17.2 Abscisic acid

- Growth-inhibiting compounds were isolated from plants in the early 1950s.
- Experiments detected compounds that inhibited coleoptile elongation, referred to as the β-inhibitor complex.
- In the early 1960s, US scientists isolated an abscission-accelerating compound called “abscisin II” from young *Gossypium hirsutum* fruits and simultaneously researchers in the UK isolated a dormancy-inducing factor called “dormin.”
- The structure of abscisin II, later called **abscisic acid** (ABA), was determined in 1965 and dormin was subsequently shown to be ABA.

2.1 Contrary to its name, ABA does not induce abscission

- As its name indicates, ABA was originally thought to induce abscission, however, abscission is regulated by ethylene rather than ABA.

- Increased internal concentrations of ABA are unlikely to impose dormancy of *S. tuberosum* tubers or resting buds of deciduous trees.

- No support for conjecture that asymmetric distribution of ABA is involved in the negative geotropic response of roots.

FIGURE 17.15 *Precocious germination (vivipary) of immature* Z. mays *seeds homozygous for* vp1. *The color-less* vp1 *kernels lack anthocyanins in the aleurone layer, and germinate before maturity because of ABA insensitivity. The seed is viable if transplanted directly from immature ears.*
Source: S. McCormick, University of California, Berkeley; previously unpublished.

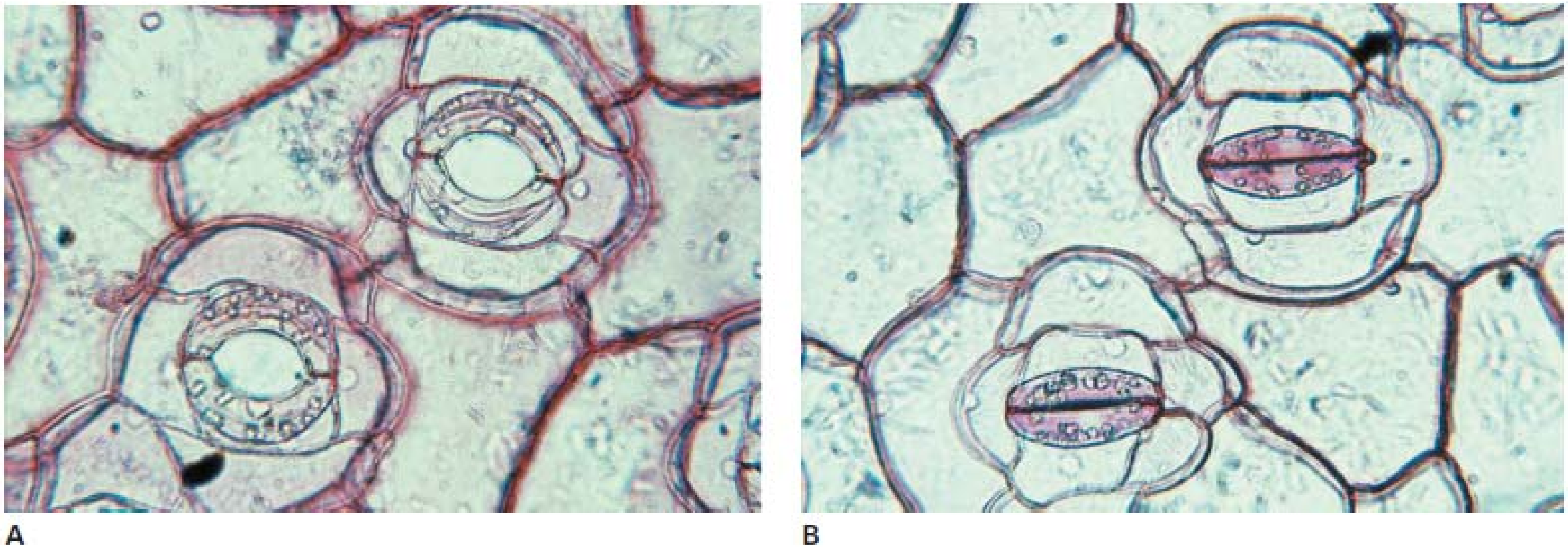


FIGURE 17.16 *ABA-induced stomatal closure. Epidermal strips of* Commelina communis *L. incubated in buffer (10 mM Pipes, pH 6.8) containing 50 mM KCl and supplied with CO_2-free air. The stomata are open after two to three hours (A). When transferred to the same solution plus 10 μM ABA, the pores close within 10 to 30 minutes (B).*

Source: J. Weyers, University of Dundee, UK; previously unpublished.

Effects

- Stomatal closure
- Inhibits shoot growth
- Protein synthesis
- Counteracts the effect of gibberellin
- Some aspects of dormancy
- Response to wounding

2.2 Many fungi, including fungal pathogens of the genera *Cercospora* and *Botrytis*, synthesize ABA from farnesyl diphosphate

By the early 1970s, two pathways leading to ABA had been proposed. The "direct C_{15}" route envisaged ABA production from the C_{15} precursor farnesyl diphosphate, while the "indirect C_{40}" route postulated oxidative cleavage of a putative C_{40} intermediate, such as violaxanthin, to yield a C_{15} intermediate of ABA.

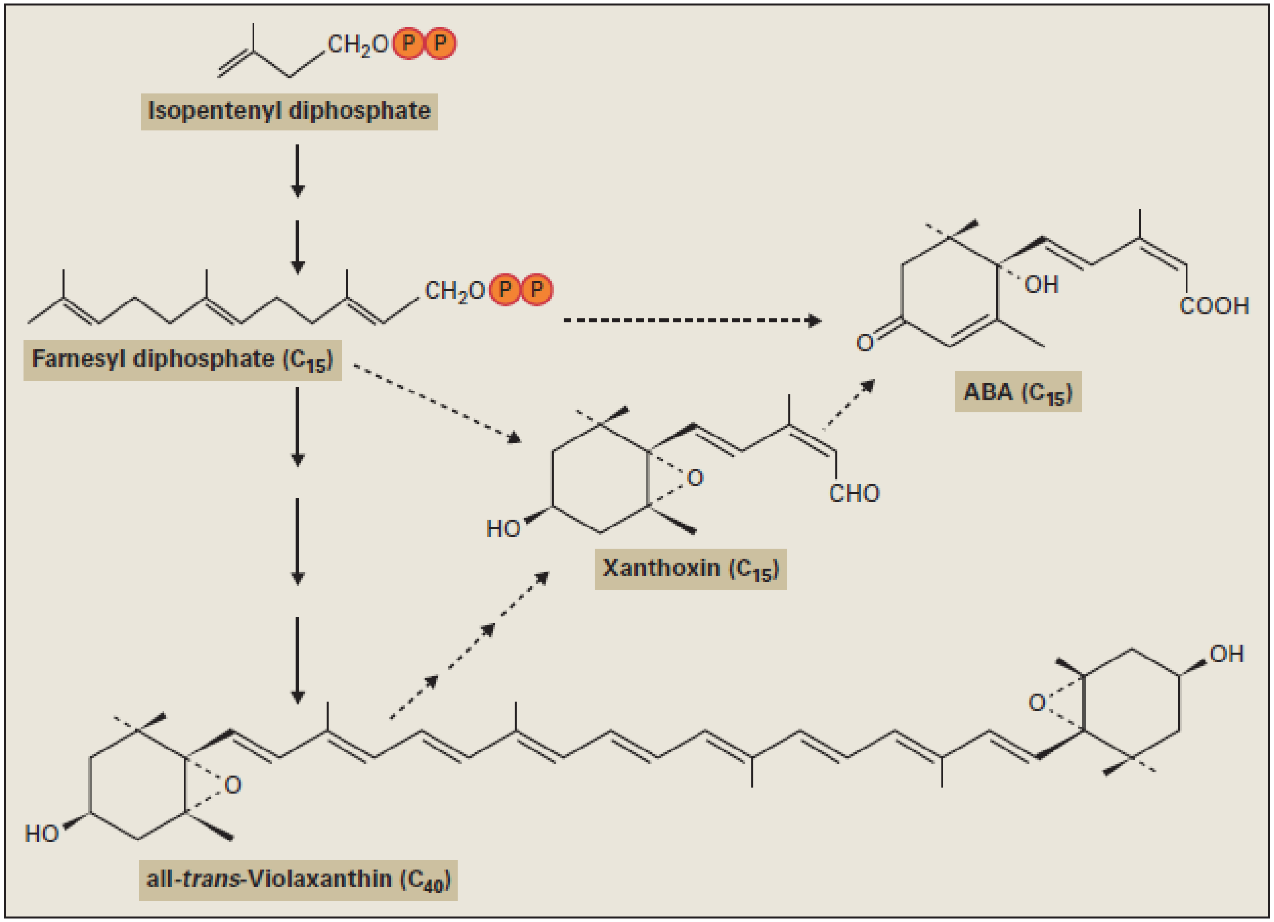


FIGURE 17.17 *Summary of two possible ABA biosynthetic routes. In the direct C_{15} pathway, farnesyl diphosphate is modified to yield ABA. Alternatively, in the indirect C_{40} pathway, a carotenoid, 9-*cis*-violaxanthin, is cleaved to form a C_{15} ABA precursor, xanthoxin.*

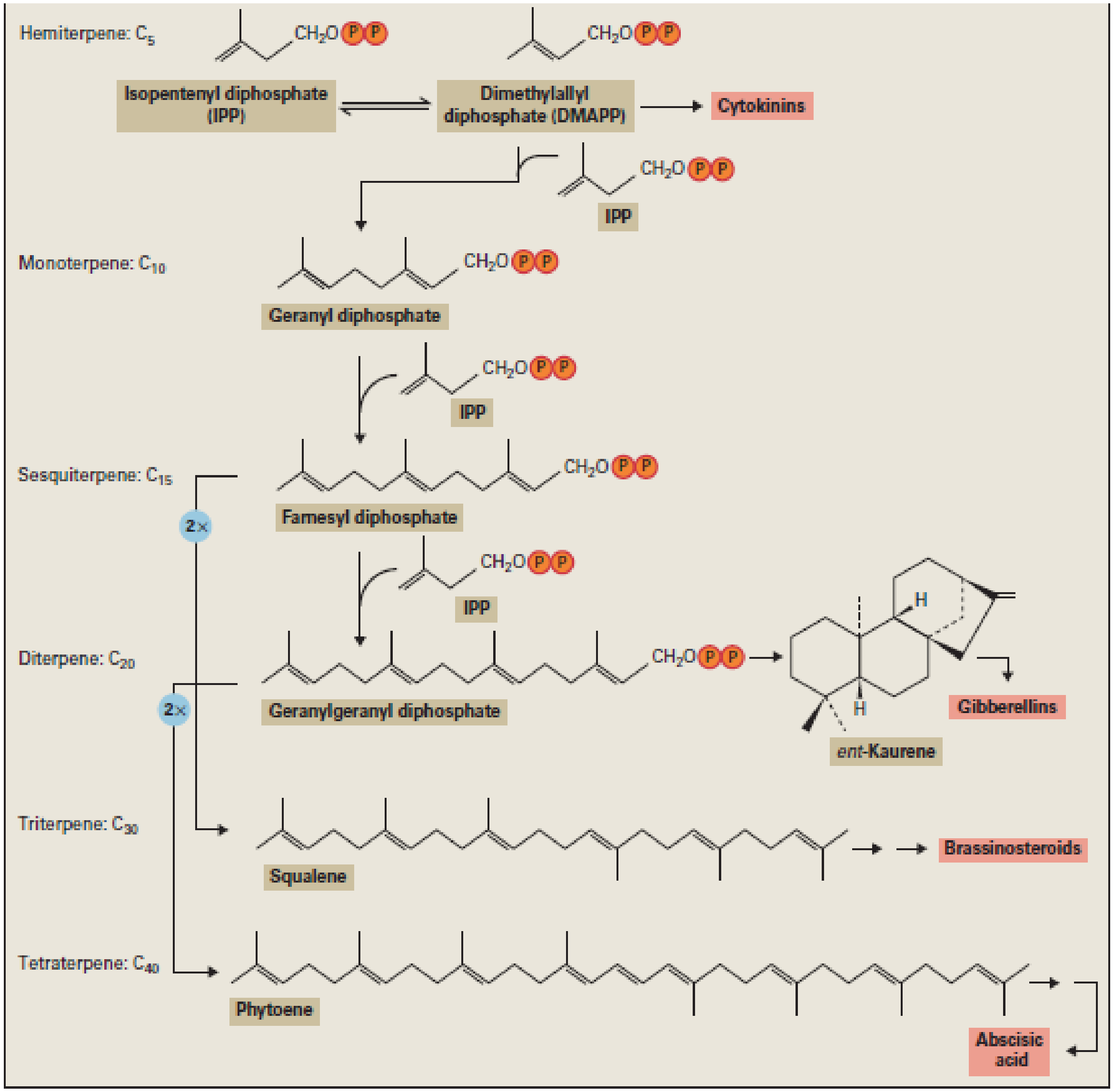


FIGURE 17.6 *Terpenoid biosynthesis pathway, showing biosynthetic origins of GAs as well as CKs, BRs, and ABA.*

2.3 Gas chromatography–mass spectrometry showed that plants synthesize ABA from a C_{40} precursor

Experiments with mutants and with inhibitors of carotene synthesis were keys to understanding ABA biosynthesis.

Firm evidence that C_{40} xanthophylls are intermediates in ABA biosynthesis was obtained in 1984.

1′-deoxy-ABA was not the immediate ABA precursor in water-stressed leaves.

ABA was synthesized from a preformed precursor with oxygen atoms at the 1′ and 4′ positions.

This was consistent with a C_{40} pathway to ABA, with $^{18}O_2$ cleaving violaxanthin to form xanthoxin labeled at the 1-CHO group, followed by oxidation of the labeled xanthoxin to yield ABA with one ^{18}O atom in the carboxyl group.

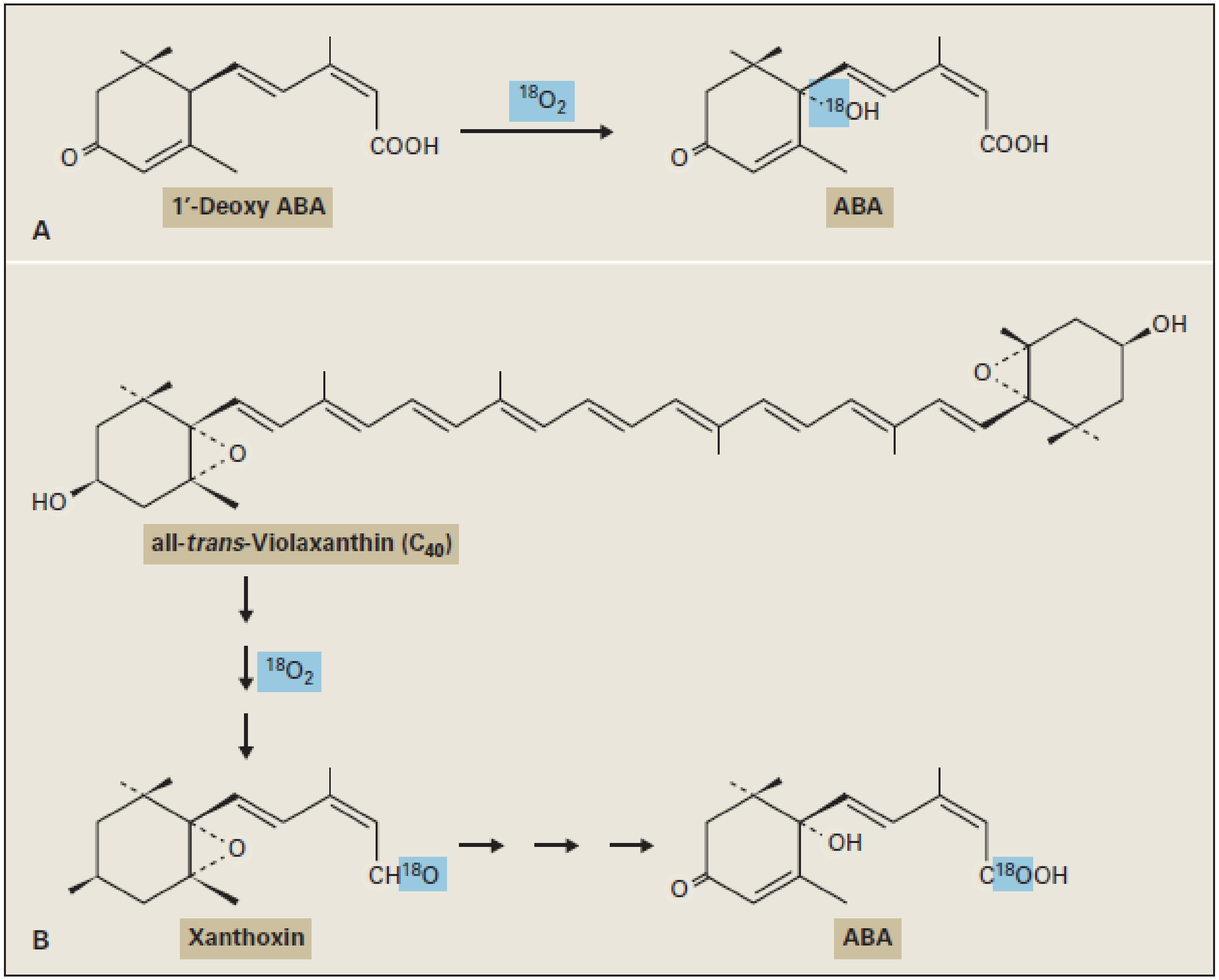


FIGURE 17.20 *Isotopic labeling experiments confirmed the existence of an indirect C_{40} plant ABA biosynthetic pathway. GC-MS revealed that ABA synthesized by plant tissues in the presence of $^{18}O_2$ was not labeled at the 1′-hydroxyl group, as would be expected if ABA were generated directly from 1′-deoxy-ABA (A). However, the appearance of label in the ABA carboxyl group was consistent with oxidative cleavage of all-trans-violaxanthin and subsequent conversion of xanthoxin to ABA (B).*

2.4 ABA synthesis is regulated by a cleavage reaction that generates the first C_{15} intermediate

Many ABA-deficient viviparous (*vp*) mutants of *Z. mays* are blocked at various points in the terpenoid and carotenoid biosynthesis pathways.

GGDP synthase, a key enzyme in the terpenoid pathway, catalyzes the three successive condensations with isopentenyl diphosphate that convert dimethylallyl diphosphate to the C_{20} compound GGDP.

As a consequence of GGDP deficiency, seedlings of the *vp*12 mutant have low chlorophyll content and reduced capacity for synthesis of carotenoids and ABA.

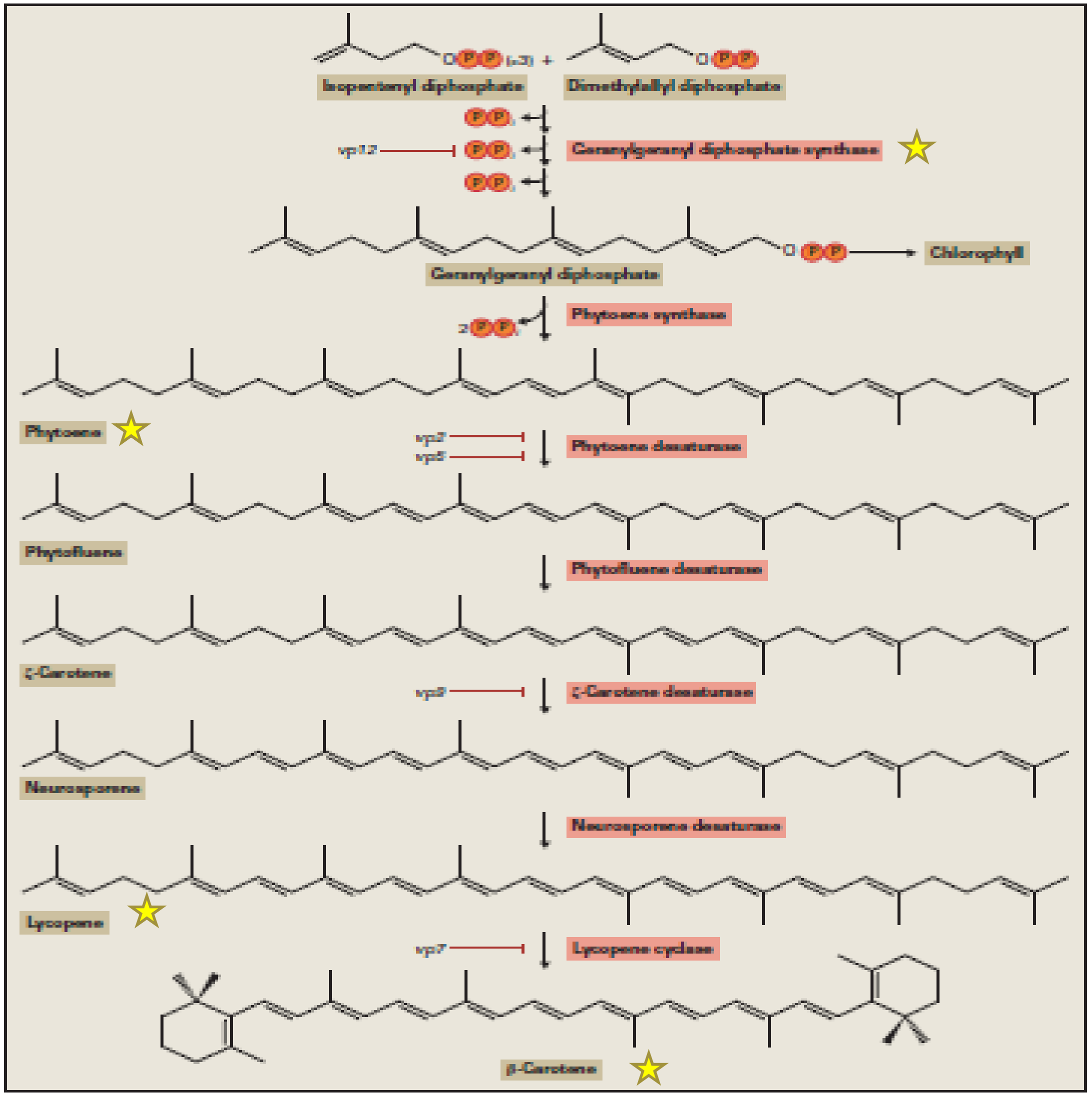


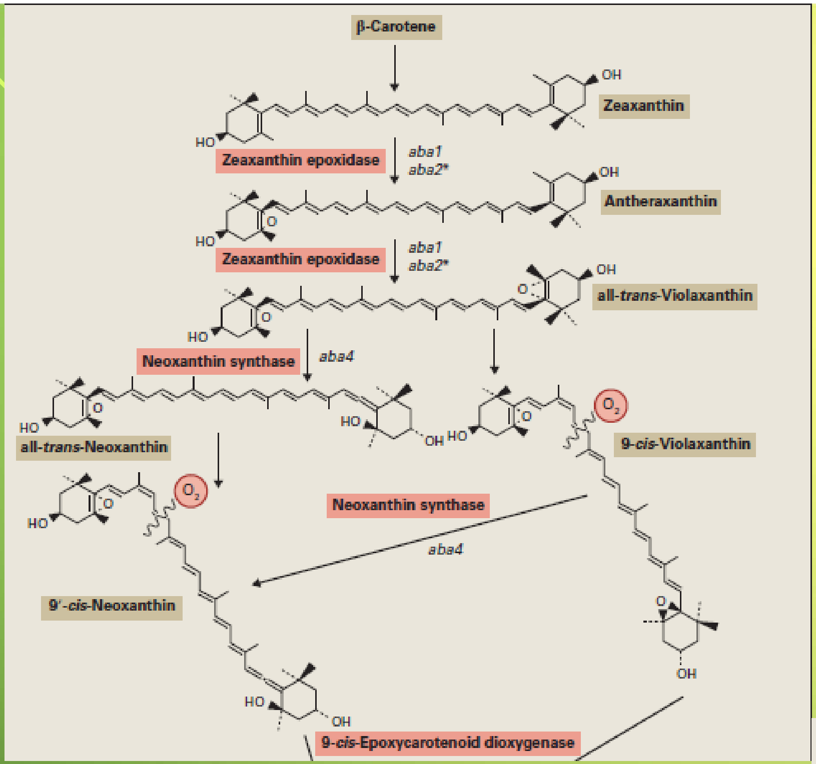
FIGURE 17.19 *Early stages in the indirect C_{40} ABA biosynthesis pathway: production of geranylgeranyl diphosphate (GGDP) and synthesis of β-carotene. Enzymes deficient in Z. mays vp mutants are indicated. The chemical inhibitors fluridone and norflurazon block conversion of phytoene to phytofluene (not shown).*

The first committed step in the ABA biosynthesis pathway, oxidative cleavage of 9′-*cis*-neoxanthin and/or -cis-violaxanthin, yields the first C15 intermediate, xanthoxin.

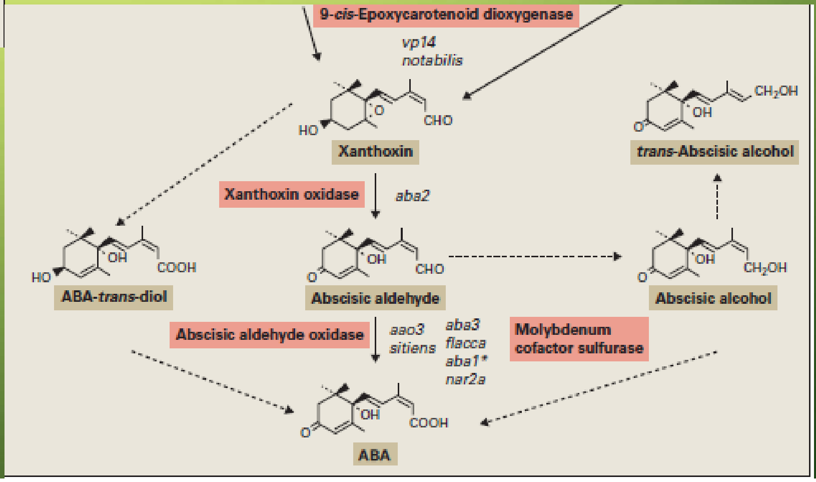
9-*cis*-epoxycarotenoid dioxygenase that catalyzes this conversion, is encoded by a multigene family.

- 9-*cis*-epoxycarotenoid dioxygenase encoded by *AtNCED3* gene is responsible for ABA production upon water stress,
- while *AtNCED6* and *AtNCED9* gene products are involved in ABA production in seeds.

This conversion appears to be a rate-limiting step in ABA biosynthesis;



- FIGURE 17.21 Later stages in the indirect C40 ABA biosynthesis pathway: β-carotene to ABA. Biosynthetic steps blocked in the following mutants are indicated. *Arabidopsis mutants*: aao3, aba1, aba2, aba3, aba4; *H. vulgare*: nar2a; *S. lycopersicum mutants*: flacca, notabilis, sitiens; *N. plumbaginifolia mutants*: aba1*, aba2*; *Z. mays mutant*: vp14



The penultimate step of the ABA biosynthesis pathway converts xanthoxin to abscisic aldehyde.

Oxidation of abscisic aldehyde, catalyzed by abscisic aldehyde oxidase, leads to formation of ABA.

- There are many mutants defective in conversion of abscisic aldehyde to ABA.
- The activity to produce ABA from xanthoxin via abscisic aldehyde is not increased following the onset of water stress

2.5 ABA is metabolized to several compounds, including phaseic acid, dihydrophaseic acid, and glucose conjugates

- The main route involves hydroxylation of the 8′ carbon, spontaneous rearrangement of the resulting 8′-hydroxy-ABA to form phaseic acid (PA), and reduction to dihydrophaseic acid and *epi*-dihydrophaseic acid.
- The alternative routes in ABA metabolism involve conversion to 7′- and 9′-
- hydroxy-ABA.
- conjugation to form ABA-β-glucosyl ester and ABA-1′-O-glucoside.

ABA 8′-hydroxylase is a membrane bound CYP450 monooxygenase classified as *CYP707A*. Single or multiple mutants defective in these genes contain elevated endogenous ABA levels.

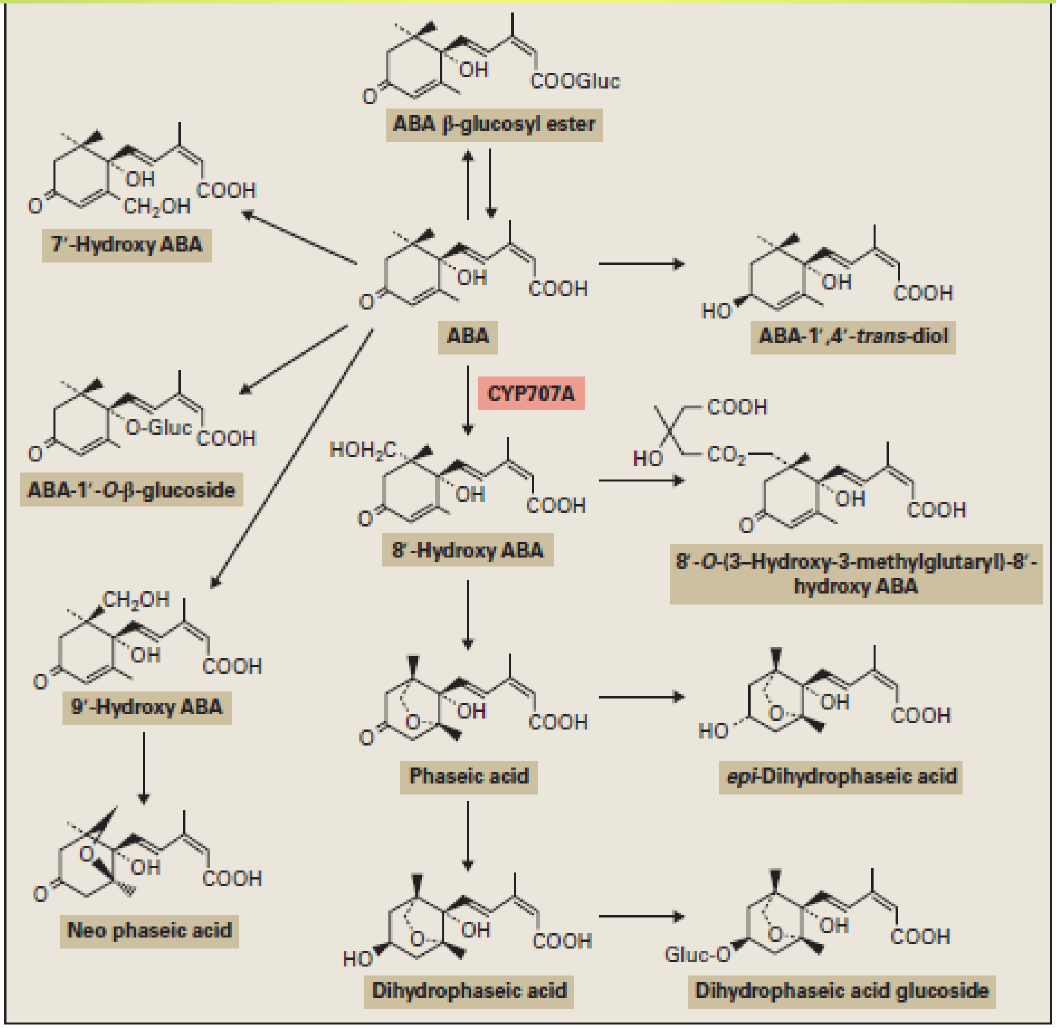


FIGURE 17.22 *ABA metabolism pathways. The major route proceeds by way of 8′-hydroxy ABA, which is rapidly converted to phaseic acid; this in turn, is reduced to epi-dihydrophaseic acid and dihydrophaseic acid. Dihydrophaseic acid undergoes conjugation to yield dihydrophaseic acid -4′-O-glucoside. ABA can also be conjugated, forming ABA β-glucosyl ester and ABA-1′-O-β-glucoside.*

17.3 Cytokinins

The discovery of CKs has its roots in the 1930s quest for chemical factors that would allow the culturing of plant tissues in synthetic media.

In the 1950s, a substance that strongly stimulated cell proliferation in *Nicotiana tabacum* tissue culture was first purified and crystallized from autoclaved herring sperm DNA extracts. This growth-stimulating compound *N6*-furfuryl aminopurine was named kinetin.

Kinetin has not been found in living plants and is believed to be an artificial by-product of DNA breakdown.

Kinetin, in combination with auxin, was found to promote the initiation and maintenance of cell division in cultured *N. tabacum* parenchyma.

A naturally occurring kinetin-like substance was first isolated from immature *Z. mays* endosperm in the early 1960s and named zeatin (*trans*-zeatin, tZ).

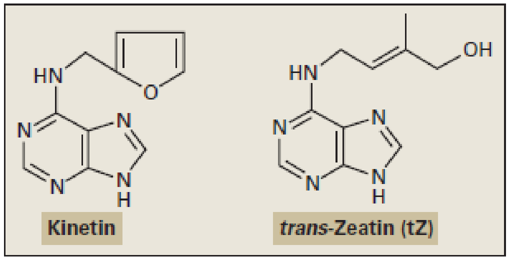


FIGURE 17.23 *CK structures. The first CK identified was the synthetic compound Kinetin. The first plant CK isolated was trans-Zeatin.*

- Usage of the term “cytokinins” for kinetin-like compounds was defined as a “generic name for substances which promote cell division and exert other growth regulatory functions in the same manner as kinetin.”

FIGURE 17.24 Arabidopsis *callus production is induced by auxin (IBA) and CK (tZ). Callus subcultured on auxin medium produces only roots (left); but on medium containing a high ratio of CK to auxin produces shoots (right).*
Source: T. Kakimoto, Osaka University, Japan; previously unpublished.

- *Effects*

✓Cell division

✓Morphogenesis

✓Growth of lateral buds

✓Leaf expansion

✓Senescence

✓Stomatal opening

✓Chloroplast development

3.1 Structural variations occur in the side chains of CKs

- Isoprenoid CKs have an isoprene-derived side chain and the common active forms include isopentenyladenine (iP; *N6*- (Δ2-isopentenyl)adenine), *trans*-zeatin (tZ), *cis*-zeatin (cZ), and dihydrozeatin (DZ).
- In general, iP and tZ are active, and their derivatives are abundant, but they are susceptible to degradation by CK oxidase (CKX).
- cZ is less active and relatively more stable than tZ and iP because of its low affinity for CKX.
- DZ appears to be biologically stable because of its resistance to CKX and is generally found in small quantities except for some legumes.

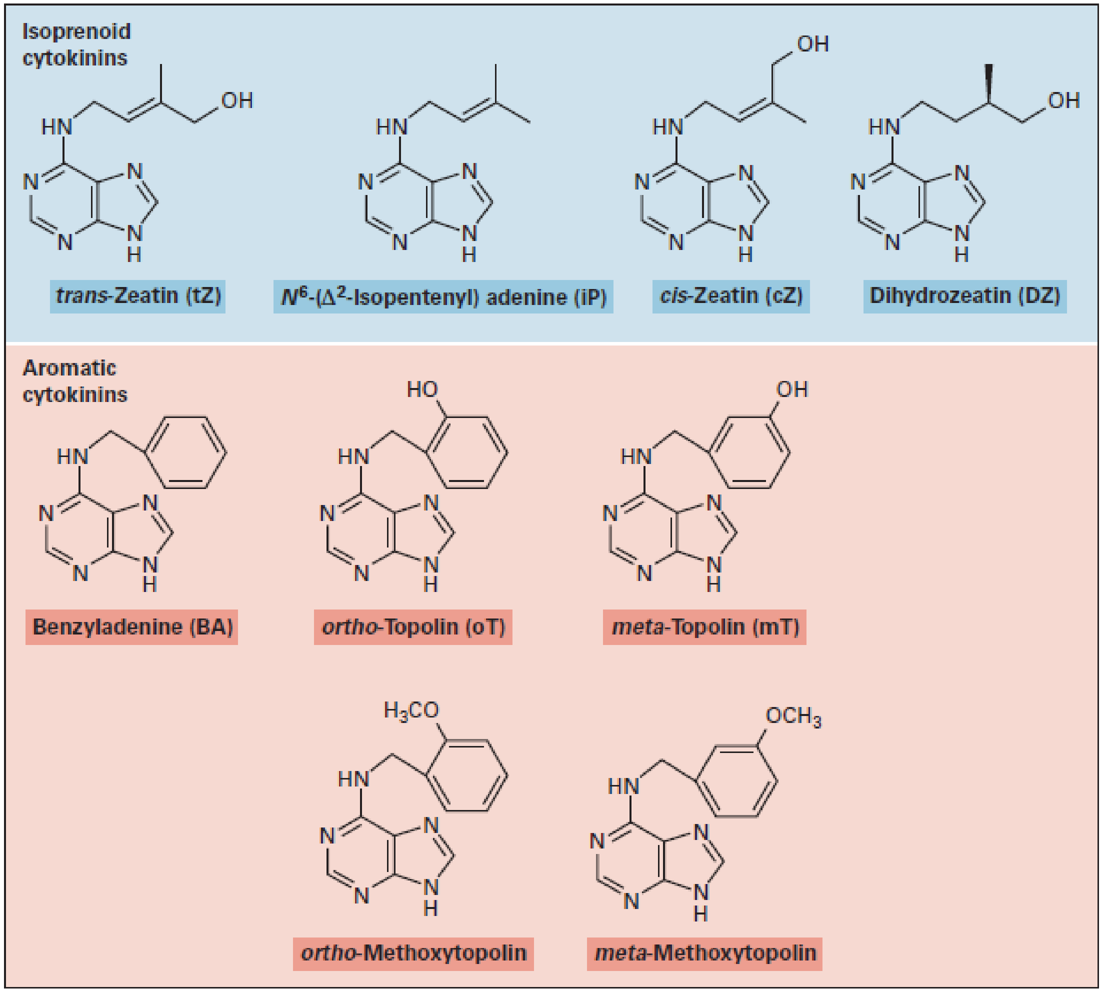


FIGURE 17.25 *Structures of naturally occurring CKs. In general, isoprenoid CKs are in greater abundance than aromatic CKs. Aromatic CKs have been described in only some plant species. Benzyladenine is found in* P. patens.

3.2 CKs are present as conjugates

- CKs are present in plants as nucleoside, nucleotide, and glycosidic conjugates.

- Glucosylation of CK occurs at the *N3*, *N7*, or *N9*-positions of the purine moiety to form *N*-glucosides.
- Amino acid conjugates of CK have also been isolated from some organisms. Lupinic acid, which was first found in lupine seedling, is an alanine onjugate of tZ.

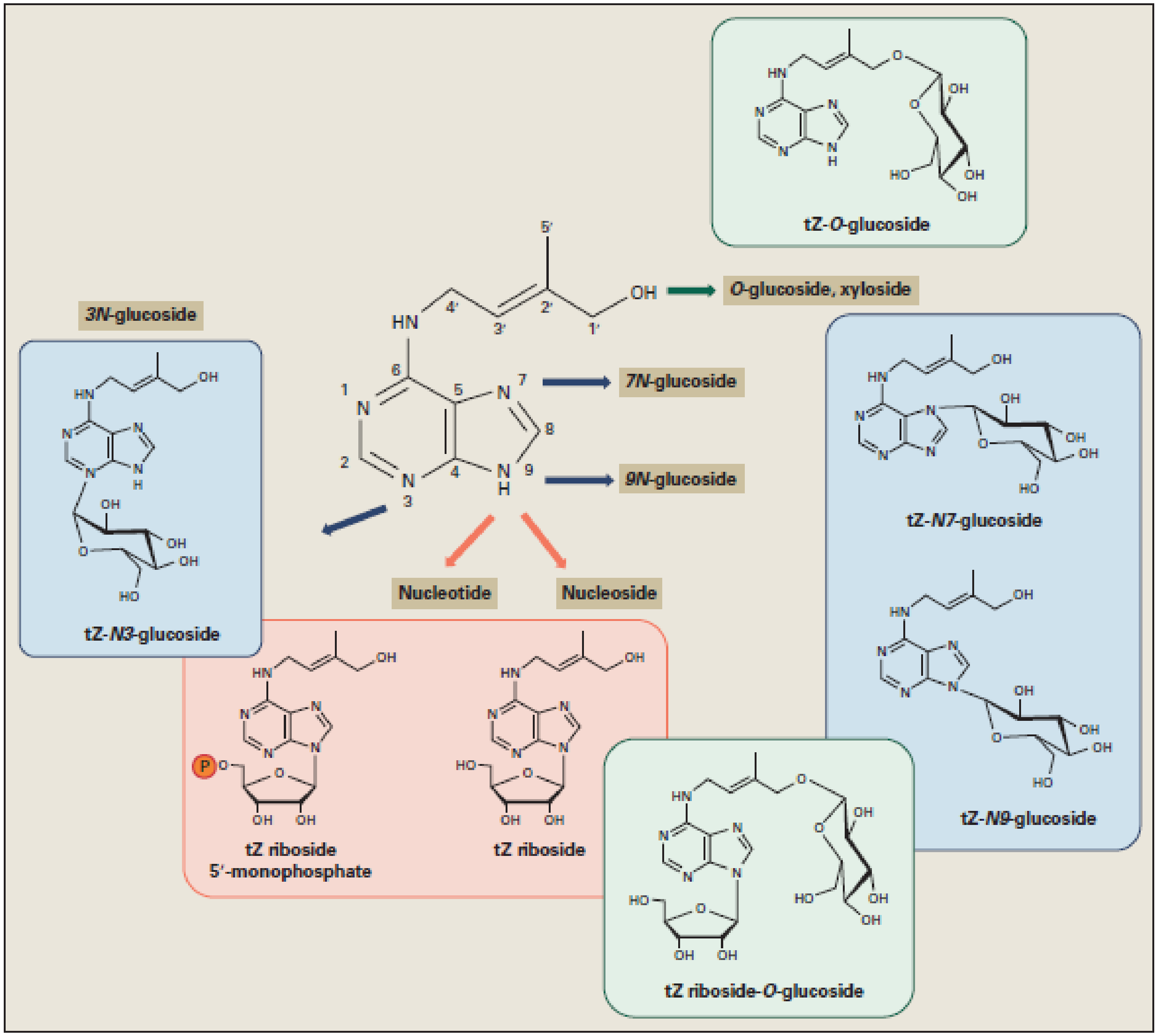


FIGURE 17.26 *Conjugates of tZ. Only representative structures are shown. Conjugation of iP, cZ, and DZ occurs in the same manner, but iP conjugates do not include O-glycosides because they lack a hydroxyl group at the end of the side chain. P represents a phosphate group.*

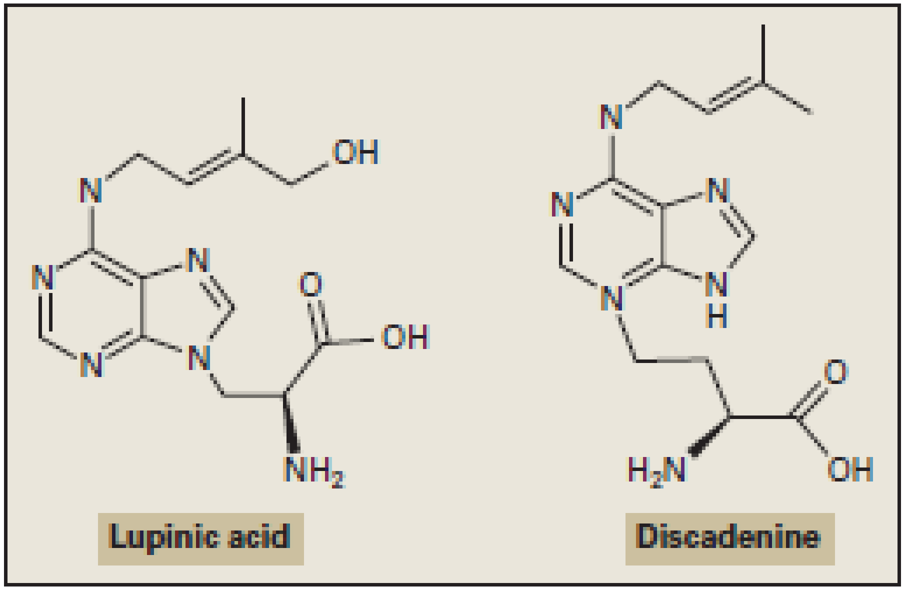


FIGURE 17.27 *Structures of amino acid CK conjugates. Discadenine is only found in slime molds.*

3.3 The initial step of isoprenoid CK biosynthesis is catalyzed by: adenosine phosphate-isopentenyltransferase (IPT)

The major isoprenoid CK biosynthesis step in plants is conjugation of adenine nucleotide and the prenyl-moiety of dimethylallyl diphosphate (DMAPP), catalyzed by adenosine phosphate-isopentenyltransferase (IPT) (Fig. 17.28). This reaction requires a divalent metal ion, such as Mg2+. Plant IPTs predominantly use ATP or ADP rather than AMP as a prenyl-acceptor.

Prenylation is the covalent attachment of a lipid consisting of either three (farnesyl) or four (geranylgeranyl) isoprene units to a free thiol of a cysteine side chain at or near the C-terminus of a protein.

Prenylation is the addition of hydrophobic molecules to a protein or chemical compound. It is usually assumed that prenyl groups facilitate attachment to cell membranes.

3.4 *trans*-Hydroxylation in tZ biosynthesis is catalyzed by a CYP450 monooxygenase

In *Arabidopsis* the *trans*-hydroxylation of the prenyl side chain in tZ biosynthesis is catalyzed by CYP450 monooxygenases CYP735A1 and CYP735A2.

- Expression of *CYP735A1* and *CYP735A2* is upregulated by CK itself and downregulated by auxin or ABA.

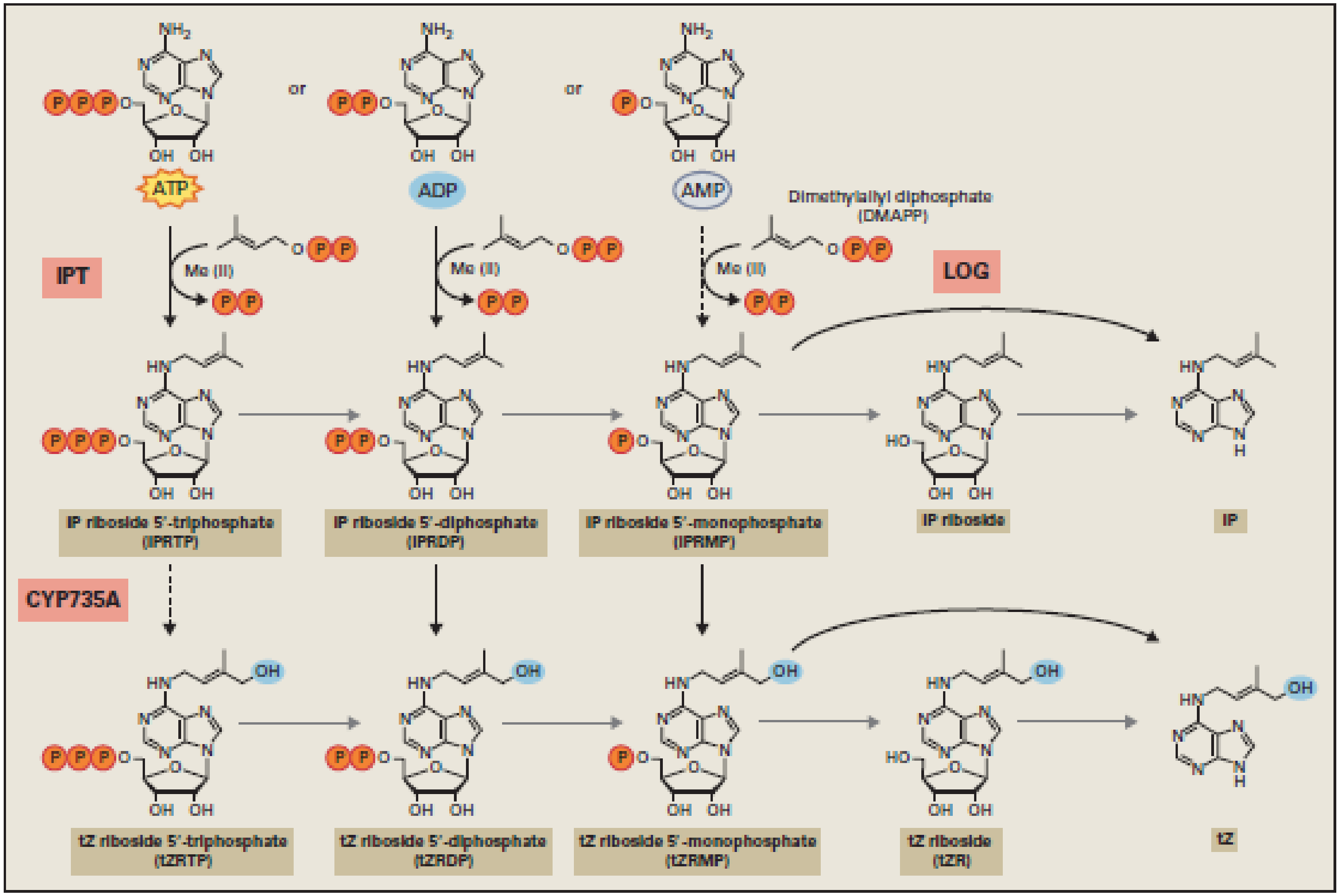


FIGURE 17.28 *Plant CK de novo biosynthesis pathway. Plant IPTs preferably utilize ATP or ADP as isoprenoid acceptors to form iPRTP and iPRDP, respectively. CYP735A preferentially utilizes iPRMP and iPRDP as substrate. Synthetic route indicated by gray arrows are not well characterized at the genetic level. For enzymes involved in the two-step pathway, see Fig. 17.33. P, phosphate group. Me (II), a divalent metal ion.*

3.5 There are two pathways for formation of active CKs

3.6 LONELY GUY catalyzes the direct activation pathway

3.7 *Agrobacterium* IPT has distinct substrate specificity

FIGURE 17.29 *Three-month-old crown gall tumor on a* Rosa spp. *stem inoculated with wild-type Agrobacterium tumefaciens.*

<u>Some plant pathogenic bacteria utilize CKs to influence plant growth</u>. *Agrobacterium tumefaciens* infects eudicots as well as some monocots and induces the formation of crown galls.

- *A. tumefaciens* integrats the T-DNA (Transfer-DNA) region of the Ti-plasmid into the plant nuclear genome.
- Ti-plasmids commonly contain an *IPT* (*Tmr*) within the T-DNA.
- Ti- plasmids contain another *IPT* gene (*Tzs*) in a region that is not transferred to the host plant.
- Tmr and Tzs are structurally related to higher plant IPTs.
- Tmr and Tzs can utilize substrates to produce iP and tZ.
- *Agrobacterium* IPTs can synthesize tZ nucleotide directly in the absence of host plant CYP735A when HMBDP is the substrate.

3.8 *Agrobacterium* IPT creates a bypass of direct tZ-type CK synthesis in host plastids

In *Agrobacterium*-infected plant cells, Tmr and Tms, encoded on the T-DNA, overproduce CK and auxin, respectively, resulting in hypertrophic and hyperplastic cell growth.

- *Tmr*-overexpressing transgenic plants almost exclusively contain tZ-type CKs.
- Tmr is targeted to and functions in plastids of infected host plant cells.
- In plastid stroma, Tmr allows the cell to synthesize CK from HMBDP without CYP735A-mediated hydroxylation.
- This bypass enables *A. tumefaciens* to produce large amounts of tZ in order to induce gall formation.

3.9 Structural studies reveal the molecular mechanism of the initial step in CK biosynthesis

3.10 CKs are also produced through degradation of tRNA

In addition to prenylation of adenine nucleotide by IPT, plants can produce CK through degradation of tRNA.

Organisms such as mammals and bacteria, in addition to plants, contain prenylated adenine in a subset of tRNA species.

Several CK species derived from tRNA-degradation have been reported, such as iP riboside (iPR), cis-zeatin riboside (cZR), tZR and their 2-methylthio derivatives.

The first step of modification is catalyzed by tRNA-IPT.

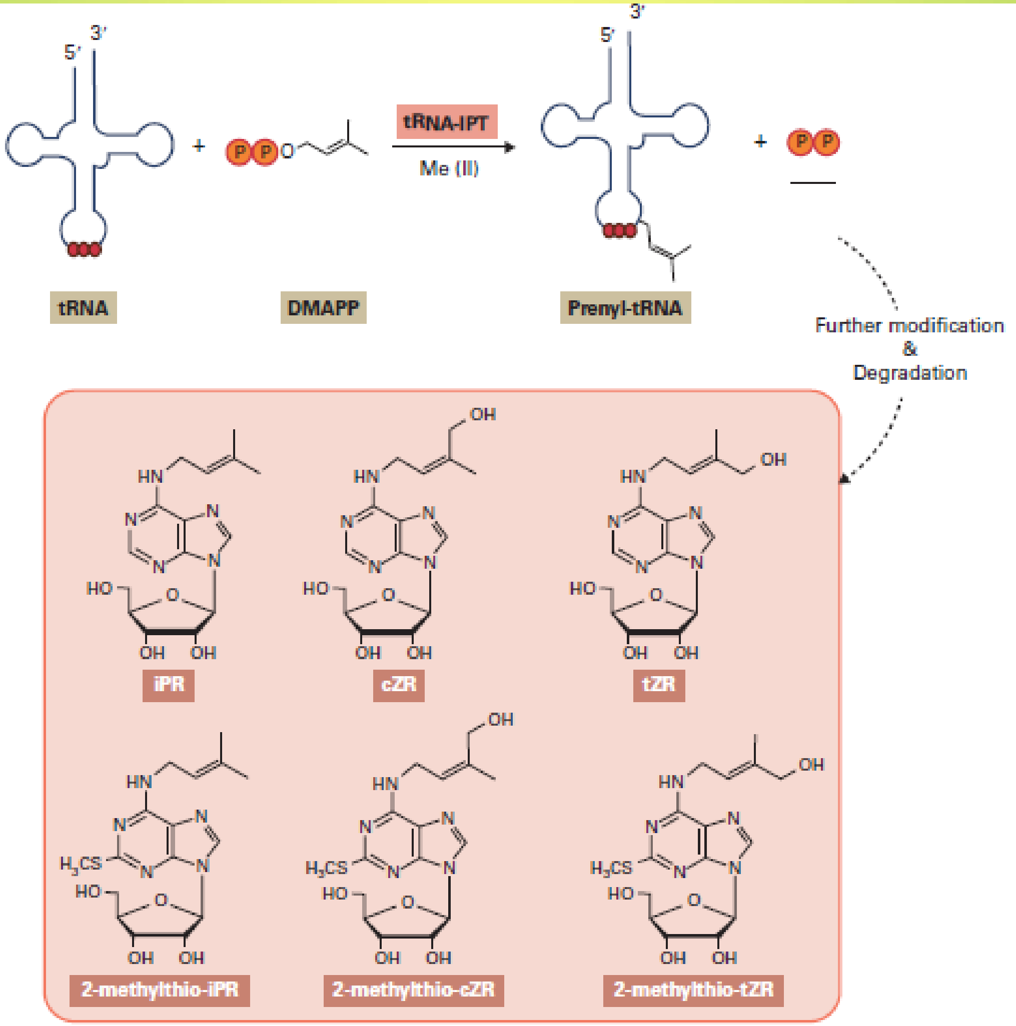


FIGURE 17.32 *CK production by tRNA degradation. tRNA-IPT conjugates DMAPP to adenine in a subset of tRNA species. Further modification and degradation results in the release of CKs. Structures of CKs derived from tRNAs are shown.*

3.11 The initial step of CK biosynthesis occurs in multiple subcellular compartments using DMAPP from different origins

DMAPP is produced through the MEP (The non-mevalonate pathway) and mevalonate (MVA) pathways.

In general, the MEP pathway is found in bacteria and plastids, and the MVA pathway in the cytosol of eukaryotes.

Application of lovastatin, an inhibitor of the MVA pathway, leads to a decrease in tZ-type CK accumulation in tobacco BY-2 cell cultures, suggesting that the MVA pathway can be the predominant origin of tZ-type CKs.

3.12 tZ can be converted to DZ and cZ

- The double bond of the tZ side chain can be enzymatically reduced to DZ by a zeatin reductase.
- In addition to degradation of tRNA, cZ-type CKs can be formed by isomerization of tZ.

3.13 Interconversion of CK nucleobase, nucleoside and nucleotide are partially shared with the purine salvage pathway

3.14 Degradation by CKX plays a key role in regulating cytokinin activity

In addition to biosynthesis and activation, deactivation is an important step in controlling active CK levels.

CKX mediates irreversible CK degradation by cleaving the side chain of an unsaturated isoprenoid side chain.

Among the naturally occurring CKs, iP, iPR, tZ, tZR, cZ, cZR, *N*-glucosides, and *N*-alanyl conjugates can be in vitro substrates, but generally CKX has a higher affinity for iP, tZ, and their nucleosides than others.

Aromatic CKs are resistant to CKX.

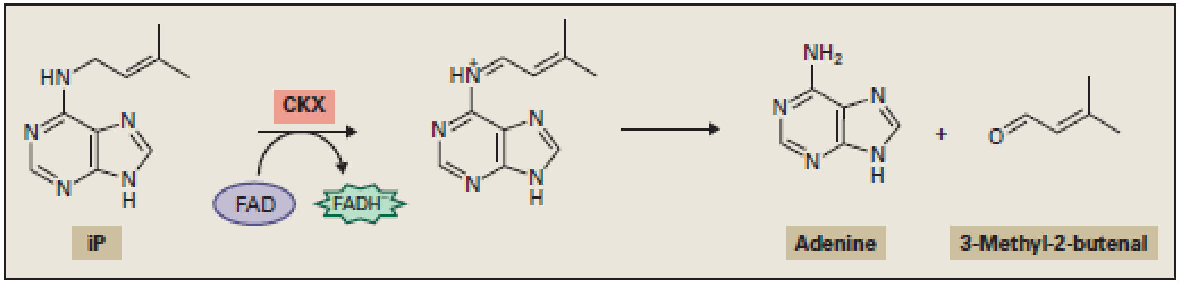
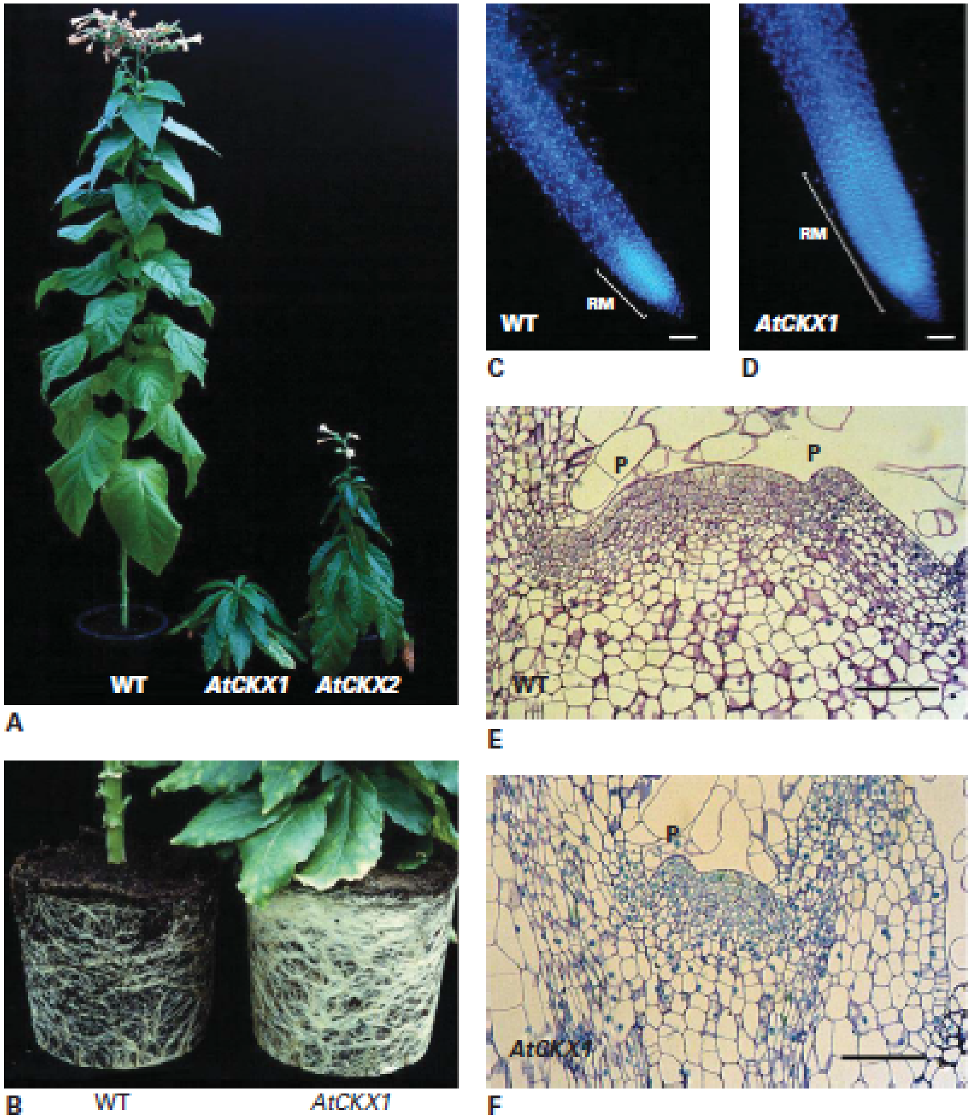


FIGURE 17.34 *CKX removes the side chain from CK molecules. CKX catalyzes the oxidation of the secondary amine group on the side-chain of the adenine ring. The resulting imine product is nonenzymatically hydrolyzed, producing adenine and an aldehyde.*

FIGURE 17.35 *Opposite phenotypes resulting from reduction of CKs in* N. tabacum *shoot and root. (A) Shoot phenotype of wild-type (WT) and transgenic lines overexpressing two different constructs of the* Arabidopsis *CKX genes:* AtCKX1 *and* AtCKX2*. (B) Root phenotype of WT and* AtCKX1*. (C), (D) Root apices of WT and* AtCKX1 *stained with DAPI. (E, F) Longitudinal section through shoot apical meristem (SAM) of WT and* AtCKX1*. The SAM is reduced in CK-deficient plants (*AtCKX1*). RM, root meristem; P, leaf primordia. Bar represents 100 µm.*



Reduction of CKX2 expression by natural variation increases CK levels in O. *sativa* (Fig. 17.36), indicating that control of CK activity in shoot apical meristems at the degradation step is mportant for agricultural productivity.

FIGURE 17.36 O. sativa CKX *gene mutation increases grain number. Panicles of* Koshihikari *(left) and* Habataki *(right). Natural variations within the* Habataki CKX2 *gene reduce expression levels in shoot meristems, resulting in increased grain numbers.*

3.15 Local CK metabolism plays an important role for regulating apical dominance

The mutual regulation of auxin and CKs plays a central role in the control of axillary bud outgrowth and dormancy (Fig. 17.37).

This regulatory system consists of the auxin-dependent expression of CKX and PIN, which is a component of auxin polar transport, and repression of IPT by auxin.

3.16 Glucosylation of the side chain hydroxyl group is catalyzed by zeatin O-glucosyltransferase

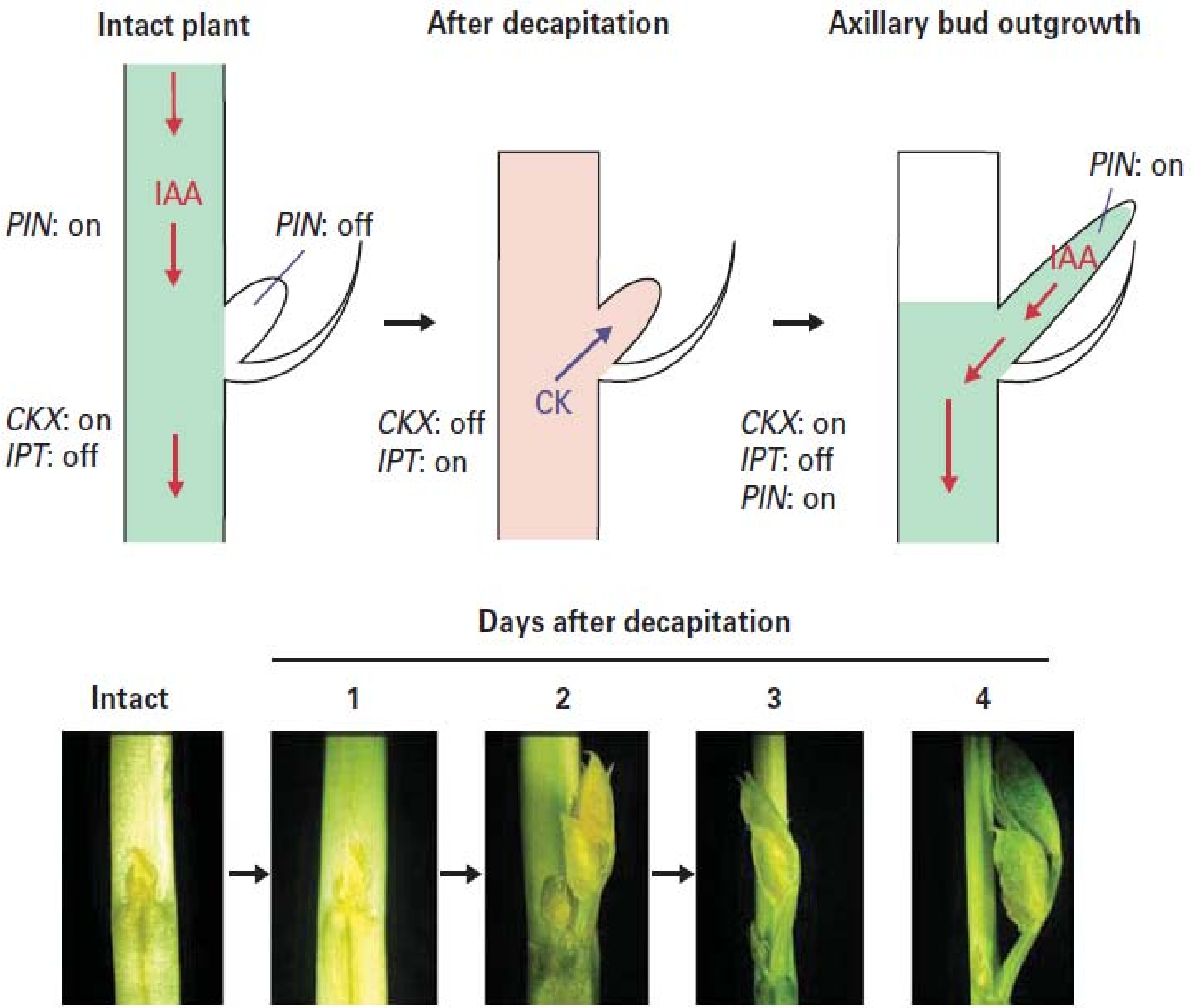


FIGURE 17.37 *A model of the interactions between auxin and CKs in controlling apical dominance. In an intact plant, auxin derived from the shoot apex maintains* PIN1 *and* CKX *expression and represses* IPT *expression. Since CK level is kept lower, axillary bud outgrowth does not occur. After decapitation (1 day after decapitation in photo), the auxin level in the stem decreases, resulting in the release of repression of* IPT *expression and down-regulation of* CKX *and* PIN1 *expression. The de novo synthesized CK in the stem is transported into dormant axillary buds and initiates their outgrowth. After axillary bud outgrowth (2 day and after in photo), de novo synthesized IAA from the new shoot apex again represses* IPT *expression and induces* CKX *and* PIN1.

4 Auxins

✓During the 19th century, Theophili Ciesielski studied the geotropic responses of plants.

✓Charles Darwin and his son Francis investigated phototropism as well as geotropism.

✓These investigations laid the groundwork for Fritz Went, who in 1926 obtained from oat coleoptiles a diffusible growth- promoting factor subsequently named “auxin.”

✓ The primary auxin present in most plants was eventually identified as **indole-3-acetic acid** (IAA).

✓Indole-3-butyric acid (IBA), 4-chloroindole-3-acetic acid, and phenylacetic acid, which are also known as naturally occurring auxins.

✓Synthetic auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthalene-1-acetic acid (NAA) are used extensively in horticulture to induce rooting and to promote the set and development of fruit. At high concentrations the synthetic auxins are effective herbicides against broad-leaved plants.

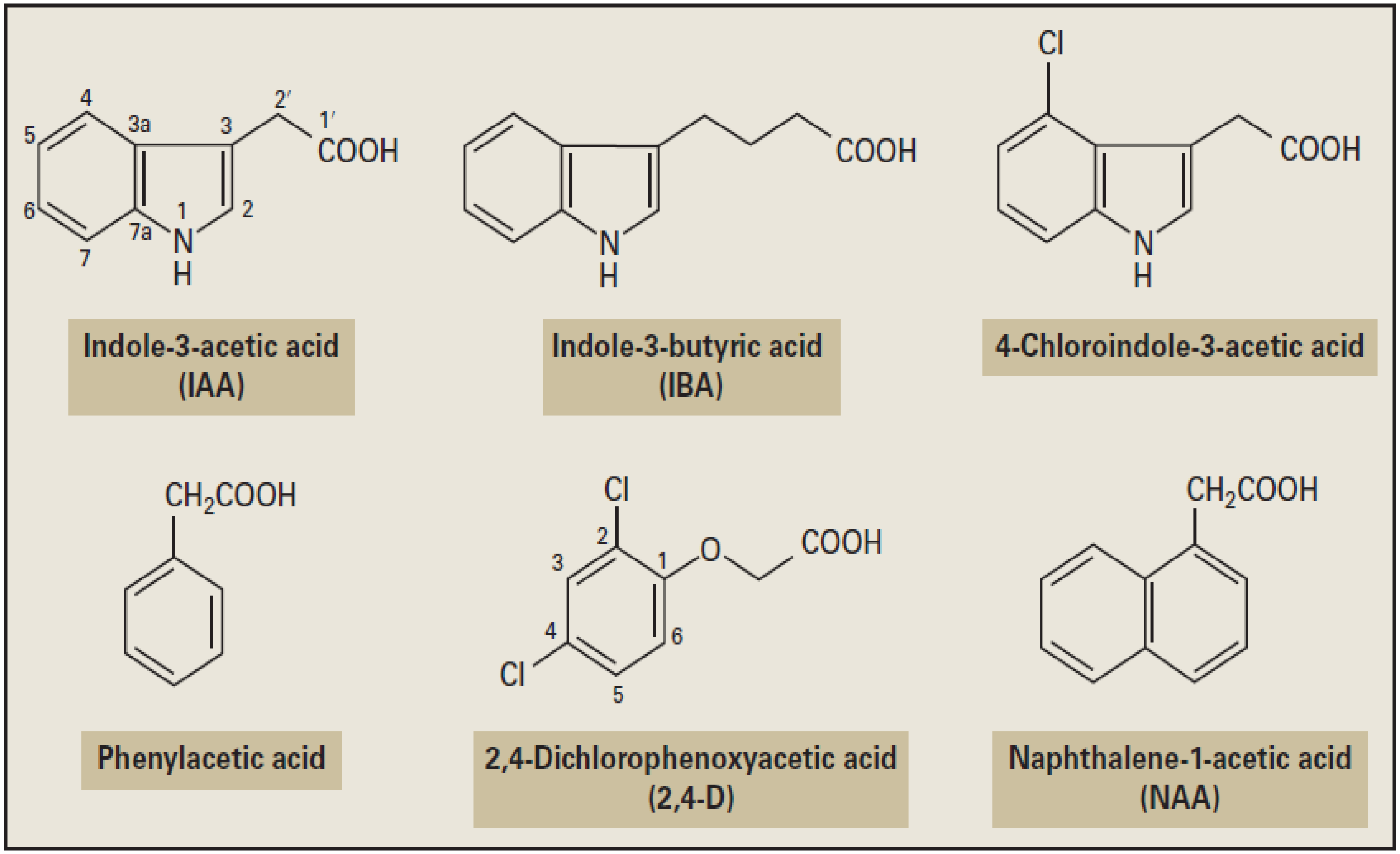


FIGURE 17.38 *Structures of auxins. IAA is the most widely distributed plant auxin. IBA, 4-chloroindole-3-acetic acid, and phenylacetic acid also naturally occur, but are less prevalent. 2,4-D and NAA are synthetic auxins.*

- *Effects*

- Cell enlargement
- Cell division
- Vascular tissue
- Root initiation
- Tropistic responses
- Apical dominance
- Leaf senescence
- Leaf and fruit abscission
- Fruit setting and growth
- Assimilate partitioning
- Fruit ripening
- Flowering
- Growth of flower parts
- Promotes femaleness in dioecious flowers

The auxin activity in plants is primarily regulated by control of IAA content via several processes:

- de novo biosynthesis,
- inactivation by various conjugation
- catabolic pathways.

There are two de novo IAA biosynthesis pathways:

- one of which is dependent on the amino acid precursor l-tryptophan
- l-Trp-independent,

Hydrolysis of IAA conjugates also releases active auxin.

IAA content in individual tissues can also be influenced by basipetal polar transport that results in the downward movement of IAA from apical meristems and young leaves towards the root system.

4.1 Multiple routes are employed in the L-Trp-dependent auxin Biosynthesis

In plants, multiple routes have been identified in l-Trp dependent IAA biosynthesis:

their first products are:

- indole-3- pyruvic acid
- tryptamine.

Indole-3-pyruvic acid is formed by Trp aminotransferase, followed by conversion to IAA by YUCCA, (a Flavin-containing Monooxygenase).

- Tryptamine is produced by decarboxylation of l-Trp, catalyzed by Trp decarboxylase. Tryptamine is then converted to indole-3- acetoaldehyde, and then to IAA.

Indole-3-ethanol and its conjugates are produced in a side shunt from indole-3-acetaldehyde. These compounds may have a storage role, given that they can be rapidly reconverted to indole-3-acetaldehyde and used as a substrate for IAA biosynthesis.

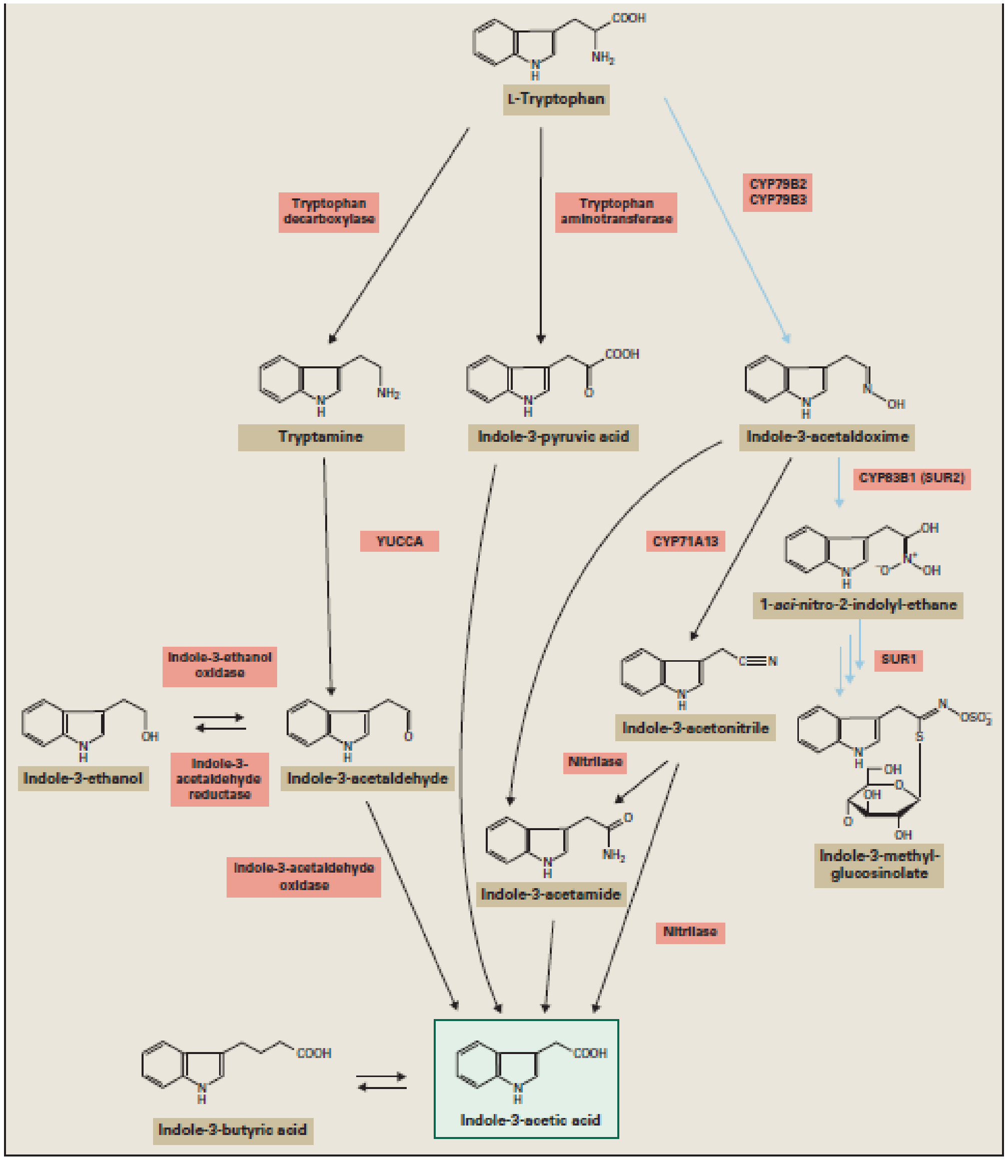


FIGURE 17.39 *L-Trp-dependent auxin biosynthesis pathway. Blue arrows indicate metabolic pathway shared with glucosinolate biosynthesis.*

- IBA has been found in a number of plants. It has auxin activity and is used to induce root formation on cuttings.
- IAA is converted to IBA in *Z. mays* and *Arabidopsis.*
- IBA synthase uses acetyl-CoA and ATP as cofactors.
- Exogenous IBA is conjugated rapidly by plants.
- Conversion of IBA to IAA has also been reported.

4.2 YUCCAs play a key role in auxin biosynthesis and plant development

- several lines of evidence from studies on gain of function and loss-of-function mutants demonstrates a key role for YUCCAs in auxin biosynthesis.
- *YUCCA* is encoded by a small multigene family, members of which are differentially expressed according to tissue.
- The expression of *YUCCAs* is temporally and spatially restricted and they are expressed in shoot and inflorescence apices, cotyledon tips in mature embryos, stamen, pollen, and stipules.
- de novo IAA biosynthesis mediated by YUCCA has a crucial role in the formation of floral organs and vascular tissues.

4.3 IAA biosynthesis pathway is shared with glucosinolate biosynthesis pathway in a few plant families

4.4 TAA1s also play a role in auxin biosynthesis and plant development and are required for shade avoidance

- Identification of genes for Trp aminotransferase (TAA1) in *Arabidopsis* revealed that auxin biosynthesis via indole-3- pyruvic acid is involved in shade avoidance responses and in ethylene–auxin interactions.
- Multiple mutants of *TAA1* family of genes show severe auxin related phenotypes, such as defects in root gravitropism, vasculature organization, and shoot and flower development.

4.5 Tryptophan-independent IAA biosynthesis was demonstrated, but the biological importance remains unclear

Evidence for IAA biosynthesis independent of l-Trp has been obtained with the orange pericarp (*orp*) mutant of *Z. mays.*

Seeds of the *orp* mutant contain increased concentrations of two l-Trp precursors, anthranilate and indole.

Despite their diminished capacity for l-Trp synthesis, *orp* mutants are rich in IAA.

Although *orp* seedlings contain roughly one-seventh the l-Trp present in wild-type *Z. mays*, IAA contents are increased 50-fold.

FIGURE 17.40 Z. mays *ear segregating kernels for the two-gene recessive trait orange pericarp* (orp)*; the orange kernels are homozygous for both mutant genes.*
Source: J. Cohen, University of Minnesota, MN, and A.D. Wright, University of Missouri, Columbia; previously unpublished.

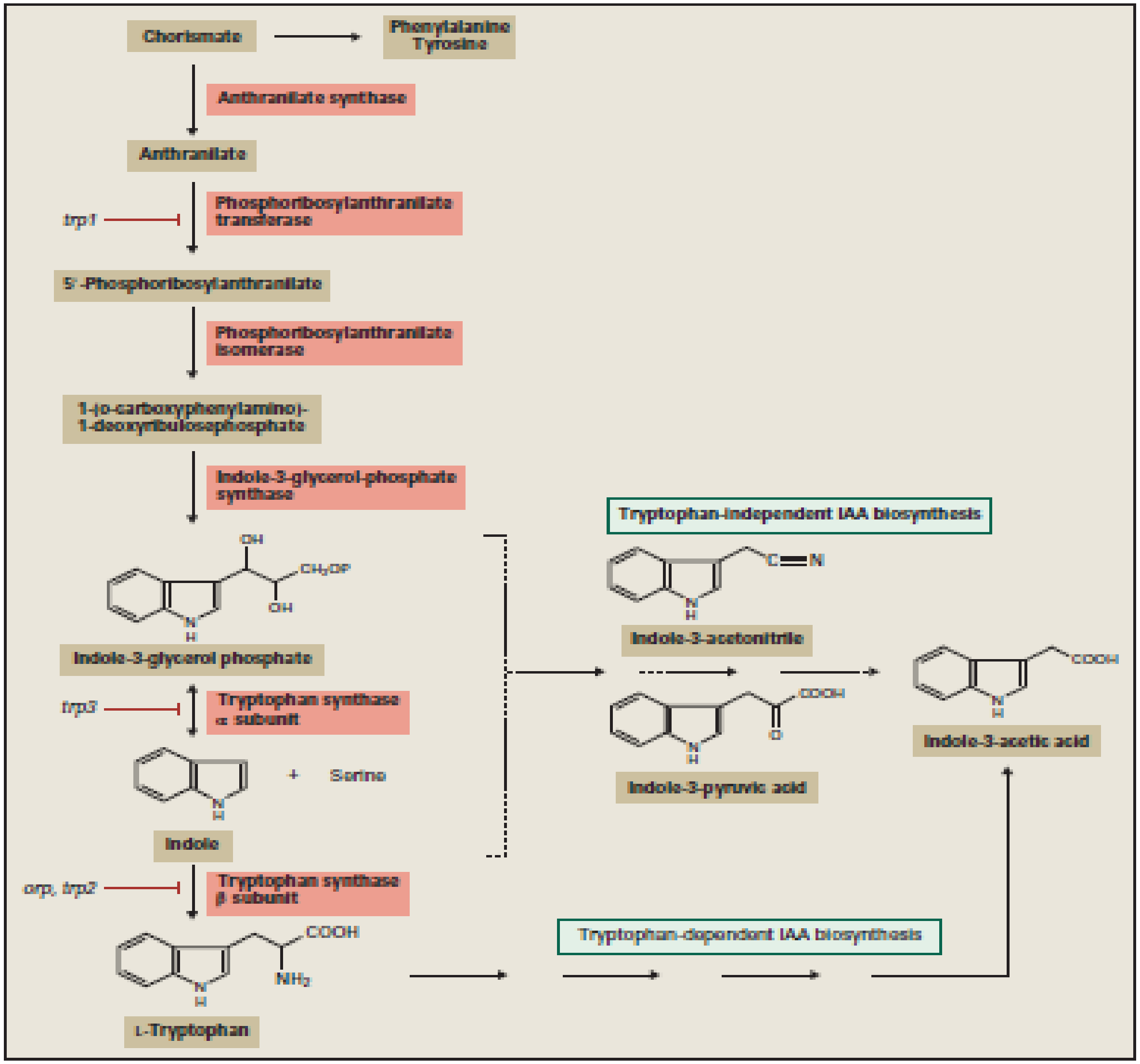


FIGURE 17.41 *Biosynthesis of L-Trp and L-Trp dependent and independent IAA biosynthesis. The enzymes affected in Z. mays orp and Arabidopsis trp1, trp2, and trp3 mutants are indicated.*

4.6 Several pathways for IAA conjugation and catabolism have been elucidated

IAA catabolism results in loss of auxin activity and irreversibly decreases the size of the IAA pool.

Catabolism can proceed by:

decarboxylative or

nondecarboxylative pathways,

and in either case can involve oxidation of the indole ring.

As with CKs, catabolism of IAA sometimes involves conjugation reactions.

For many years, the decarboxylative catabolism was thought to represent the major IAA degradation pathway in plant tissues. However, evidence obtained with Z. *mays*, tomato and pea indicates that peroxidases have only a minor role in the regulation of endogenous IAA pools. IAA–amino acid conjugates that were once perceived as storage products have been identified as intermediates in nondecarboxylative catabolic pathways that deactivate IAA irreversibly.

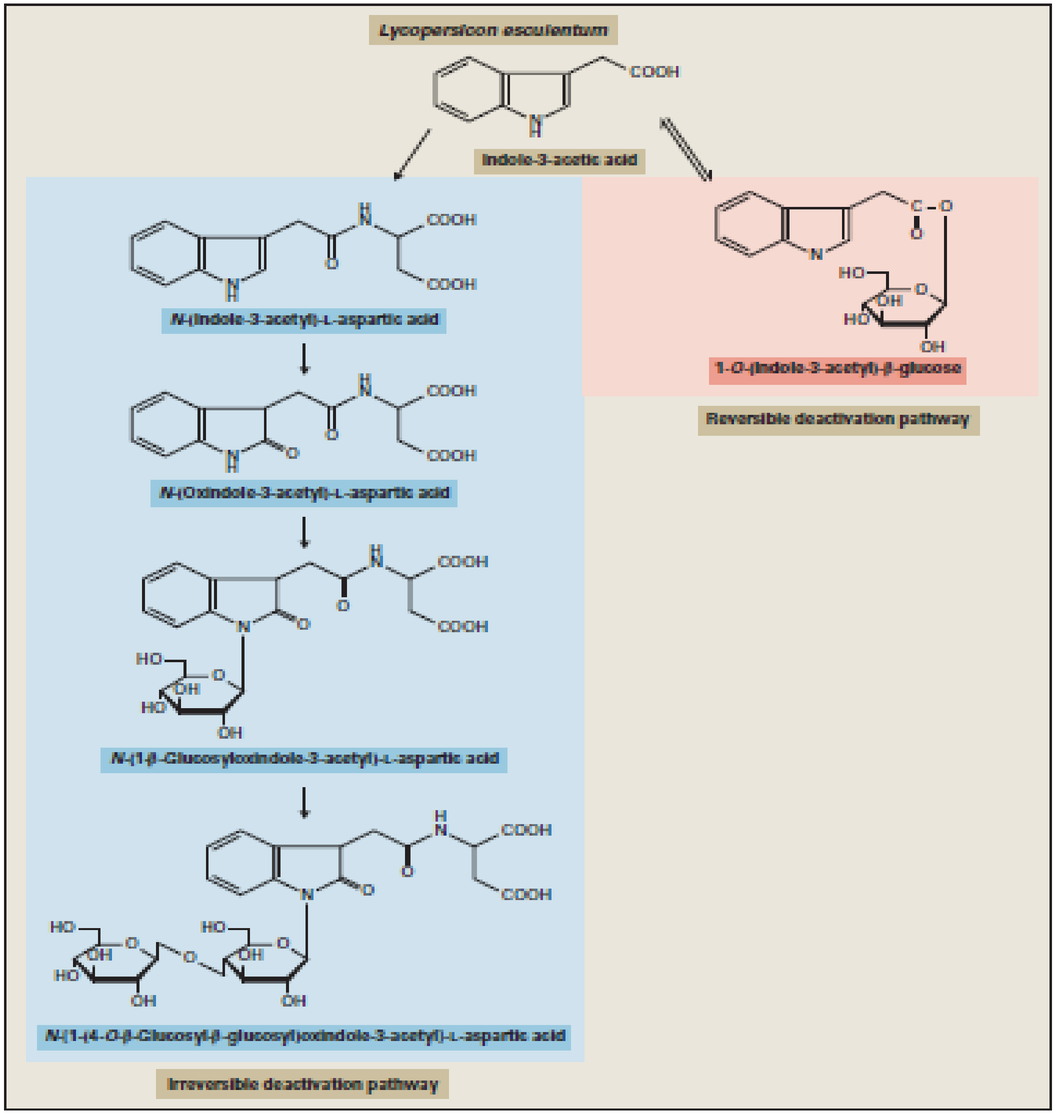


FIGURE 17.42 *Nondecarboxylative catabolism and conjugation of IAA in S. lycopersicum pericarp discs. N-(1-β-Glucosyloxindole-3-acetyl)-L-aspartic acid and N-[1-(4-O-β-glucosyl-β-glucosyl)oxindole-3-acetyl]-L-aspartic acid are permanently deactivated IAA conjugates formed in both green and red S. lycopersicum fruits, whereas the 1-O-(indole-3-acetyl)-β-glucose formed by red pericarp tissues can be converted back to IAA.*

4.7 IAA ester conjugates serve as storage products in Z. *mays* seeds

The conjugation reactions, including aspartylation or glutamylation of the 1′ carboxyl, *N*-glycosylation of the indole ring, and glycosylation of either the 3 or 7 hydroxyl groups, appear to permanently deactivate IAA.

O-glycosylation of the 1′ carboxyl is typically reversible, so IAA–ester conjugates may function as storage products.

❖During the first days of germination, the Z. *mays* embryo derives most of its IAA from these three conjugates. However, the supply of hydrolyzable IAA conjugates declines as the seedling grows, and the young plant rapidly develops the capacity to synthesize IAA.

4.8 Amino acid conjugation of IAA is catalyzed by enzymes of the GH3 family and together with IAA-amido hydrolases are involved in IAA homeostasis

A subset of GH3 family proteins as IAA-amido synthetase, which catalyzes amino acid conjugation of IAA in the presence of ATP and Mg2+.

GH3.2 to GH3.6 and GH3.17 conjugate various l-amino acids, such as l-Ala l-Asp, l-Glu, l-Met, and l-Tyr to IAA in vitro, and can utilize indole-3-pyruvic acid, indole-3-butyric acid, phenyl acetic acid, and naphthalene-1-acetic acid as substrates.

- *Most of the IAA-amido synthetase genes are responsive to auxin treatment, suggesting that plants use feedback regulation to control the amounts of active auxin*

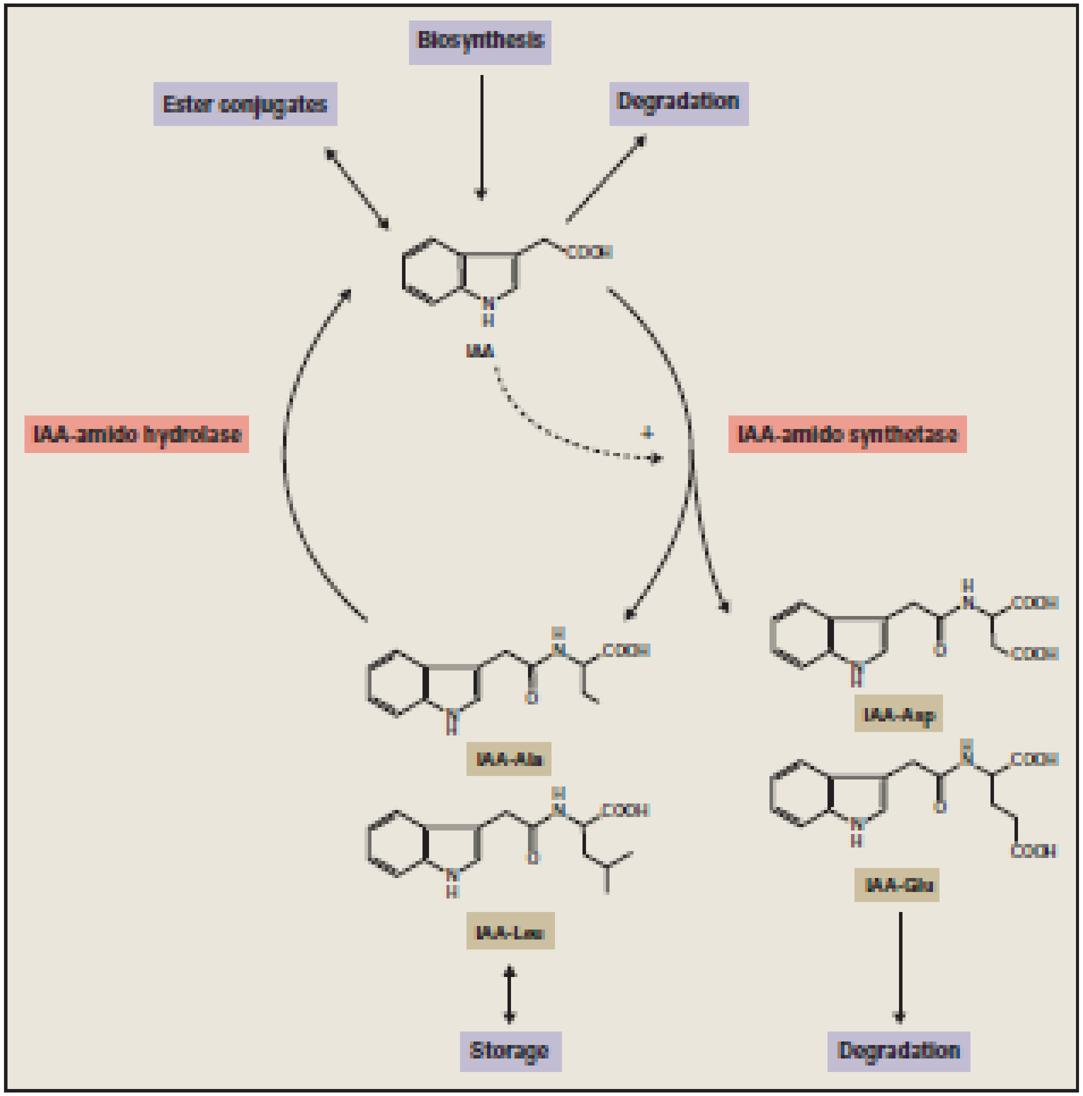


FIGURE 17.45 *Metabolic homeostasis regulating IAA concentration by IAA-amido synthetases and hydrolases. Dotted arrow shows transcriptional activation for several IAA-amido synthetase genes.*

4.9 Some bacterial pathogens encode novel IAA synthesis and conjugation pathways

some bacterial enzymes catalyze the production of plant hormones. The enhanced synthesis of IAA in *A. tumefaciens*-induced galls and tumors results from expression of two bacterial genes that are transferred to the plant when the T-DNA integrates into the host genome. These genes are associated with a unique two-step Trp-dependent pathway to IAA.

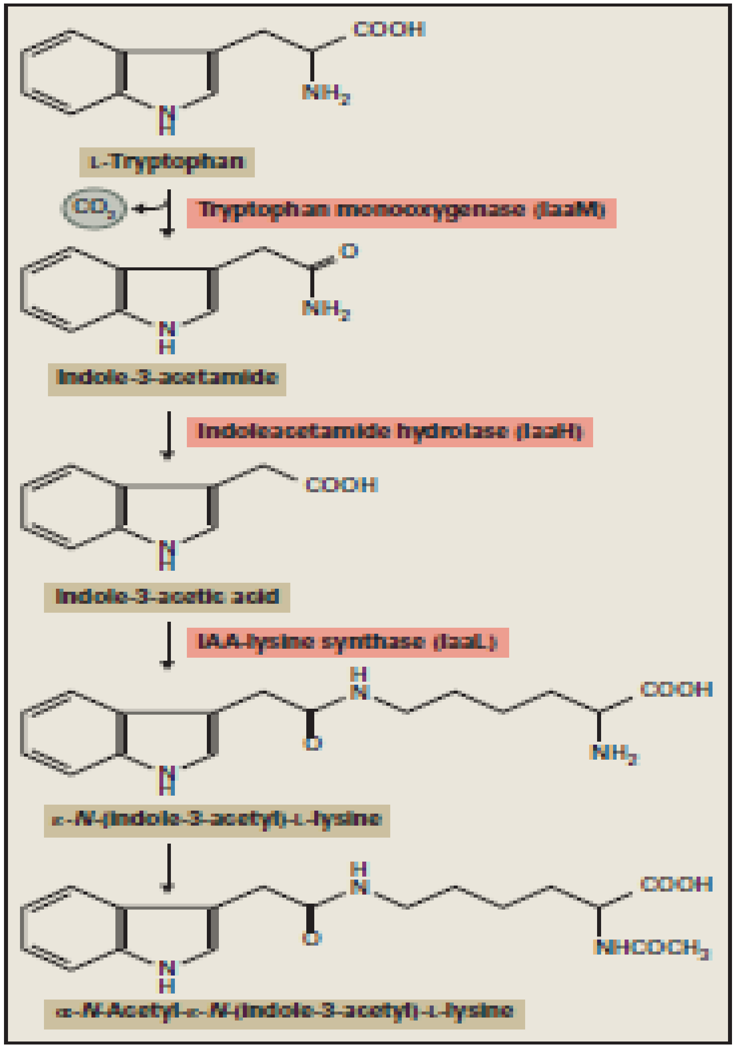


FIGURE 17.46 *IAA biosynthesis and conjugation pathways in A. tumefaciens and Pseudomonas savastanoi. IaaL does not occur in A. tumefaciens.*

4.10 Transgenic plants expressing IAA biosynthesis genes have been used to study the effects of excess amounts of endogenous IAA

- when IAA production is enhanced, conjugation appears to play a key role in regulating the size of the endogenous IAA pool and maintaining an almost normal phenotype.

High concentrations of IAA are typically accompanied by an increased rate of ethylene biosynthesis, and it was not initially possible to determine whether the phenotypic effects of IAA overproduction were a direct result of IAA or a consequence of increased ethylene levels.

major phenotypic effects, including:

- pronounced apical dominance,
- dwarfism,
- excess adventitious root formation,
- increased phloem and xylem formation,
- excess lignification,
- leaf epinasty,
- and abnormal flower production

FIGURE 17.47 *Eight-week-old tobacco plants,* N. tabacum cv. Petit Havana SR1*: wild-type plant (left); IAA-overproducing plant expressing* A. tumefaciens iaaH *and* iaaM *genes under the control of the CaMV 35S promoter (right). Note the severe stunting associated with production of IAA at about 500% of wild-type concentrations.* Source: Adapted from Nilsson et al. (1993). Plant J. 3:681–689.

The phenotype of the Samsun double-transformants, in which IAA overproduction is not accompanied by increased ethylene biosynthesis, shows that apical dominance and leaf epinasty are controlled primarily by IAA, whereas reduced stem elongation is an indirect consequence of high ethylene concentrations

FIGURE 17.48 *Uncoupling of auxin and ethylene effects in eight-week-old transgenic* N. tabacum *cv.* Samsun. *(Left) An ethylene-deficient plant expressing a* Pseudomonas *ACC deaminase gene under the control of the figwort mosaic virus 19S promoter. The phenotype is indistinguishable from wild-type plants. (Middle) A double transformant with increased IAA content and decreased ethylene production. (Right) An IAA-overproducer expressing the* A. tumefaciens iaaM *gene under the control of the CaMV 35S promoter. The phenotype indicates that apical dominance and leaf epinasty are primarily controlled by IAA, whereas ethylene is partially responsible for the inhibition of stem elongation observed in IAA-overproducing plants.*
Source: Romano et al. (1993). Plant Cell 5:181–189.

4.11 GAs increase IAA pools, whereas CKs may down-regulate IAA synthesis and turnover

Application of GA3 to Little Marvel dwarf pea seedlings enhances shoot growth with a concomitant eightfold increase in IAA content of elongating tissues. Conversely, the size of the endogenous IAA pool is reduced in the Alaska pea seedlings dwarfed by treatment with uniconazole.

In contrast to GA3, CK reduce the size of endogenous IAA pools. Transgenic tobacco plants that express the A. tumefaciens ipt gene overproduce CKs. Compared with wild-type plants, these transgenics contain significantly lower concentrations of free IAA and in most cases IAA conjugates. Rates of IAA biosynthesis also are reduced.