Auxins

15

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The term "auxin" is derived from the Greek word *auxein* which means "to increase." Auxins constitute an important group of naturally occurring hormones which have been detected practically in all land plants and in several soil- or plant-associated microbes. Naturally occurring auxins include indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), phenyl acetic acid, and 4-chloroindole-3-acetic acid (4-Cl-IAA). Of all these, IAA is the most extensively studied auxin (Fig. 15.1). Chemically, a common feature of all these molecules exhibiting auxin activity is the presence of acidic side chain on the aromatic ring. All the above natural auxins, except for phenyl acetic acid, are also indole derivatives.

15.1 Discovery of Auxin

Auxin was the first hormone discovered in plants with history dating back to the days of De Candolle (1832) who made the first observations on phototropism. He observed that indoor plants turn themselves toward the windows since they seek light (and not air). Ciesielski (1872) observed loss of gravitropism upon removal of root tips and its restoration by the replacement of severed tip. From this he concluded that some "influence" is transmitted from the root tip to the bending part of the root before it is amputated. Subsequent detailed investigations by Charles Darwin and his son Francis, published in their book entitled *The Power of Movement in Plants* in 1881, showed that when coleoptiles of the dark-grown canary grass, *Phalaris canariensis*, are exposed to unilateral light, some influence is transmitted from the upper part of the coleoptile downward causing the lower part to bend. When the seedling coleoptile is decapitated or an opaque cap is placed on the tip, the bending response to unilateral light is prevented. It was obvious that the tip of the coleoptile was the site of light perception. Paal (1914–1919) showed that removal of the coleoptile tip in dark stops growth and replacing it on one side causes curvature

S. C Bhatla, M. A. Lal, *Plant Physiology, Development and Metabolism*,
https://doi.org/10.1007/978-981-13-2023-1_15

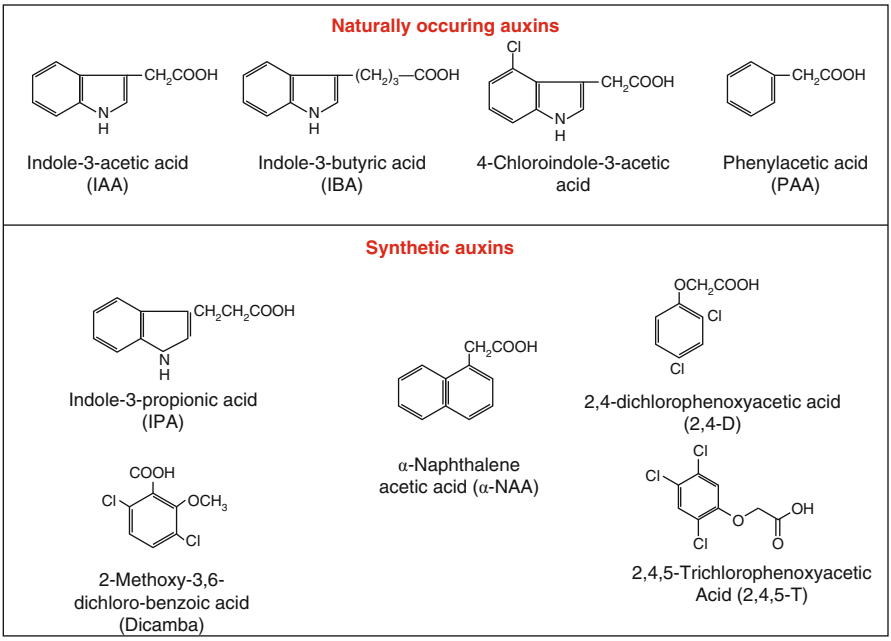
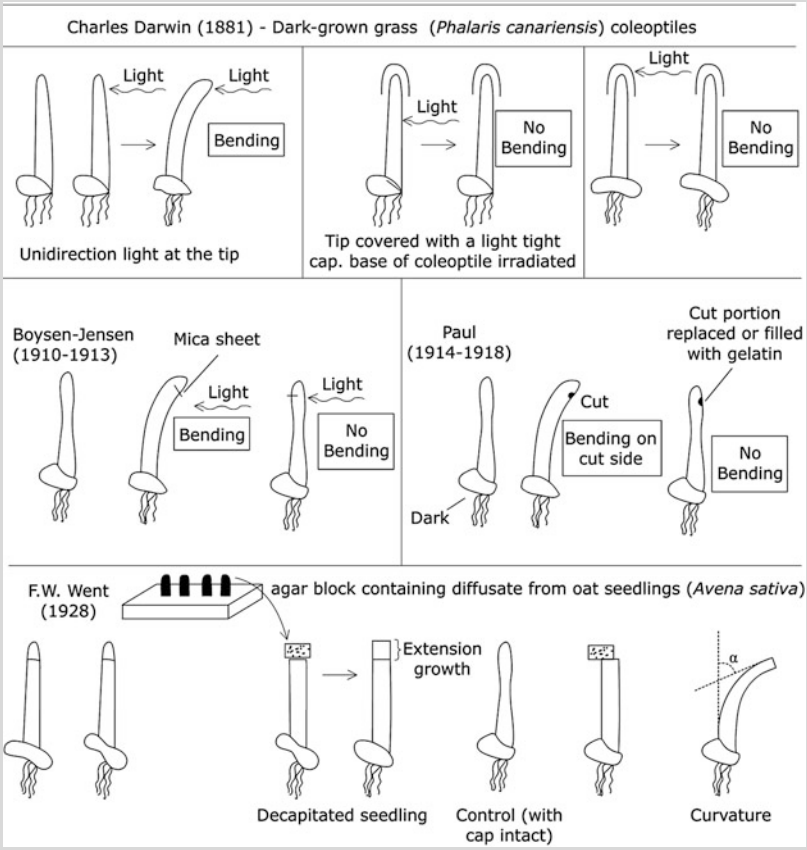


Fig. 15.1 Structures of some naturally occurring and synthetic auxins

(Box 15.1). Boysen-Jensen (1910–1913) demonstrated that phototropism would fail when the downward flow of material on the shaded side is interrupted by a piece of mica, but not when mica is placed on the lighted side, indicating the presence of a growth **promoter**, rather than an **inhibitor**. Later workers proposed the presence of a growth-inducing substance (*Wuchsstoff*) which was proposed to exhibit polar movement from the tip to the zone of elongation, thereby causing asymmetric growth. Söding (1923) and Cholodny (1924) were the first to call the "Wuchsstoff" a hormone. In 1928, F. W. Went reported a quantitative assay for the diffusible substance responsible for curvature response in *Avena sativa* (oat) coleoptiles. The assay was referred as *Avena* curvature assay, and it established that the extent of curvature is proportional to the amount of growth substance diffusing from the tip into the lower region (Box 15.2). This concept is still called the **Cholodny-Went theory**. Went's experimental approach was to place coleoptile tips on agar blocks to let the "Wuchsstoff" accumulate in the agar block. These "Wuchsstoff"-containing agar blocks, when placed asymmetrically on the cut surface of decapitated coleoptiles, induced curvature. This was, in fact, the turning point in auxin research. Went's work is significant because (1) it confirmed the existence of a regulatory substance in the coleoptile apex, and (2) a means for isolation and quantitative analysis of the active substance was established. Subsequently, Kögl and Haagen-Smit (1934) proposed the term "**auxin**" for the "Wuchsstoff" crystalized from human urine. They reported isolation of auxin a and auxin b and heteroauxin from human urine. Almost during the same period, Thimann group (1935) reported auxin from *Rhizopus* culture medium. Investigations in

Box 15.1: Classical Experiments Leading to Discovery of Auxins



Box 15.2: Bioassay for Auxins

Bioassay: An assay using biological samples to estimate the concentration or activity of a substance.

1. Avena Curvature Test: In this bioassay, an auxin-containing agar block is placed on one side of a decapitated oat coleoptile. As auxin from the agar block diffuses down through the coleoptile, it causes rapid growth of cells in the region just below the agar block (auxin source). This results in bending of the coleoptile to the opposite side. Degree of bending can be measured by placing the coleoptiles on a graph paper. Up to 200 μg. litre^{-1}, the degree of coleoptile bending shows linearity with increasing IAA concentration. Using this bioassay, unknown auxins removed from a plant can be estimated.

(continued)

Box 15.2 (continued)

2. Straight Growth Test: This bioassay is based on the ability of auxins to cause elongation of coleoptile or stem cells. Dark-grown coleoptiles are decapitated to remove endogenous auxin source. An equal number of 5 mm long sections of the remaining coleoptile stump are placed in petri dishes, each containing solution of known IAA concentration. The length of coleoptile increases with increasing concentration of IAA in the incubation medium over a defined period (24–36 h). A standard curve thus generated (IAA concentration vs increase in coleoptile length in mm) can be used to estimate IAA concentration in a plant tissue exudate homogenate (unknown sample), and it can be expressed as units of IAA equivalents. Alternatively, sections of dark-grown pea seedling stem can also be used for this bioassay. It may be noted that stem segments obtained from light-grown pea seedlings are less effective since they exhibit lesser extension growth in response to added auxin. A control solution (-IAA) will not result in extension growth.

subsequent years, however, revealed that auxin a and b did not contain any auxin activity. First isolation of IAA from a plant was achieved by Haagen-Smit et al. (1942) from the alkaline hydrolysate of corn meal (*Zea mays*). This was soon further confirmed by Berger and Avery (1944) and Haagen-Smit (1946) from their isolation of IAA from immature maize kernels. Subsequently, between 1944 and 1974, indole-3-acetic acid was isolated by various investigators from coleoptile tips, roots and seedlings of maize, seedlings of *Avena sativa* and *Phaseolus mungo*, *Pinus radiata* buds, and phloem sap of *Ricinus communis*. All current investigations on the isolation, characterization, and quantification of endogenous auxins from plant tissues rely on techniques like liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and immunoassays, such as ELISA (enzyme-linked immunosorbent assay) and RIA (radioimmunoassay), which possess variable sensitivity for auxin detection.

15.2 Synthetic Auxins

After the discovery of indole-3-acetic acid and establishment of its role in cell elongation, attempts were made to examine the biological activity of a number of compounds with substituted indoles, such as indole-3-propionic acid (IPA). Like natural auxins, IPA is biologically active and is commonly used as a rooting hormone in horticultural practices. Like IAA, it has an indole ring and a terminal carboxyl group but differs in its side chains (Box 15.3). Compounds such as α-naphthalene acetic acid (NAA) are also biologically active and are used as rooting hormone for certain plants. NAA lacks indole ring but retains acetic acid side chain present in IAA. Another biologically active synthetic auxin is 2,4-dichlorophenoxy acetic acid (2,4-D) (Box 15.4). Like NAA, it also lacks indole ring.

Box 15.3: Synthetic Auxins

Synthetic auxins include 2,4-dichlorophenoxyacetic acid (a herbicide), naphthalene acetic acid (a component of commercially available rooting chemical), 2-methoxy-3,6-dichlorobenzoic acid (dicamba, a herbicide), and indole butyric acid (IBA). Propagation of plants by vegetative means is commonly practiced in horticulture. Auxin applications are generally beneficial in bringing about rooting of cuttings for vegetative propagation. Another property of auxins is their ability to initiate development of fruits without pollination (**parthenocarpy**). Because of the difficulty of satisfactory pollination in some plants as well as to improve the quality of the fruits, auxin treatment in the form of spray or aerosol is commonly practiced. Fruits so induced are seedless. So, besides increasing yield, auxin treatment also makes parthenocarpy feasible.

In pineapple, flower induction at the appropriate time can be a problem. It can, however, be made to flower at any time of the year by the application of synthetic auxins. In the USA and Russia, growers of citrus, apple, and pear extensively use auxin spray for the prevention of premature fall of fruits. Premature fruit fall can lead to extensive damage to crop. Thus, growers must either harvest fruits before the best quality is obtained or else risk a heavy loss. Auxin sprays have proved highly successful in preventing premature fruit fall, and now growers can obtain reasonable assurance against loss of apple and pear crops by using auxin sprays.

Box 15.4: 2,4-D: The First Selective Herbicide

During Vietnam War (1961–1971), the US Air Force sprayed "Agent Orange" over Vietnamese agricultural land with the intention of destroying crops. Agent Orange or Herbicide Orange (HO) is one of the herbicides and defoliants used by the US military as a part of its herbicidal warfare program. HO is a mixture of equal parts of two herbicides, 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) and 2,4-dichlorophenoxy acetic acid (2,4-D). The mission to destroy crop, however, failed. 2,4-D is a selective herbicide which kills dicots while leaving monocots alone. In other words, it can be sprayed on grasses (like wheat, rice, corn, and other cereal crops) without any major harm while killing broad leaf weeds (dicots). It gives dicots a form of symbolic cancer, and the plants grow uncontrollably (the plant grows itself to death). It affects only dicots because the two classes of plants (monocots and dicots) transport these herbicides using different mechanisms. Broad-leaved weeds have their growing point at the tips of the leaves, while grasses grow from their base.

(continued)

Box 15.4 (continued)

2,4-D works by interfering with growth, either by blocking photosynthesis and protein synthesis or by destroying or inhibiting root formation. 2,4-D gets absorbed through stomata and is transported to the meristems of the plant. This causes uncontrolled and unsustainable growth, and the plants wilt and die. The underlying molecular mechanism regarding selectivity of this synthetic auxin is because of either limited translocation or rapid degradation, altered vascular anatomy, or altered perception of auxin in monocots. Also, auxin transport is influenced by plant vascular systems. The differences in vascular tissue structure between dicots and monocots may contribute to the selectivity of auxinic herbicide. In monocots stems, the vascular tissues are scattered in bundles and lack vascular cambium. In dicot stems, the vascular tissues are formed in rings and possess a cambium. To conclude, in monocots, general mode of action of auxin is not affected but an accessory pathway clears up auxin in levels excess of any normal amounts that may be present.

Differences/similarities in response of dicots and monocots to synthetic auxin application (spray or through soil)

Dicots (e.g., weeds)	Monocots (crops)
Accumulates in free form; so remains active (e.g., 2,4-D)	Conjugates with other biomolecules, so becomes inactive soon after absorption
Slow transport (due to lack of compatible transporter proteins), thus acting longer	Slow transport
Stays longer in metabolically active form, hence causes toxic effects due to accumulation	Low affinity with auxin-binding proteins, hence less tissue sensitivity

15.3 Auxin Distribution and Biosynthesis

The amount of IAA present in the tissue will depend on the type of tissue and its age. In vegetative tissues, IAA concentration varies between 1 and 100 μg (5.7–570 nanomoles) kg^{-1} fresh weight. Seeds, however, generally have an abundance of IAA. Thus, the endosperm of a maize seed may contain up to 308 picomoles of IAA. On the contrary, maize shoot contains much less IAA (in the range of 27 picomoles). High level of auxin has also been reported in legume seeds. Cereal grains mainly contain esterified IAA, while legume seeds largely contain peptidyl IAA. Although most plant tissues are able to biosynthesize low levels of IAA, it is largely produced in shoot apical meristem, developing fruits and seeds and in young leaves. High amount of IAA present in root tips is due to its transport from other sites of production rather than its biosynthesis in the root tip alone. Auxin biosynthesis has also been demonstrated in the tips of dicot leaves (Fig. 15.2). With the passage of

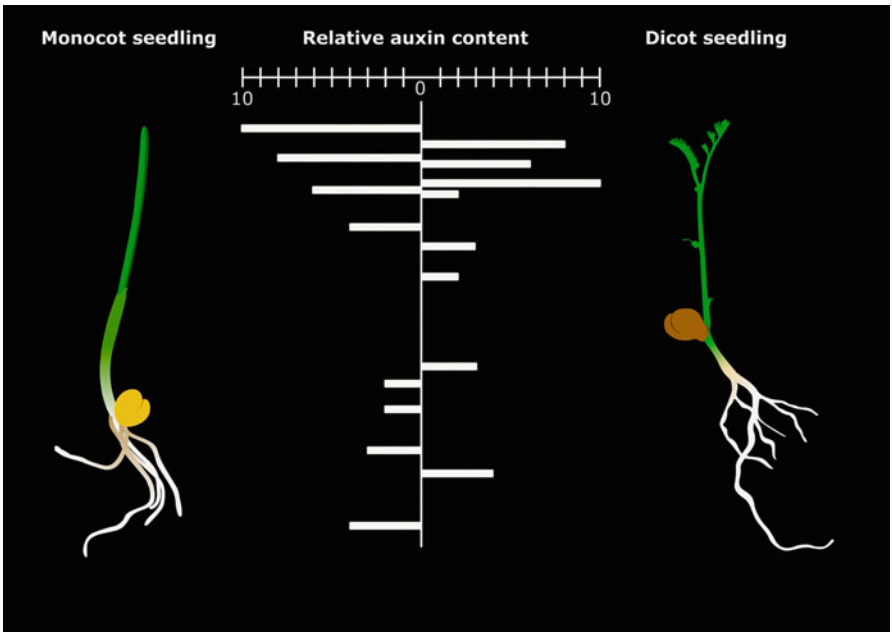


Fig. 15.2 Relative auxin distribution in a monocot and a dicot seedling. Relative units of auxin: 10=high; 1=low

development of leaves, the site of IAA biosynthesis gradually shifts toward the base of the leaves and then to the central region representing the vascular system. Intense auxin biosynthesis is also evident in the cells that differentiate into hydathodes (gland-like structures through which water is released as a result of positive pressure). A decreasing auxin gradient from the tip of the hydathodes to the developing vascular strand down below indicates the role of auxin in vascular tissue differentiation.

Biosynthesis of tryptophan (auxin precursor) principally takes place in chloroplasts. Subsequent formation of IAA may occur both in cytosol and chloroplasts. About one-third of IAA in a cell is found in chloroplasts and rest in the cytosol. IAA biosynthesis can take place both via tryptophan-dependent and tryptophan-independent pathways.

15.3.1 Tryptophan-Dependent Pathways

Tryptophan (an aromatic amino acid) is the precursor of IAA. Plants convert tryptophan to IAA through several pathways (Fig. 15.3).

1. *Bacterial pathway*: In some pathogenic bacteria, such as *Agrobacterium tumefaciens* and *Pseudomonas savastanoi*, tryptophan is converted into indole-3-acetamide (IAM) in the presence of enzyme tryptophan monooxygenase. IAM is then hydrolyzed to IAA in the presence of enzyme IAM hydrolase.

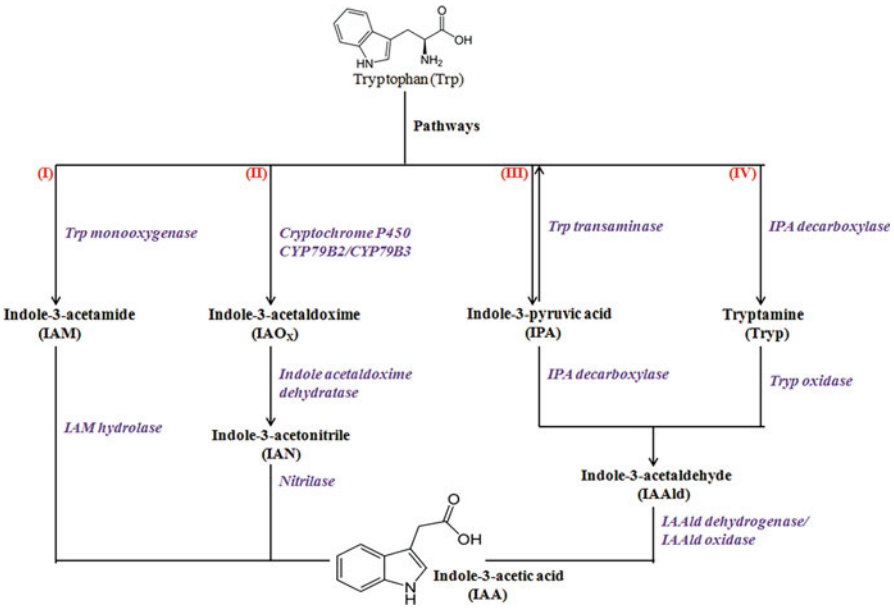


Fig. 15.3 Pathways of indole-3-acetic acid biosynthesis (I) pathway operative in bacteria. (II) to (IV) pathways operative in plants—(II) Indole-3-acetonitrile pathway. (III) Indole-3-pyruvic acid pathway. (IV) Tryptamine pathway

2. *IAN (indole-3-acetonitrile) pathway*: It is prevalent in members of Brassicaceae, Poaceae, and Musaceae. Tryptophan is converted to IAA in the presence of the enzyme nitrilase. Indole-3-acetaldoxime and indole-3-acetonitrile are the intermediates.
3. *IPA (indole-3-pyruvic acid) pathway*: Tryptophan →IPA →Indole-3-acetaldehyde →IAA.
4. Tryptophan is deaminated to form indole-3-pyruvic acid followed by decarboxylation, resulting in the formation of indole-3-acetaldehyde. The enzymes involved are transaminase and IPA decarboxylase, respectively.
5. *TAM (tryptamine) pathway*: Tryptophan is decarboxylated to form tryptamine (TAM), followed by deamination to form indole-3-acetaldehyde (IAld). The enzymes involved are tryptophan decarboxylase and tryptamine oxidase, respectively. IAld is readily oxidized to form IAA by the enzyme IAld dehydrogenase.

15.3.2 Tryptophan-Independent Pathways

In this pathway IAA is also synthesized from indole or indole-3-glycerol phosphate. Evidence for the same was obtained from orange pericarp mutant of maize. In this mutant, both subunits of the enzyme tryptophan synthase are inactive. It requires

exogenous tryptophan to survive, and it is unable to convert tryptophan to IAA. Despite this, the mutants contain high level of auxin. Thus, it was concluded that IAA could be synthesized from indole or indole-3-glycerol phosphate (Fig. 15.4).

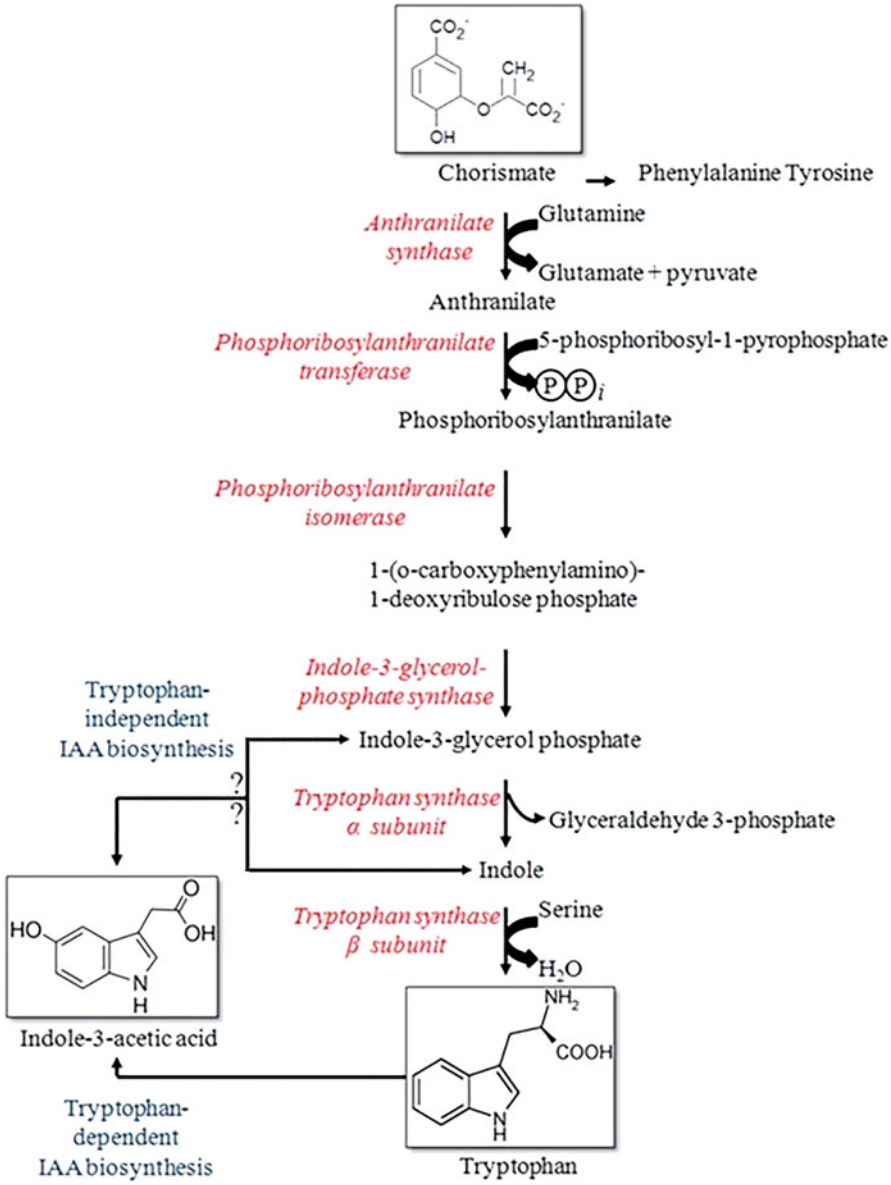


Fig. 15.4 Indole-3-acetic acid synthesis in plants

15.4 Conjugation and Degradation of Auxins

At high concentrations, intracellular auxin can be toxic. Therefore, its homeostatic control through conjugation and degradation is also necessary in addition to regulation of its biosynthesis. Such a process ensures removal of active auxin when available at supraoptimal concentrations. These processes (conjugation and/or degradation) are also operative within the cells when auxin action is completed. Conjugation of IAA to glucose, alanine, and leucine is a reversible process, and most conjugated forms are sequestered within the vacuoles. Myoinositol derivative of IAA derived from IAA-glucose also leads to reversible release of free IAA via formation of IAA peptides (Fig. 15.5). Indole-3-butyric acid (IBA), like IAA, also exhibits reversible conjugation with molecules like alanine and glucose. Conversion of IBA to IAA is also possible through β-oxidation of IBA-CoA derivative. This process of IBA to IAA conversion holds significance in view of the fact that IBA is routinely used in horticulture to promote rooting of cuttings. Oxidative catabolism of IAA is an irreversible process for permanent removal of intracellular auxin not required by the cells. It refers to chemical modification of indole nucleus or the side chain of IAA, resulting in loss of auxin activity. As early as in 1940s, an enzyme

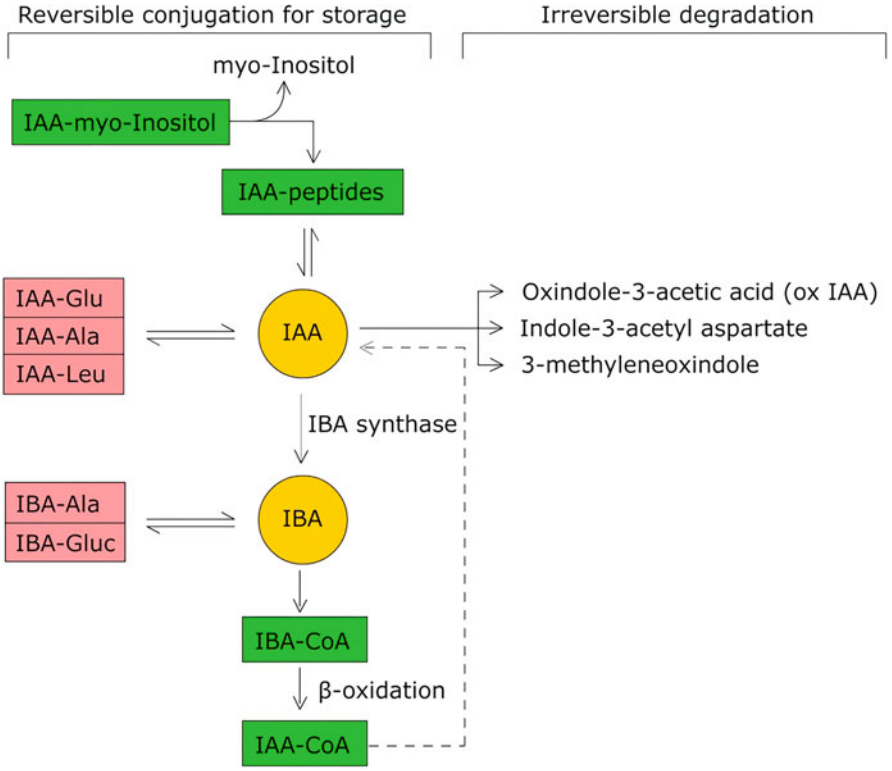


Fig 15.5 Conjugation and degradation of IAA and IBA

responsible for inactivation of IAA was isolated from plant extracts and was called **IAA oxidase**. IAA catabolism is now known to be mediated by specific isoforms of **peroxidase**, resulting in the formation of 3-methyleneoxindole derivative. IAA can also be irreversibly oxidized to oxindole-3-acetic acid (ox-IAA) or indole-3-acetyl aspartate (Fig. 15.5).

15.5 Auxin Transport

Auxin is the only plant hormone which exhibits polar transport in almost all plants, including bryophytes and ferns. Polar transport of auxin is always basipetal, i.e., toward the base, both in shoots and roots. Thus, it is transported from shoot tip and leaves downward in the stem and from the root tip toward the base of the root to the root-shoot junction (Fig. 15.6). The rate of polar auxin transport varies from 5 to 20 cm/h. Auxin biosynthesized in the leaves has been reported to be transported via the phloem cells to the other parts of the plant. Most of the auxin transport within the plant occurs through vascular parenchyma (specially xylem parenchyma) and also through sieve-tube elements. Polar auxin transport proceeds in a cell-to-cell fashion. It exits the cell through the plasma membrane, diffuses across the middle lamella, and crosses the next plasma membrane of the next cell (*apoplastic movement*). Auxin entry is referred as influx and its exit is efflux.

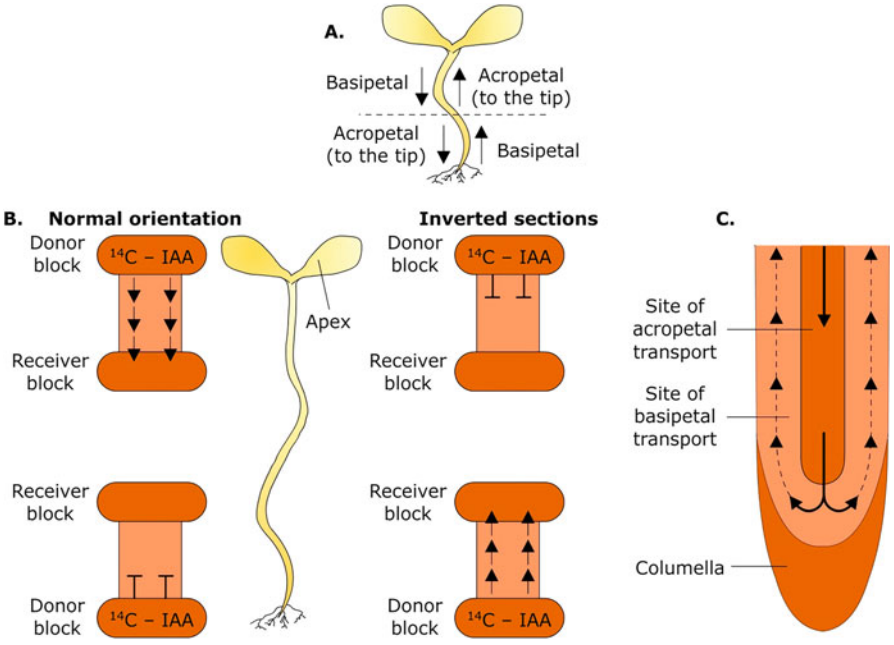


Fig 15.6 (**a**) Nomenclature used to define the direction of auxin transport. (**b**) Demonstration of polar auxin transport in the hypocotyl segments of a dicot seedling. (**c**) Direction of auxin transport in root

15.5.1 Auxin Influx

Two mechanisms are operative for auxin influx.

1. Passive diffusion of protonated form (IAAH, a lipophilic molecule) from any direction across the phospholipid bilayer
2. Secondary active transport of dissociated form (IAA^-) using symporter protein, AUX1

The plasma membrane H^+-ATPase activity normally maintains the cell wall pH around 5.5. As a result, about 25% of IAA in the apoplast remains in protonated form (IAAH) which diffuses passively across the plasma membrane according to concentration gradient. The secondary active transport of auxin allows greater auxin accumulation than simple diffusion does because it is driven across the membrane by proton motive force (pmf). An auxin uptake carrier protein (AUX1) functions in leaf vascular tissue and root apices. AUX1 belongs to a family of proteins similar to permeases in prokaryotes. [*Permeases are a group of membrane-bound carriers (enzymes) that affect solute transport across the semipermeable membrane.*] AUX1 exhibits polar localization at the ends of protophloem cells in root apex where it is thought to function in the process of unloading phloem-derived auxin, moving acropetally toward the tip. It also exhibits a polar localization in root cap for basipetal transport (Fig. 15.7).

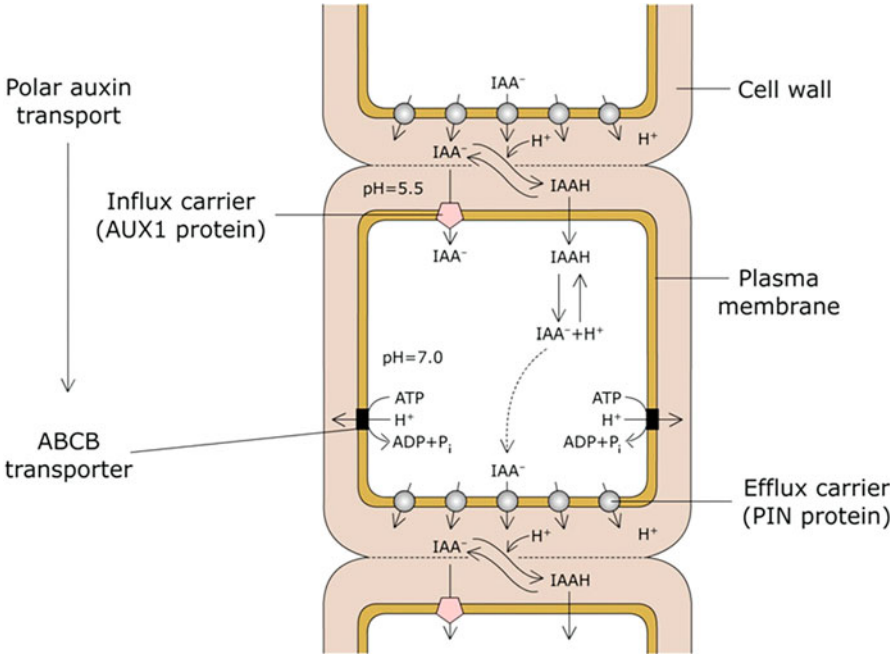


Fig. 15.7 Schematic chemiosmotic model for polar transport of auxin

15.5.2 Auxin Efflux

Is largely brought about by a set of PIN proteins which are mainly located in the basal region of the cells. PIN derives its name from the *Arabidopsis pin 1* mutant because of the needle-like shape of inflorescence and the stem which lacks leaves, buds, and flowers (Fig. 15.8). At least eight PIN genes have been isolated from *Arabidopsis.* Polar auxin transport is significantly reduced in *pin* mutants, and this characteristic feature of these mutants can be mimicked by blocking polar auxin transport using certain compounds categorized as **phytotropins**. Phytotropins are noncompetitive inhibitors of polar auxin transport. These include TIBA (2,3,5-triiodobenzoic acid), morphactin (9-hydroxyfluorine-9-carboxylic acid), and NPA (N-1-naphthylphthalamic acid). PIN1 is mainly located in xylem parenchyma both in shoot and root and is crucial for polar auxin transport (Fig. 15.8). PIN2 is localized in the root, cortex, and epidermis and regulates basipetal movement of auxin in roots. PIN3 is localized in endodermis in the shoot and in columella in the root. In both the tissue systems (shoot and root), PIN3 is involved in lateral distribution of auxin. PIN4 is located in the quiescent center and appears to function in the establishment of auxin sink below the quiescent center in the root apical meristem. PIN7 has been reported to play a role in forming and maintaining apical-basal auxin gradient in the

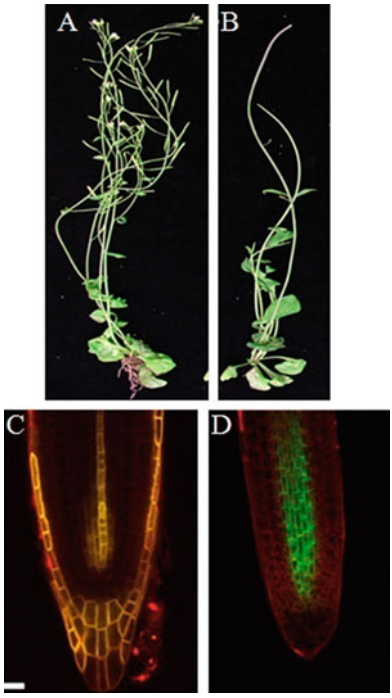


Fig. 15.8 Phenotypes of wild-type *Arabidopsis* (**a**) and PIN1 mutant (**b**) plants. Note the lack of inflorescence in the mutant (**b**) giving it a pin-like appearance. AUX1 (**c**) and PIN1 (**d**) proteins localized in the vascular parenchyma cells in the roots of *Arabidopsis*

Table 15.1 Various types of PIN proteins found in plants

Auxin efflux protein	Location	Role
PIN1	Xylem parenchyma in shoots and roots	Basipetal IAA transport in shoots and acropetal transport in roots
PIN2	Roots	Basipetal redistribution of auxin through cortical and epidermal cells
PIN3	Cells of shoot epidermis; columella of roots and pericycle	Lateral redistribution of auxin
PIN4	Quiescent center	Establishment of auxin sink below the quiescent center in root apical meristem
PIN7	Embryo	In forming and maintaining apical-basal gradients for embryonic polarity

embryos (Table 15.1). Polar auxin transport is largely controlled through the recycling of PIN proteins between the plasma membrane and various endomembrane compartments by an actin-dependent mechanism. This involves secretion of vesicles carrying PIN proteins from their site of biosynthesis and their migration to specific sites on the plasma membrane of auxin-conducting cells. This secretion is regulated by endocytotic cycling of vesicles along the actin track.

15.5.3 Chemiosmotic Model for Auxin Transport

The model for auxin transport proposed by Rubery, Sheldrake, and Raven in the mid-1970s has three principle features:

1. The existence of a pH gradient or proton motive force (pmf) as the driving force for IAA transports across the plasma membrane.
2. Activity of auxin influx carrier.
3. Preferential location of auxin efflux carrier proteins in the basal region of the cells.

On the basis of these three features, chemiosmotic model of auxin transport can be summarized as follows (Fig. 15.7):

1. IAA is a lipophilic weakly acid molecule. The apoplast/cell wall space is moderately acidic with a pH of about 5.5. At this pH, about 25% of IAA remains protonated (IAAH), and the rest exists in anionic form (IAA^-). Thus, the cell wall region contains both protonated (IAAH) and anionic (IAA^-) forms of indole-3-acetic acid (Box 15.5).

Box 15.5: Dissociation Pattern of IAA in the Cells

Auxins, gibberellins, abscisic acid (ABA), and jasmonic acid (JA) are weak acids, which in solution dissociate into anions and protons (*weak acids and bases are those acids which are not completely dissociated in solution. They exist in solution as an equilibrium mixture of undissociated and dissociated species*). pKa value of IAA, ABA, and JA at 20 °C is about 4.7–4.8 (pKa of GA_1 is lower, 3.85). Accordingly, at neutral pH in free solution, these hormones exist predominantly in dissociated form, but at pH closer to pKa, the proportion of undissociated form increases. The equilibrium constant (K) for the ionization of an acid (Ka) is

$$K = \frac{[H^+][A^-]}{[HA].}$$

The pKa of IAA is defined as: $pKa = -\log K = \log 1/K$

At pH 4.7 $\frac{[IAA^-][H^+]}{[IAA]} = 1$

In undissociated form, the carboxyl group of IAA is protonated and is lipophilic. It readily diffuses across the membrane. Dissociated form is negatively charged (IAA^-) and, therefore, does not cross plasma membrane unaided.

At pH 5.5, the two forms maintain a balance as:

IAA^-	⟷	$IAAH^+$
(75%)		(25%)

Driving force for auxin uptake is proton motive force (pmf) across the plasma membrane (*proton motive force refers to the gradient of electrochemical potential for H^+ across the plasma membrane. It is the sum of proton chemical potential and transmembrane electric potential*). Driving force for auxin efflux is membrane potential. Auxin efflux is driven by the inside negative membrane potential.

2. Because of its lipid solubility, IAAH slowly diffuses across the plasma membrane from the cell wall region into the cytosol.
3. Most of the IAA, however, enters the cell across the plasma membrane as IAA- using AUX1 symporter proteins which are uniformly distributed along the plasma membrane.
4. Inside the cells, at the cytoplasmic pH which is generally close to 7, IAAH dissociates into IAA− and H+. Thus, auxin gets trapped inside the cell because IAA- cannot spontaneously diffuse across the plasma membrane.

5. The critical component of the chemiosmotic model is, however, the basal location of PIN proteins which is responsible for efflux of IAA- from the cells. It is this unique basal location of PIN proteins which is responsible for establishing polarity in auxin transport.
6. The major driving force causing efflux of IAA− from the cell is the negative plasma membrane potential, which varies between −200 and −300 mV.

Another set of auxin efflux proteins recently reported from some "loss of function" mutants of *Arabidopsis* is referred as **ABCB transporters** (Fig. 15.7). They have been reported to facilitate IAA^- efflux or influx across the plasma membrane (and also tonoplast), but unlike PIN proteins, they do not show preferential basal localization in the cells. So, although these transporter proteins are involved in auxin efflux or influx, they do not play any significant role in maintaining polar transport of auxin. Furthermore, they require energy in the form of ATP hydrolysis to transport IAA^-.

15.6 Physiological Effects of Auxins

15.6.1 Cell Expansion (Acid Growth Hypothesis)

A common feature of the cell wall in growing cells is that they extend much faster at acidic pH than at neutral pH. This phenomenon is called **acid growth**. Cleland and Rayle proposed in 1970 a theory to explain auxin-stimulated cell wall extension. According to them, auxin causes acidification of cell wall environment through the release of protons from the cells. Lower (acidic) apoplastic pH conditions so created then activate the process of cell wall loosening through enzymatic action. During the same period in 1970s, another scientist named Hager from Germany further proposed the role of plasma membrane-bound H^+-ATPases in auxin-stimulated proton release. These two proposals earlier referred as Cleland-Hager proposal are now known as **acid growth hypothesis** for plant cell enlargement. This hypothesis proposes that:

1. Auxin activates plasma membrane-localized H^+-ATPase resulting in cell wall acidification.
2. Lower/acidic cell wall pH enhances the activity of **expansins** which results in breakage of hydrogen bonds between the cellulose microfibrils.
3. This loosening of cellulose microfibrils in the cell wall allows turgor-induced cell expansion.

Auxin brings about activation of H^+-ATPases through its binding with a membrane-associated protein called **ABP1** (auxin-binding protein 1). ABP1 is thus an auxin receptor which has been localized in the ER and plasma membrane.

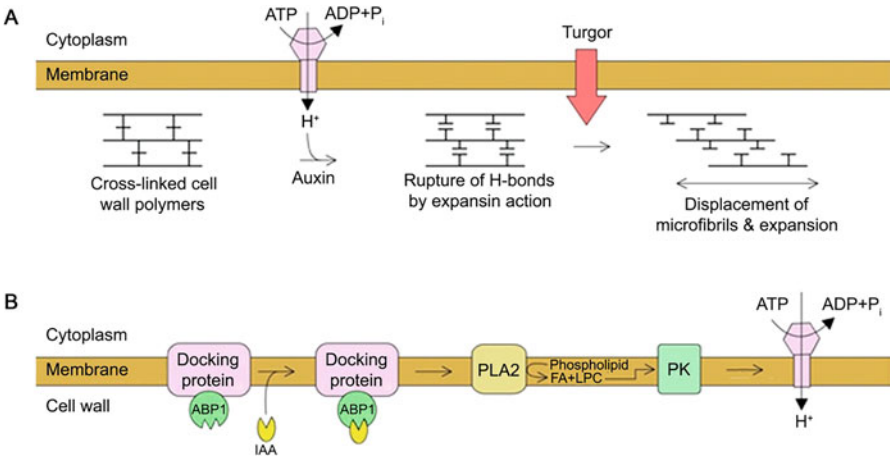


Fig. 15.9 Mechanism of cell enlargement. (**a**) Cell wall polymers (cellulose microfibrils) are extensively cross-linked which limits their capacity to expand. An auxin-activated ATPase proton pump acidifies the cell wall space by pumping protons from the cytoplasm. The lower pH activates expansins which loosen the load-bearing bonds. Turgor pressure causes the polymers to displace and cell enlarges. (**b**) Proposed signal transduction chain linking auxin with ATPase proton pump activity. *ABP1* auxin-binding protein 1, *PLA2* phospholipase A2, *FA* fatty acids, *LPC* lysophosphotidylcholine, *PK* protein kinase

It is a 43 kDa glycoprotein dimer of two subunits of 22 kDa each. It is proposed that ABP1 forms a complex with a transmembrane docking protein which provides lipid solubility to anchor ABP1 to the membrane. This complex (ABP1-docking protein complex) then migrates from ER to the plasma membrane where ABP1 gets lodged facing outside (apoplastic side). In this position, ABP1 attaches itself to an auxin molecule. This auxin-ABP1-docking protein complex initiates a signal transduction pathway leading ultimately to activation of ATPase proton pumps. There is evidence to implicate phospholipase A2 (PLA2) in this auxin-induced signal transduction chain. It is suggested that PLA2 follows ABP1 in this chain of events followed by formation of lysophospholipids and fatty acids through the cleavage of phospholipids by PLA2 action. The lysophospholipids have been reported to activate H^+-ATPase through the involvement of protein kinase cascade (Fig. 15.9).

15.6.2 Apical Dominance

Removal of the shoot apex is a common horticultural practice to produce bushy plants by stimulating the growth of axillary buds. Apical dominance refers to the inhibition of axillary bud growth by the continued meristematic activity in the shoot

apex. This process can be easily demonstrated by the decapitation of the shoot apices. Apical dominance is best observed in herbaceous plants and also in trees during first year of growth. This state of arrest of growth of axillary buds (quiescence) is different from bud dormancy, in physiological terms. In case of bud dormancy (in contrast with apical dominance), both terminal as well as lateral buds remain dormant in plants native to temperate and colder regions of the earth. Their growth activity is regulated by temperature and photoperiod. Apical dominance, on the other hand, determines the branching patterns of plants.

Herbaceous plants can be grouped into three categories with reference to the extent of apical dominance exhibited by them.

1. Those plants which show strong apical dominance include *Helianthus annuus* (sunflower) and *Tradescantia* sp. such plants show little or no lateral branching unless decapitated.
2. The second category of herbaceous plants exhibits intermediate or partial apical dominance. Thus, they exhibit some branching. For example, *Phaseolus vulgaris* (common bean), *Pisum sativum* (pea), *Ipomoea nil* (Japanese morning glory), and *Vicia faba* (broad bean).
3. The third category of plants shows very weak or no apical dominance. These plants continue to exhibit substantial branching even when intact. For example, *Coleus* and *Arabidopsis*.

Plants with strong to moderate apical dominance exhibit greater inhibition of axillary buds close to the apex, and the impact of apical dominance is gradually released on the axillary buds at a greater distance from the apex where branching becomes more common. A change in the reproductive phase of the plant also brings about significant changes in their branching patterns. Thus, for example, in the case of oats, growth inhibition of lateral buds is nullified with the onset of flowering. In contrast, in plants such as *Phaseolus*, fruiting and fruit set can reimpose inhibition of growth of lateral buds.

Attempts to understand the physiology of apical dominance began with the observations of Thimann and Skoog in 1933 whereby they showed that application of lanolin paste containing IAA to the cut stumps of *Vicia faba* plants inhibits the growth of lateral buds below. Subsequently, various investigators demonstrated similar response using both IAA and the synthetic auxin-NAA, thereby highlighting that auxins can substitute for shoot apex and result in inhibition of axillary bud growth (Fig. 15.10). Application of other hormones, such as cytokinins, gibberellins, or abscisic acid to the cut stumps, does not bring about similar inhibition of lateral bud growth. Inhibition of lateral bud growth is released upon application of compounds, such as TIBA or NPA (auxin transport inhibitors), in the lanolin paste placed over the decapitated stump (Fig. 15.10). All these observations lead to conclusions that auxin produced in the shoot apex inhibits lateral bud growth

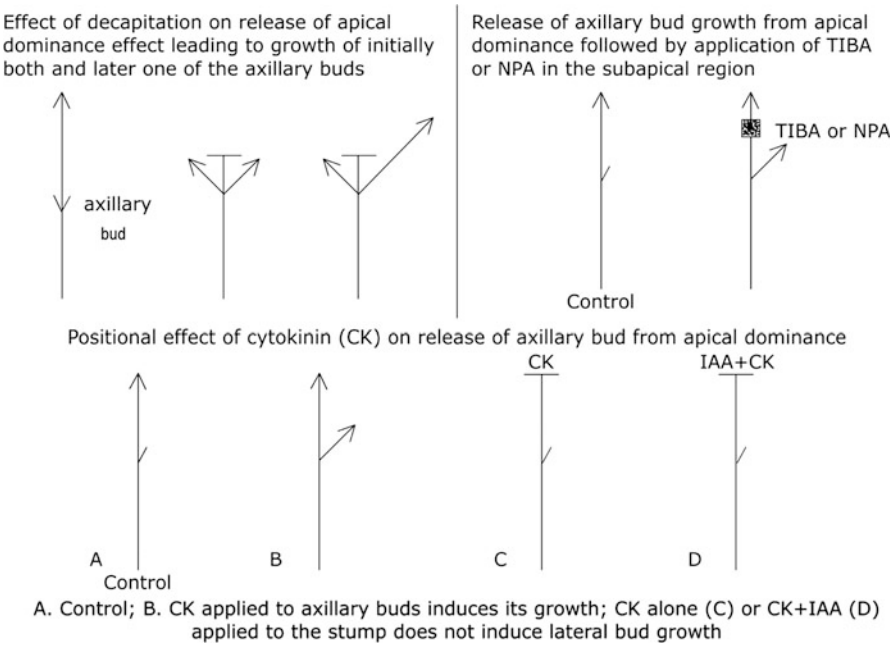


Fig. 15.10 Experiments demonstrating the effect of decapitation, auxin transport inhibitor, and cytokinin in the release of axillary bud growth from apical dominance effect in pea plants

through its transport downward. The most recent model to understand the mechanism of apical dominance proposes as follows (Figs. 15.11 and 15.12):

1. The initial signal for axillary bud growth has been found to be an increase in sucrose availability to the bud. The use of radioactively labeled sucrose has demonstrated that the concentration of sucrose derived from leaves decreases in the stem region adjacent to the axillary bud as early as within 2 h of decapitation. This decline in sucrose in the stem is due to its uptake by the axillary bud. It is thus evident that following decapitation, sucrose depletion in the stem adjacent to the bud takes place prior to auxin depletion leading to bud outgrowth. Apical dominance is thus regulated by limiting sugar availability to the axillary buds in intact plants, and sustained growth of the axillary bud requires depletion of auxin in the stem adjacent to the bud as well.
2. Auxin itself does not accumulate in the cells of axillary buds whose growth is suppressed. It, in fact, acts via intermediary signals.
3. Activation of axillary bud growth following decapitation of the apical bud depends on the supply of cytokinins and on the ability of the axillary bud to export auxin.

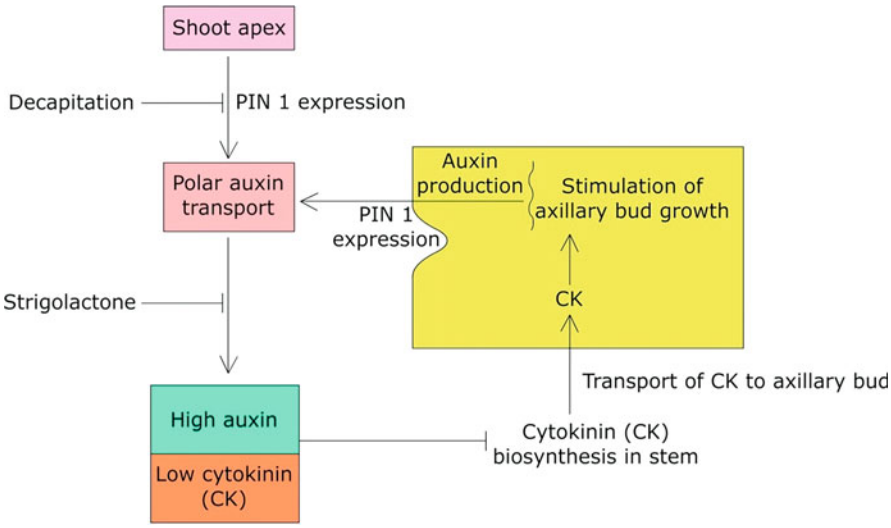


Fig. 15.11 Model depicting interaction among auxin, strigolactone, and cytokinin in controlling apical dominance

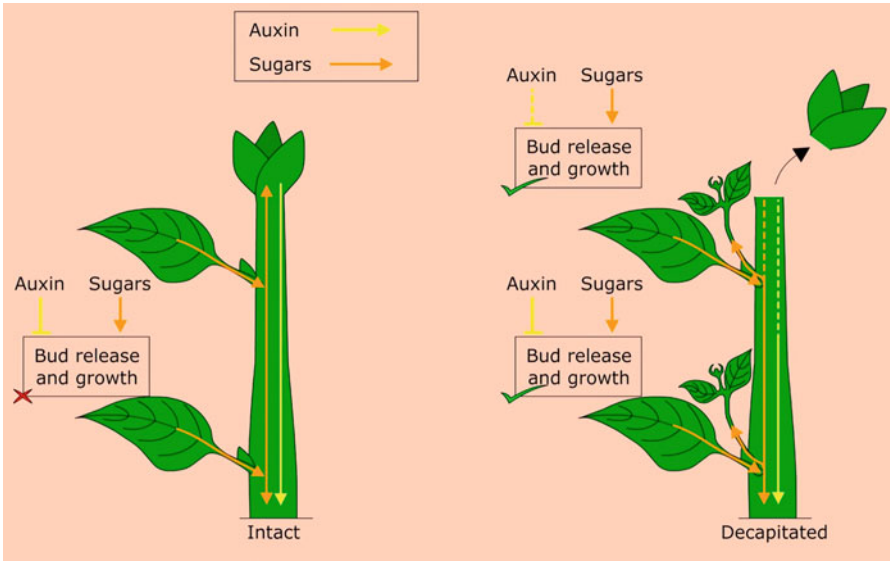


Fig. 15.12 Regulation of apical dominance by sugar availability

4. Strigolactones (a family of terpenoid derivatives) negatively regulate basipetal auxin transport from the shoot apex. In this way, strigolactones reduce the sink strength of the stem for auxin.
5. Auxin is a positive regulator of strigolactone synthesis, and it also inhibits cytokinin synthesis in the stem. As a result of decapitation of the shoot apex, auxin source is lost. Consequently, suppression of cytokinin biosynthesis genes is reversed, and they become active.
6. Enhanced cytokinin biosynthesis in the suppressed axillary buds initiates meristematic activity in them resulting in axillary bud growth. Thus, a new cycle of apical dominance/axillary bud growth is established.

Strigolactones are believed act in coordination with auxin during apical dominance. It has been observed that *Arabidopsis* mutants deficient in strigolactone biosynthesis (*max1* [*more axillary growth 1*], *max3* and *max4*) or strigolactone signaling mutant (*max2*) exhibit increased shoot branching without decapitation. If cytokinin is applied directly to axillary buds in intact plants, it stimulates their growth, indicating the involvement of cytokinin in breaking apical dominance. It has been shown that the cytokinins involved in breaking apical dominance are synthesized locally in the axillary buds.

15.6.3 Floral Bud Development

Polar auxin transport is required for floral development in the inflorescence meristem. *pin1* mutant of *Arabidopsis* lacks auxin efflux carrier and exhibits abnormal flowers. This shows that in the absence of auxin efflux carriers, the meristem is starved of auxin, and normal floral development is disrupted. PIN1 encodes a transmembrane protein that has been shown to localize with polarity in cells and to participate in auxin efflux. Interestingly, disruption of auxin polar transport does not appear to prevent the transition from vegetative growth to reproductive growth as *pin1* still forms an inflorescence. However, converting an inflorescence meristem to a floral meristem apparently requires normal polar auxin transport. Furthermore, treatment of plants with polar auxin transport inhibitor, naphthylphthalamic acid (NPA), also leads to the formation of pin-shaped inflorescence. It is possible that inhibition of auxin transport leads to accumulation of auxin in the meristem to levels that are inhibitory for the formation of new primordia. Alternatively, blocking of auxin transport may also lead to depletion of auxin in the meristem depending on where auxin is synthesized. Furthermore, disruption of auxin transport may not change the overall auxin levels. Rather, the phenotypes may be the result of a change in the local auxin gradients, which are presumably essential for organogenesis. Two other *Arabidopsis* mutants, *pinoid*, and *monopteris* (mp) also fail to initiate floral buds. PINOID encodes a serine/threonine protein kinase and is suggested to participate in auxin signaling and polar auxin transport, supporting the theory that auxin plays an essential role in the formation of floral buds. The role of Yucca (YUC)

family of flavin monooxygenases in auxin biosynthesis provides further evidence regarding the role of auxin in flower formation. Overexpression of YUC1 gene leads to auxin overproduction, and mutations in some members of this gene lead to development of inflorescence without flower primordia.

15.6.4 Vascular Differentiation

Both localized and transported auxins play crucial role in the differentiation of the vascular tissues. Since differentiation of phloem tissue is usually difficult to follow, therefore, most reports on the effect of auxins on vascular tissue differentiation are confined to differentiation of xylem elements, a process called as **xylogenesis**. In a growing shoot or root, the procambial strands invariably differentiate in an acropetal order toward the shoot and root apices, respectively. Within the procambial strands as well, the primary xylem and phloem elements also differentiate acropetally. The effect of auxin on xylogenesis is twofold: (1) it acts as a vascular inducing signal and (2) "canalization" of newly differentiated vascular strand leading to polar transport of auxin.

1. Localized effect of auxin in the induction of xylogenesis is evident from the application of auxin to explants derived from cortex or pith or in callus tissues. Under such situations, addition of suitable concentrations of IAA or NAA to the culture medium containing the explants of lettuce (*Lactuca sativa*), *Coleus blumei*, tobacco (*Nicotiana tabacum*), or pea (*Pisum sativum*) leads to differentiation of tracheary cells. Likewise, differentiation of tracheary elements is also released in the mesophyll cells derived from *Zinnia* leaves in the presence of suitable concentrations of NAA. From this study, it has been observed that the first step of vascular differentiation is dedifferentiation of mature cells which requires no hormone. Both auxin and cytokinin regulate the second phase, i.e., the phase of induction. In the examples discussed so far, auxin has been found to be necessary for xylogenesis without involving its long-distance polar transport.
2. Xylogenesis in wounded tissue in an intact plant best depicts the impact of polarly transported auxin on xylogenesis. Auxin gradient created by its basipetal transport regulates vascular tissue differentiation in the shoot. New vascular elements usually develop toward the preexisting vascular strands and ultimately unite with them (Fig. 15.13). It is thus evident that wounded site acts as auxin source and the existing vasculature in the stem acts as auxin sink. This source-sink model is also referred as **canalization model** for auxin flow leading to reestablishment of continuous strands of vascular elements.

Xylogenesis proceeds in well-defined stages. These include radial expansion of cells, deposition of secondary wall, lignification, and, finally, sequential degradation of nucleus and cytoplasmic contents. Whereas the involvement of auxin in radial expansion of cells is well established, it is also linked to the process of lignification.

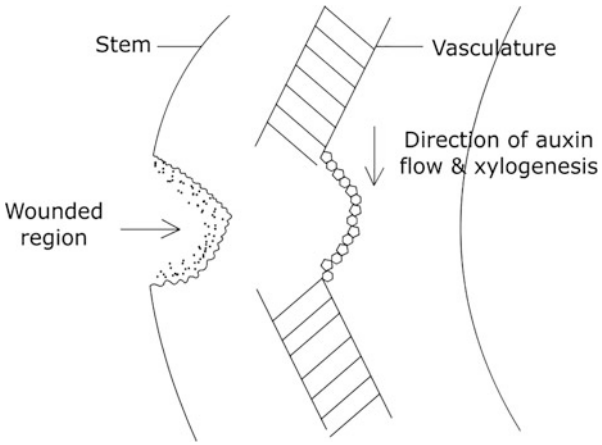


Fig. 15.13 Effect of wounding on auxin-induced xylogenesis

It is further expected that auxin may also play a role in the terminal stage of xylogenesis (i.e., loss of contents/autolysis or cell death) through induction of ethylene biosynthesis.

15.6.5 Origin of Lateral and Adventitious Roots

Lateral root (LR) primordia are initiated through localized cell divisions in the pericycle which results in a protrusion in the otherwise differentiated pericycle zone. A lateral root primordium thus formed penetrates through the cells of the cortex by developing its own root apex and root cap and emerges out of the root epidermis as a lateral root. Likewise, adventitious roots are also initiated by cell divisions in the parenchyma cells close to the vascular tissues in the stem (note: stem and leaves of seed plants generally do not have pericycle). A root primordium so organized subsequently proliferates as a new adventitious root.

Creation of local auxin gradient through equilibrium between auxin biosynthesis and transport has been observed to be critical for LR formation (Fig. 15.14). Auxin seems to act through regulation of cell cycle during LR initiation. In addition to the promotive role of auxin in LR initiation, certain other hormones also interplay in its regulation. Thus, increased ethylene levels have been reported to inhibit LR initiation. Auxin effect is also antagonized by abscisic acid by blocking cell cycle during G1 to S transition. On the other hand, LR initiation is inhibited by cytokinins by blocking G2 to M transition. It has further been observed that expression of cytokinin biosynthesis genes in the pericycle cells reduces LR formation and also disturbs cell division patterns. On the other hand, cytokinin degradation in the pericycle cells enhances LR initiation. Expression of PIN is negatively influenced by cytokinins,

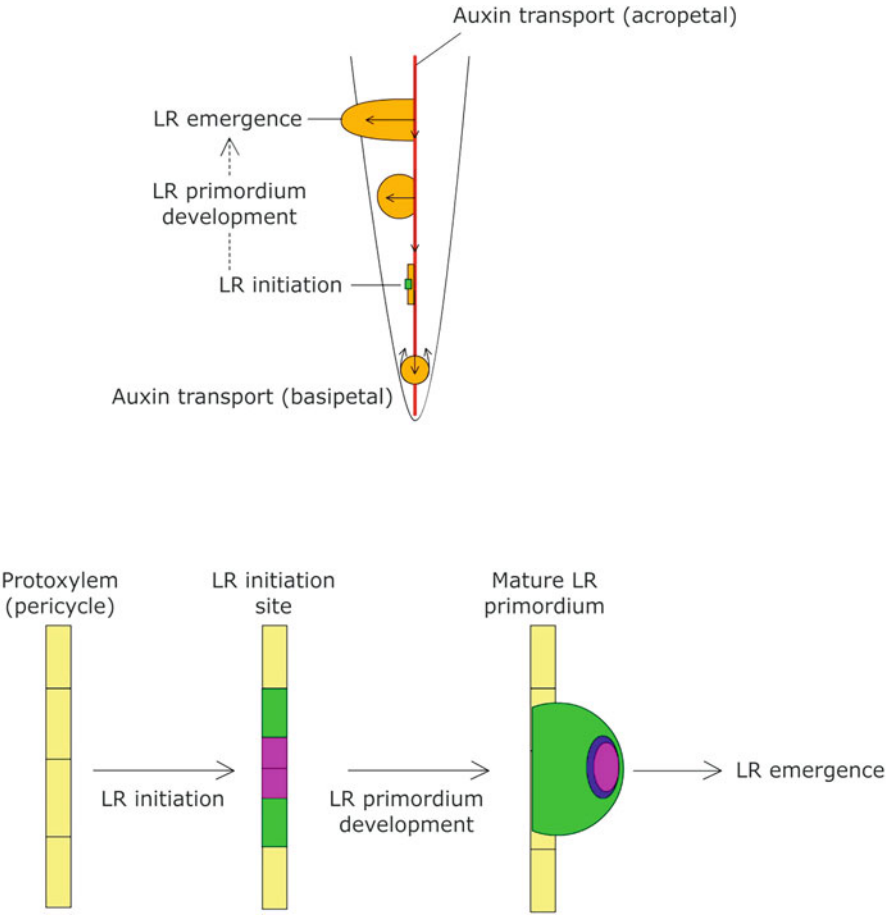


Fig. 15.14 Process of lateral root (LR) formation

which also induces degradation of PIN1 proteins resulting in lowering of auxin levels in the meristematic cells.

15.7 Signaling Mechanisms Associated with Auxin Action

Auxin action in plant cells is brought about in two major ways: (1) fast, non-transcriptional responses and (2) changes in gene expression. The non-transcriptional responses include activation of plasma membrane proton pump

and ion channels, thereby elevating the apoplastic hydrogen ion concentration which will ultimately bring about loosening of cell wall through the activation of expansins. The non-transcriptional auxin response also brings about reorientation of microtubules.

Two major categories of **auxin receptors** are known. These include auxin-binding proteins (ABPs) and F-box protein, which is a component of E3 ligase involved in ubiquitin-mediated proteolysis. During auxin action through non-transcriptional means, auxin (IAA molecule) binds ABPs located either on the plasma membrane or on the ER. This binding (IAA-ABP complex) triggers enhanced trafficking of vesicles carrying newly synthesized H^+-ATPase from the ER through the vesicles, and they are transported to the plasma membrane to elicit enhanced H^+-ATPase activity (Fig. 15.15). Thus, IAA-ABP complex not only facilitates faster migration of H^+-ATPase to the requires site on the plasma membrane, it also further enhances its activity on the membrane so long as H^+-ATPase has the proximity of IAA-ABP complex on the membrane. Enhanced H^+-ATPase activity results in greater release of H^+ ions into the apoplast leading to cell wall loosening process.

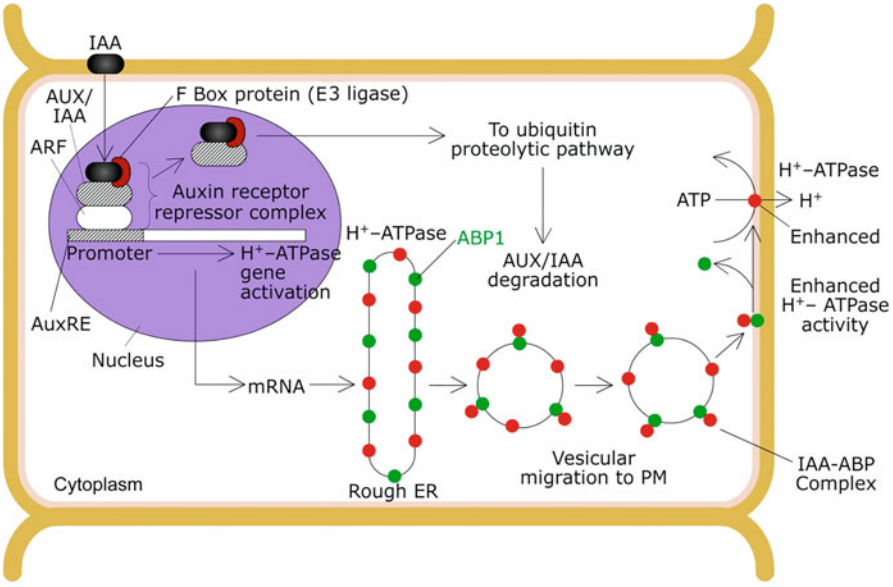


Fig. 15.15 Auxin-modulated gene expression leading to activation and enhanced activity of PM-associated H^+- ATPase. *IAA* indole-3-acetic acid, *AUX/IAA* transcription repressor, *ARF* auxin response factor, *AuxRE* auxin sensitive promoter region, *ABP1* auxin-binding protein 1

15.7.1 Changes in Gene Expression

A typical auxin responsive gene has auxin response element (AuxRE) which is the binding site located in the promoter region. Certain transcription factors known as auxin response factors (ARFs) bind to these AuxRE motifs to stimulate or repress transcription. The third component of an auxin-inducible gene is a proteinaceous repressor of the transcription factor. These repressors, referred as AUX/IAA, remain associated with ARFs when auxin concentrations are low, thereby repressing transcription activity (Fig. 15.16). In the presence of auxin, AUX/IAA repressors get separated from the auxin-inducible gene and get tagged with ubiquitin for degradation through 26S proteasome pathway. [*Ubiquitin is a small (76 kDa), single peptide protein that is found in almost all living cells and plays an important role in degradation of defective and superfluous proteins.*] **Ubiquitination**, i.e., addition of ubiquitin to the target protein, marks AUX/IAA for degradation via the proteasome pathway. As a result of this dissociation of AUX/IAA repressors from the ARFs (transcription factors), the ARFs are able to dimerize or even oligomerize leading to gene activation.

15.7.2 The Process of AUX/IAA Degradation

This is brought about by polyubiquitination of AUX/IAA, leading to its release from auxin gene. Auxin molecules interact with a protein called transport inhibitor

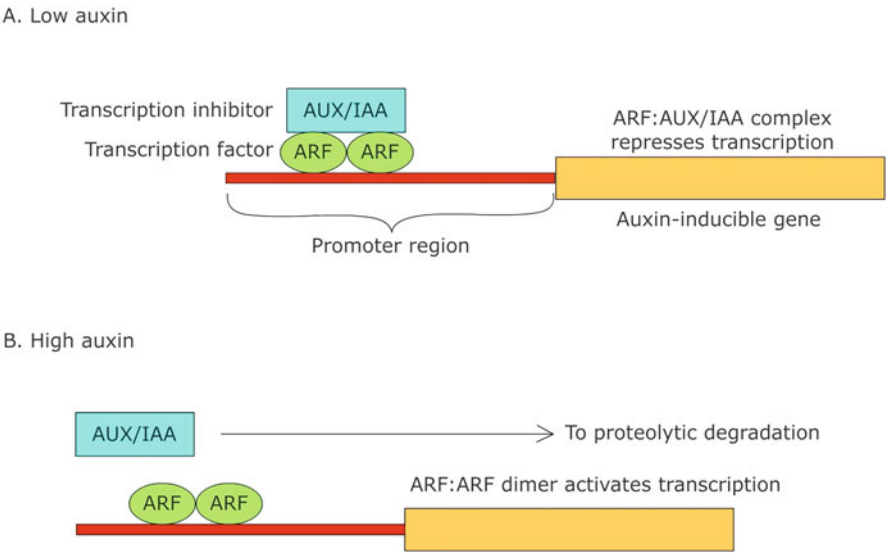


Fig. 15.16 Activation of auxin responsive gene by auxin

response protein 1 (TIR1) in the SCF complex, which is now known to be the auxin receptor associated with auxin-induced gene action. TIR1, therefore, functions as an auxin receptor with auxin acting as a molecular glue. TIR1 is a part of a protein complex called SCF. SCF refers to the three polypeptides found in the complex: *Skp*, *Cullin*, and *F-box*. SCF catalyzes ATP-dependent covalent addition of ubiquitin molecules to proteins targeted for degradation. Auxin binds to TIR1 at the conserved leucine-rich repeats. Four steps lead to auxin perception through auxin-induced gene expression: (1) Binding of TIR1 to IAA; (2) attachment of AUX/IAA proteins with the activated TIR1, i.e., IAA/TIR1 complex leading to its ubiquitination; (3) degradation of AUX/IAA proteins by proteasome pathway; and (4) activation of transcription factor ARF due to non-availability of transcription inhibitor (AUX/IAA) which has been degraded facilitating transcription of auxin response gene (Figs. 15.16 and 15.17).

Auxin-induced gene expression can lead to two types of responses:

1. *Early or primary response genes*: Their expression is insensitive to protein synthesis inhibitors, and their expression time is short-lived. Their expression has three main functions. These encode transcription factors for the transcription of late or secondary response genes. The expression products of these genes are

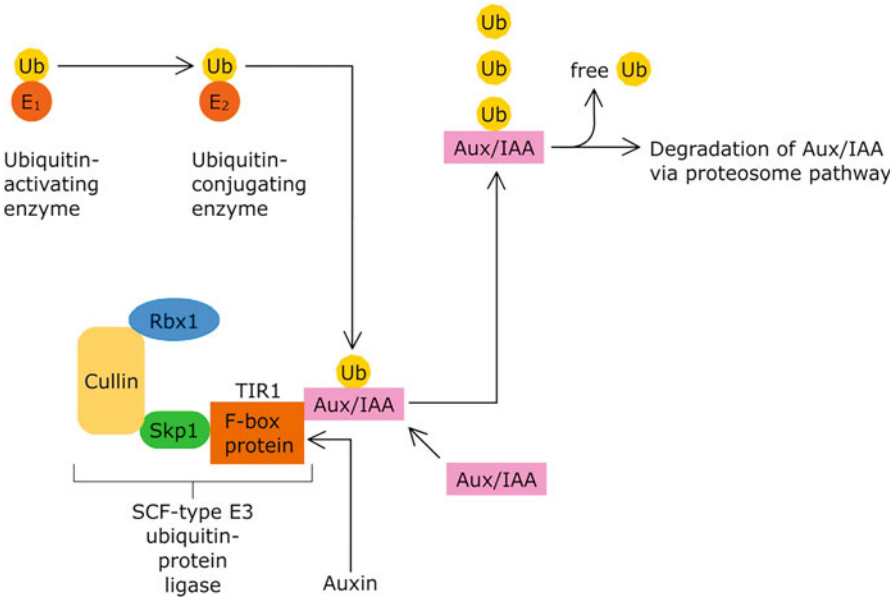


Fig. 15.17 Targeted protein degradation mediates auxin signal transduction. Auxin binding to the F-box protein TIR1 enhances interaction between TIR1 and AUX/IAA proteins. This allows polyubiquitination of Aux/IAA proteins through the sequential actions of E1, E2, and E3 enzymes and so targets Aux/IAAs for degradation
Ub Ubiquitin

involved in signaling of intracellular communication or cell-to-cell signaling. They also include genes encoding proteins involved in adaptation to stress.
2. *Late or secondary response genes*: They require de novo protein synthesis, and they trigger the expression of enzymes such as glutathione S-transferase and ACC synthase.

The highly diversified response due to auxin-induced gene action is brought about by the existence of multiple forms of the three components of auxin signaling. Thus, *Arabidopsis* is known to have 6 TIRs, 29 AUX/IAA, and 23 ARF proteins. The auxin response signaling route discussed above primarily derives information from investigations on *Arabidopsis*, maize, and rice as model systems. Similar auxin response pathway has also recently been found to operate in the earliest diverging land plants, liverworts, and mosses. Thus, auxin response in plant cells has an ancient history.

Summary

- Auxins constitute an important group of naturally occurring hormones which have been detected practically in all land plants and in several soil- or plant-associated microbes.
- Tryptophan (an aromatic amino acid) is the precursor of IAA. Biosynthesis of tryptophan principally takes place in chloroplasts. Subsequent formation of IAA occurs both in cytosol and chloroplasts. IAA is also synthesized from indole or indole-3-glycerol phosphate in tryptophan-independent pathways.
- The homeostatic control of auxin through conjugation and degradation is also necessary in addition to regulation of its biosynthesis. Conjugation of IAA to glucose, alanine, and leucine is a reversible process, and most conjugated forms are sequestered within the vacuoles. Oxidative catabolism of IAA is an irreversible process for permanent removal of intracellular auxin not required by the cells. IAA catabolism is now known to be mediated by specific isoforms of peroxidase, resulting in the formation of 3-methyleneoxindole derivative.
- Auxin is the only plant hormone which exhibits polar transport in almost all plants, including bryophytes and ferns. Polar transport of auxin is always basipetal, i.e., toward the base, both in shoots and roots. Two mechanisms are operative for auxin influx: (1) Passive diffusion of protonated form (IAAH, a lipophilic molecule) from any direction across the phospholipid bilayer. (2) Secondary active transport of dissociated form (IAA^-) using symporter protein, AUX1. AUX1 is an auxin uptake carrier protein that functions in leaf vascular tissue and root apices. It belongs to a family of proteins similar to permeases in prokaryotes. Phytotropins are noncompetitive inhibitors of polar auxin transport. These include TIBA (2,3,5-triiodobenzoic acid), morphactin (9-hydroxyfluorine-9-carboxylic acid), and NPA (N-1-naphthylphthalamic acid).

- The critical component of the chemiosmotic model is, however, the basal location of PIN proteins which is responsible for efflux of IAA^- from the cells. It is this unique basal location of PIN proteins which is responsible for establishing polarity in auxin transport.
- The major driving force causing efflux of IAA^- from the cell is the negative plasma membrane potential, which varies between −200 and −300 mV. ABCB transporters facilitate IAA^- efflux or influx across the plasma membrane (and also tonoplast), but unlike PIN proteins, they do not show preferential basal localization in the cells.
- Acid growth hypothesis proposes that auxin brings about activation of H^+-ATPases through its binding with a membrane-associated protein called ABP1 (auxin-binding protein 1). ABP1 is an auxin receptor which has been localized in the ER and plasma membrane. It is a 43 kDa glycoprotein dimer of two subunits of 22 kDa each. It is proposed that ABP1 forms a complex with a transmembrane docking protein which provides lipid solubility to anchor ABP1 to the membrane. This auxin-ABP1-docking protein complex initiates a signal transduction pathway leading ultimately to activation of ATPase proton pumps.
- Apical dominance refers to the inhibition of axillary bud growth by the continued meristematic activity in the shoot apex. It is best observed in herbaceous plants and also in trees during first year of growth.
- Auxin produced in the shoot apex inhibits lateral bud growth through its transport downward. Apical dominance is regulated by limiting sugar availability to the axillary buds in intact plants, and sustained growth of the axillary bud requires depletion of auxin in the stem adjacent to the bud as well.
- Polar auxin transport is required for floral development in the inflorescence meristem.
- Both localized and transported auxins play crucial role in the differentiation of the vascular tissues. The effect of auxin on xylogenesis is twofold: (1) it acts as a vascular inducing signal and (2) "canalization" of newly differentiated vascular strand leading to polar transport of auxin. Creation of local auxin gradient through equilibrium between auxin biosynthesis and transport has been observed to be critical for lateral root (LR) formation. Auxin seems to act through regulation of cell cycle during LR initiation.
- Auxin action in plant cells is brought about in two major ways: (1) fast, non-transcriptional responses and (2) changes in gene expression. Two major categories of auxin receptors are known. These include auxin-binding proteins (ABPs), and F-box protein, which is a component of E3 ligase involved in ubiquitin-mediated proteolysis. IAA-ABP complex not only facilitates faster migration of H^+-ATPase to the required site on the plasma membrane, it also further enhances its activity on the membrane so long as H^+-ATPase has the proximity of IAA-ABP complex on the membrane. In the presence of auxin, AUX/IAA repressors get separated from the auxin-inducible gene and get tagged

with ubiquitin for degradation through 26S proteasome pathway. Auxin-induced gene expression can lead to two types of responses. (1) *Early or primary response genes*: These encode transcription factors for the transcription of late or secondary response genes. They also include genes encoding proteins involved in adaptation to stress. (2) *Late or secondary response genes*: They require de novo protein synthesis and they trigger the expression of enzymes such as glutathione S-transferase and ACC synthase.

Multiple-Choice Questions

1. Which of the following is a natural auxin?
   (a) NAA
   (b) 2,4-D
   (c) 2,4,5,-T
   (d) IAA
2. Phytotropins refer to:
   (a) Conjugated forms of auxin
   (b) Noncompetitive inhibitors of polar auxin transport
   (c) Proteins responsible for efflux of auxin
   (d) Transporters of auxin
3. Which of the following is true regarding the auxin-induced expression of early or primary response genes?
   i. Expression of these genes is insensitive to protein synthesis inhibitors.
   ii. These encode transcription factors for the transcription of late or secondary response genes.
   iii. These encode proteins involved in adaptation to stress.
   iv. They are expressed for a rather longer period of time.
   (a) Only ii and iv
   (b) Only i, ii, and iv
   (c) Only i, ii, and iii
   (d) Only i and iv
4. Which of the following *does not* inhibit auxin transport?
   (a) TIBA
   (b) NAA
   (c) NPA
   (d) Morphactin
5. Canalization model of auxin flow states that:
   (a) Auxin flow majorly occurs through xylem.
   (b) Auxin flow majorly occurs through phloem.
   (c) Auxin is transported from the source to sink in an acropetal manner.
   (d) Auxin transport results in the reestablishment of continuous strands of vascular elements in a self-organizing pattern.

6. Which of the following is/are auxin receptor(s)?
   i. ABP
   ii. AUX/IAA
   iii. F-box proteins
   iv. ARF
   (a) Only i
   (b) Only ii
   (c) Only i and iii
   (d) Only i, ii, and iii
7. Which of the following is true regarding the role of ABP?
   (a) ABP forms complex with IAA and enhances trafficking of H^+-ATPase carrying vesicles to plasma membrane.
   (b) ABP binds with IAA and brings about degradation of AUX/IAA and subsequent transcription of auxin responsive genes.
   (c) ABP facilitates the influx of auxin into cells.
   (d) ABP is responsible for establishing polar transport of auxin.
8. Which of the following does not come under the category of tryptophan-dependent pathway of IAA production?
   (a) Tryptamine pathway
   (b) Indole-3-acetonitrile pathway
   (c) Indole-3-glycerol pathway
   (d) Indole-3-pyruvic pathway
9. Auxin transport is:
   (a) Always basipetal both in roots and shoots
   (b) Always acropetal both in roots and shoots
   (c) Acropetal in roots and basipetal in shoots
   (d) Basipetal in roots and acropetal in shoots
10. Influx of auxin:
    i. Takes place through passive diffusion of the dissociated form of IAA, i.e., IAA^-
    ii. Takes place through passive diffusion of the protonated form of IAA, i.e., IAAH
    iii. Takes place through active transport of dissociated form of IAA, i.e., IAA-
    iv. Takes place through active transport of protonated form of IAA, i.e., IAAH
    (a) Only i and ii
    (b) Only i and iv
    (c) Only ii and iii
    (d) None of the above

11. Which of the following is true for the processes of conjugation and oxidation both of which regulate auxin homeostasis?
    i. Both work to remove the active auxin when present at supraoptimal concentrations.
    ii. Both are irreversible processes, causing permanent removal of auxin.
    iii. Conjugation is reversible, while oxidation of IAA is irreversible and brings about loss of auxin activity.
    iv. Both conjugation and oxidation are reversible and allow for reversible release of free IAA when required.
    (a) Both i and ii
    (b) Both i and iii
    (c) Both i and iv
    (d) None of the above
12. PIN1 is:
    (a) A transmembrane protein that is responsible for establishing polar basipetal auxin transport.
    (b) An inhibitor of polar auxin transport.
    (c) A protein for auxin uptake.
    (d) A positive regulator of auxin biosynthesis.
13. Which of the following is responsible for establishing polarity of auxin transport?
    (a) ABP1
    (b) AUX1
    (c) TIBA
    (d) PIN1
14. Which of the following is true for apical dominance?
    i. It is the phenomenon of inhibition of lateral bud growth by continuous auxin production in shoot apex.
    ii. It is regulated by limitation of sugar availability to axillary buds.
    iii. It is due to the accumulation of cytokinins within cells of axillary buds.
    iv. It is controlled by both strigolactones and auxin.
    (a) Only i, ii, and iv
    (b) All of the above
15. Decapitation of apical bud results in overcoming apical dominance due to:
    (a) Reversion of auxin-induced cytokinin biosynthesis inhibition and initiation of meristematic activity of axillary buds
    (b) The efflux of cytokinins from the axillary buds that promotes their growth
    (c) The rapid accumulation of auxin in axillary buds that facilitates their growth
    (d) Increased meristematic activity of axillary bud resulting from efflux of growth inhibiting substances from them

Answers

1. d	2. b	3. c	4. b	5. d	6. c
7. a	8. c	9. a	9. a	11. b	12. a
13. d	14. c	15. a			

Suggested Further Readings

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