants to produce proteinase inhibitors. As a response to mechanical stress (e.g., by wind), jasmonic acid triggers the elevated growth in the thickness of stems or tendrils to make the plants more stable.

In many cases an attack by herbivores initiates defense responses not only in the wounded leaves, but also in more distant parts of a plant (**systemic response**). This requires a long-distance transport of signal substances within the plant. Recent results indicate that **jasmonic acid** and **methyl jasmonate** function as such a **systemic wound signal** in establishing a systemic acquired resistance (**SAR**). Also **methyl salicylate** is responsible for a long-distance signal transfer.

19.10 Light sensors regulate growth and development of plants

Light controls plant development from germination to the formation of flowers in many different ways. Important light sensors are the **phytochromes** which sense red light. Phytochromes are involved when light initiates the germination and greening of the seedling and in the adaptation of the photosynthetic apparatus of the leaves to full sunlight or shade. Five different phytochromes (A–E) have been identified in the model plant *Arabidopsis thaliana* (section 20.1). Plants also have photoreceptors for blue and UV light for their adaptation to the full spectrum of sunlight. So far, three proteins have been identified as blue light receptors; these are **cryptochromes** 1 and 2, each comprising a flavin (Fig. 5.16) and a pterin (Fig. 10.3); and **phototropin**, containing one flavin as a blue light-absorbing pigment.

Phytochromes function as sensors for red light

Since the structure and function of **phytochromes** have been studied extensively in the past, they offer a good example for a detailed discussion on the problems of signal transduction in plants. Phytochromes are soluble **dimeric proteins**. The monomer consists of an apoprotein (molecular mass 120–130 kDa) with six domains (Fig. 19.16A). The first three domains represent the photosensoric part of the protein. These domains are also present in bacterial and fungal phytochromes. The remaining domains 4, 5, and 6 form the regulatory part. Plant phytochromes contain a sequence at the N-terminus of the apoprotein, which is involved in the Pfr → Pr conversion. Domain 6 possesses a kinase which binds ATP and catalyzes an autophosphorylation of the phytochrome. Domain 2 contains an open chain tetrapyrrole that is linked to the protein via a sulfhydryl group of a cysteine residue. It is the chromophore of the holoprotein (Fig. 19.16B). The autocatalytic binding of the tetrapyrrole to the apoprotein results in the formation of a phytochrome

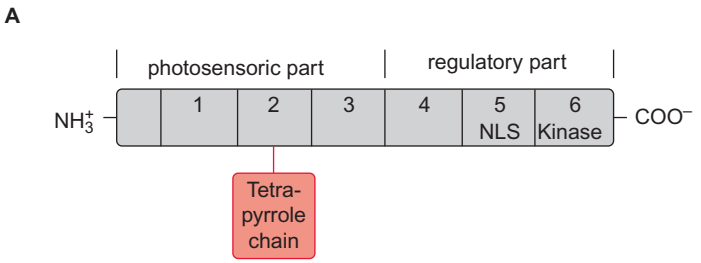


Figure 19.16A Structure of a phytochrome. The apoprotein is composed of 6 domains; domains 1–3 represent the photosensoric part and domains 4–6 the regulatory part. The chromophore, an open tetrapyrrole chain, is linked to domain 2. Domain 6 comprises a Ser/Thr kinase.

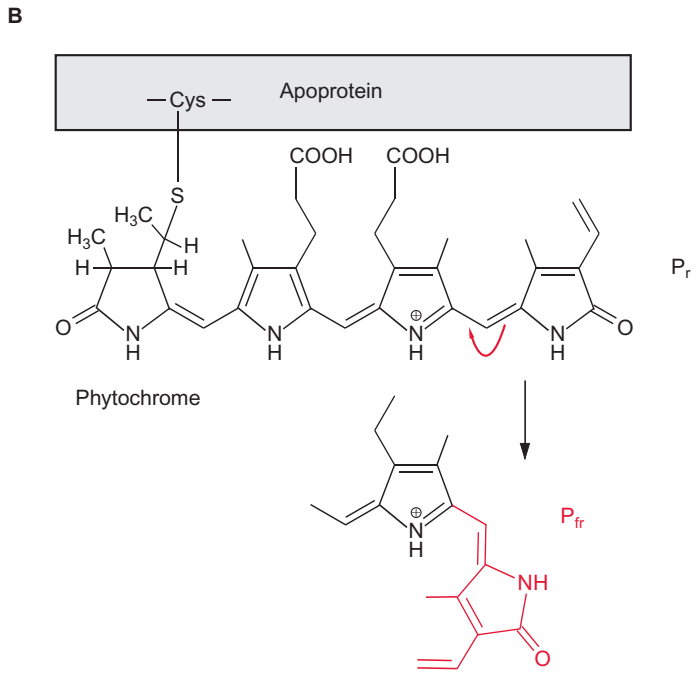


Figure 19.16B The chromophore of a phytochrome consists of an open tetrapyrrole chain, which is linked via a thioether bond to the apoprotein. The absorption of red light results in a *cis-trans*-isomerization of a double bond, causing a change in the position of one pyrrole ring (colored red).

P_r (r = red) with an absorption maximum at about 660 nm (**red light**) (Fig. 19.17). The absorption of this light results in a change in the chromophore; a double bond between the two pyrrole rings changes from the ***trans-*** to the ***cis-***configuration (colored red in Fig. 19.16B), which subsequently changes the conformation of the protein. The phytochrome in this new conformation has an absorption maximum at about 730 nm (**far-red light**) named **P_{fr}**, representing the active form of the phytochrome. It reflects the state of illumination. P_{fr} is reconverted to P_r by the absorption of far-red light. Since the light absorption of P_r and P_{fr} overlaps (Fig. 19.17), depending on the color of the irradiated light, a reversible equilibrium between P_r and P_{fr} is attained. Thus, with light of 660 nm, 88% of the total phytochromes are present as P_{fr} and at 720 nm, only 3% is in the P_{fr} form. In bright sunlight, where the red component is stronger than the far-red component, the phytochrome is present primarily as P_{fr} and indicates the state of illumination to the plant.

Whereas the inactive form of phytochrome (P_r) has quite a long lifetime (~100 h), the active form (P_{fr}) is converted within 30 to 60 minutes. P_{fr} can be recovered by a reversal of its formation (Fig. 19.18). In the case of phytochrome A, however, the light absorption can be terminated by conjugation of P_{fr} with **ubiquitin**, which marks it for proteolytic degradation by the **proteasome pathway** (section 21.4).

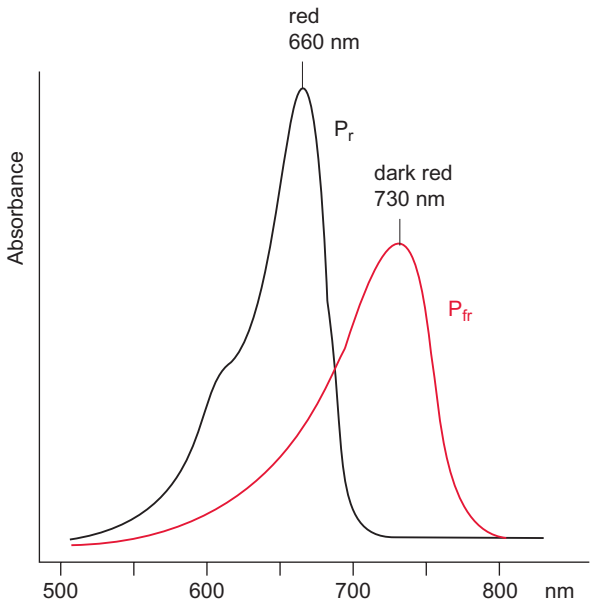


Figure 19.17 Absorption spectra of the two forms of phytochrome A, P_r and P_{fr}.

Phytochromes undergo a photoconversion upon illumination with red and far-red light. In addition to this, phytochromes are transferred between the cytosol and the nucleus. The C-terminal domain 5 (Fig. 19.16A) contains a **nuclear localization signal** (**NLS**) that is responsible for targeting to the nucleus. Under the influence of light the protein kinase of domain 6 causes an autophosphorylation of the protein. This phosphorylation releases the phytochrome from a cytosolic anchor and the NLS domain is uncovered. This allows the migration of the P_{fr} form of phytochrome into the nucleus, where it associates with factors, such as the **phytochrome interacting factor** (**PIF3**) and other transcription factors to affect **gene expression** (Fig. 19.18). It has been shown that phytochrome A affects the transcription of **10% of all genes** in *Arabidopsis*. This effect of phytochromes is modulated by phytohormones, such as cytokinins and brassinosteroids, and the signal cascades of pathogen defense. Light sensors, phytohormones, and defense reactions appear to be interwoven in a **cellular network**.

Phytochrome A, having been most thoroughly investigated, has an absorption maximum at far-red light and is instable in light, whereas the other phytochromes (B–E) are light stable. Phytochrome A often forms homodimers, while the other phytochromes aggregate to heterodimers. The functions of phytochromes B–E have still to be resolved in detail.

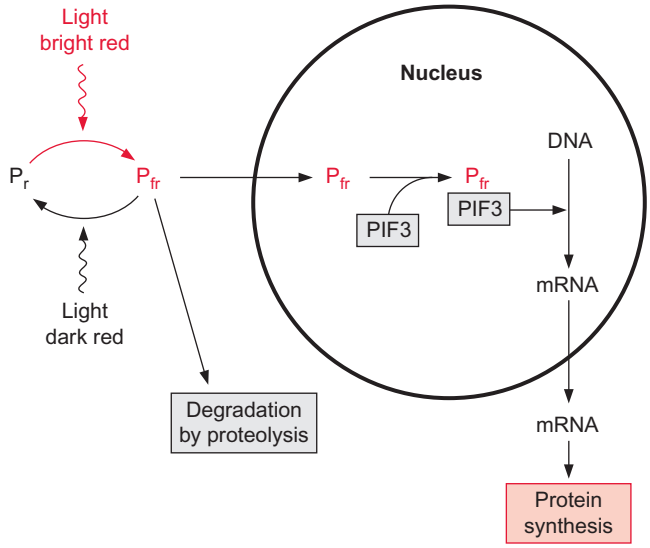


Figure 19.18 Phytochrome A influences gene expression. Phytochrome is converted by irradiation with red light to the active form P_{fr} and is reconverted by far-red light to the inactive P_r. P_{fr} enters the nucleus, where it binds to a transcription factor PIF3. The P_{fr}-PIF3-complex regulates gene expression by binding to promoter regions of the DNA. P_{fr} can also be irreversibly degraded by proteolysis.

Phototropin and cryptochromes are blue light receptors

Light sensors such as phototropin and cryptochrome are present in plant cells in order to use the blue spectrum (320–500 nm) of the sun efficiently. Blue light induces stoma opening, the elongation of the hypocotyls and the chloroplast movement. At low blue light intensity the chloroplasts move to that part of the cell exposed to the light in order to optimize light harvesting, whereas at higher blue light intensity the chloroplasts retreat from the light source to avoid destruction by excessive light.

Phototropin was first isolated in 1997 from pea cotyledons that had been irradiated with blue light, and was found to be ubiquitous in higher plants. The protein structure of phototropin shows similarities to the structure of phytochromes. As in phytochromes the photosensor is located at the N-terminal region which contains a flavine mononucleotide (FMN) (Fig. 5.16) as light absorbent. Moreover, there is a serine/threonine kinase at the C-terminal region, catalyzing the autophosphorylation of the protein. In the dark FMN is associated noncovalently (Fig. 19.19). Upon illumination the FMN is covalently bound within microseconds to a cysteine residue that changes the absorption maximum of the flavine from 447 nm to 390 nm. This induces the kinase at the C-terminal region to catalyze the autophosphorylation of 8 serine residues, converting the phototropin into an active state. In the dark the FMN is released again, the phosphate groups are split off by hydrolysis, presumably by a phosphatase, and the phototropin returns within 10–100 s to the inactive ground state.

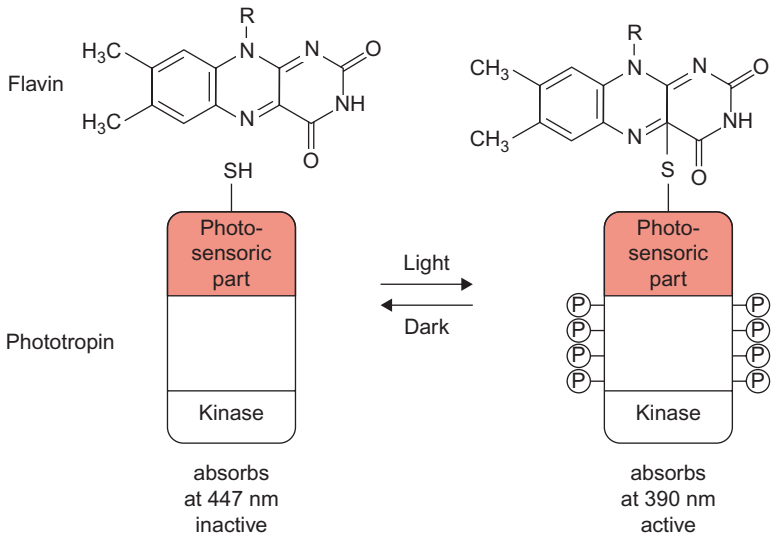


Figure 19.19 Action mode of phototropin. FMN, the chromophore of phototropin, is in the dark noncovalently bound to the protein and absorbs light of 447 nm. Illumination activates FMN to enter a covalent bond with a cysteine residue of the photosensoric part of phototropin. This induces an autophosphorylation of the protein resulting in the activation of phototropin.

Cryptochromes also absorb blue light but are not structurally related to phototropin. They derive from microbial DNA repair enzymes, so-called photolyases. They are flavoproteins influencing many processes in plants, such as hypocotyl and stem elongation, regulation of flowering time and anthocyan accumulation. Details of the interplay of the two blue light receptors, phototropins and cryptochromes, in plant development and metabolism are still largely unknown.

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