Cytokinins

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Cytokinins (CK) are a class of plant growth substances which promote cell division. The first cytokinin was discovered from Herring (an oily fish from genus *Clupea*) sperm DNA by Miller et al. in 1955. In the 1940s and 1950s, Skoog and his coinvestigators tested many substances for their ability to initiate and sustain proliferation of cultured tobacco pith tissue. They observed stimulation of cell division when cultured pith tissue was treated with autoclaved Herring sperm DNA. This indicated that DNA degradation product caused stimulation of cell division in tobacco pith culture. This compound was identified as **kinetin** since it caused cytokinesis (Fig. 16.1). It is now characterized as 6-furfurylaminopurine. Although kinetin is a natural compound, it is not synthesized in plants. It is, therefore, considered a "synthetic cytokinin" with reference to plants. Subsequently, immature endosperm from corn (*Zea mays*) was found to contain a substance with biological activity similar to kinetin. This substance stimulates mature plant cells to divide when added to a culture medium along with auxin. The active ingredient was later identified as **zeatin** [trans-6-(4-hydroxy-3-methyl-2-butenylamino) purine]. Zeatin was also the first natural cytokinin reported from unripe maize kernels by Miller and Letham in 1963. Zeatin can exist in *cis* or *trans* configuration. These forms can be interconverted by an enzyme known as *zeatin isomerase*. The *trans* form is biologically more active, although *cis* form has been found in high levels in a number of plant species. Cytokinins can be present in plants as ribosides (in which ribose sugar is attached to the 9 nitrogen of the purine ring), ribotides (in which the ribose sugar moiety contains a phosphate group), or a glycosides (in which a sugar molecule is attached to 3, 7, or 9 nitrogen of the purine ring).

Many synthetic compounds have been synthesized and tested for cytokinin activity. Some of these are benzylaminopurine (BAP); N,N′-diphenylurea; thidiazuron (TDZ); and benzyladenine. Also, a range of natural cytokinins have now been isolated like isopentenyladenine (iPA) and dihydrozeatin in addition to zeatin. With the exception of diphenylurea, all native and synthetic CKs are derivatives of the purine base adenine. Cytokinins occur both in free and bound forms. They are detectable across all plant

S. C Bhatla, M. A. Lal, *Plant Physiology, Development and Metabolism*,
https://doi.org/10.1007/978-981-13-2023-1_16

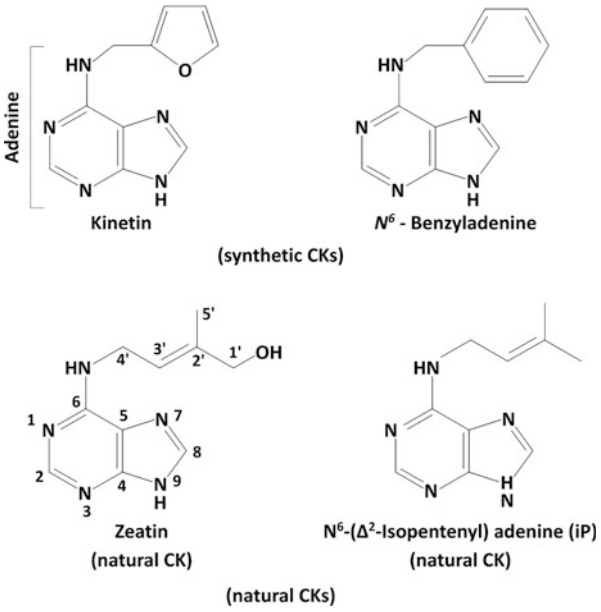

Fig. 16.1 Structures of some natural and synthetic cytokinins (CKs) in plants

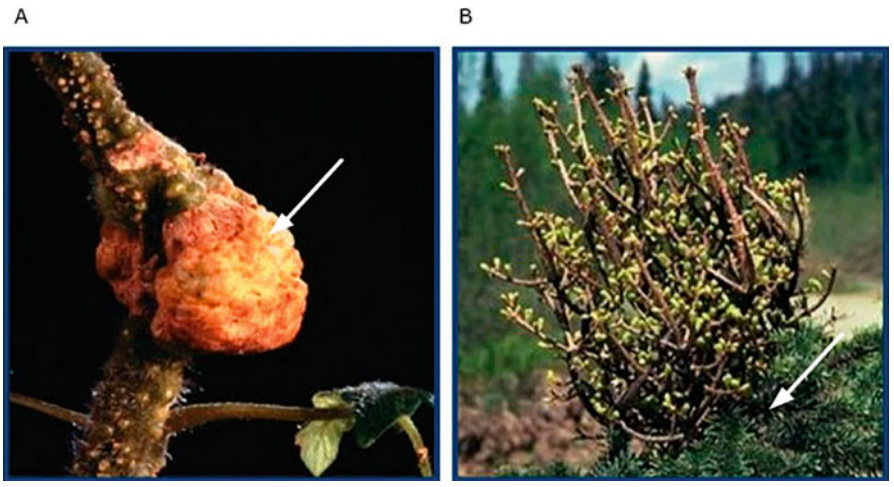

Fig. 16.2 Overexpression of cytokinins causes (**a**) tumor formation and (**b**) abnormally located meristems

groups. Some plant pathogenic bacteria, insects, and nematodes also secrete free cytokinins. For example, *Corynebacterium fascians* is a major cause of the growth abnormality known as "witches broom" in which lateral buds are stimulated to grow by the bacterial cytokinin (Fig. 16.2). Cytokinins are also known to occur abundantly in coconut milk. The most abundant cytokinin in coconut water is *trans*-zeatin riboside.

16.1 Bioassay

Cytokinin activity in stimulating cell division forms the basis of various cytokinin bioassays.

1. *Tobacco pith culture*: Tobacco pith cultures supplemented with cytokinin can be used as a bioassay for this hormone. Increase in fresh weight of the tissue over the control is a measure of cell divisions and thereby cytokinin activity.
2. *Retardation of leaf senescence*: Cytokinins are known to delay leaf senescence by inhibiting chlorophyll degradation. Leaf discs kept in cytokinin containing solution show delayed senescence against control after 48–72 h. The test is sensitive at CK concentration as low as 1 pg.litre^{-1}. All these bioassays, however, have certain limitations as they utilize relatively large volumes of solutions and/or relatively long time for completion of response.
3. *Excised cotyledon expansion*: Cotyledons when placed in cytokinin-containing solution exhibit enlargement against control. This is taken as an indication of cytokinin activity. This bioassay is considered more efficient since cytokinin response can be obtained using very small solution volume and it is sensitive at concentration as low as 10^{-8} M.

16.2 Biosynthesis

Understanding the biosynthetic route for cytokinins has been relatively more difficult as compared to other hormones since it is practically not possible to isolate cytokinin-impaired mutants. Cytokinin, being principally responsible for cell divisions, proves lethal for plants in attempts to isolate mutants lacking it. Two pathways have been proposed for CK biosynthesis. The *direct pathway* involves formation of N^6-isopentyladenosine monophosphate (iPMP) from 5′-AMP and dimethylallyl pyrophosphate (DMAPP), followed by hydroxylation of the side chain to form zeatin-type compounds (Fig. 16.3). Conversion of DMAPP and AMP to iPMP occurs in plastids because DMAPP is synthesized in plastids. This reaction is the rate limiting step in cytokinin biosynthesis. The next step is the hydroxylation of the isopentenyl side chain by a member of the cytochrome P450 monooxygenase (CYP) family of enzymes. Hydroxylation reaction occurs largely on ER membranes and is regulated by several hormones. iPMP is then converted to zeatin by unidentified hydroxylases. Various phosphorylated forms can be interconverted, and free zeatin can be formed from the riboside by the action of enzymes of general purine metabolism. Also, reduction of the double bond in isopentenyl side chain of zeatin gives rise to **dihydrozeatin (diHZ).**

The *indirect pathway* of cytokinin biosynthesis involves turnover of tRNA containing *cis*-zeatin. tRNAs with anticodons that start with a uridine and carrying an already-prenylated adenosine adjacent to the anticodon release the adenosine as a

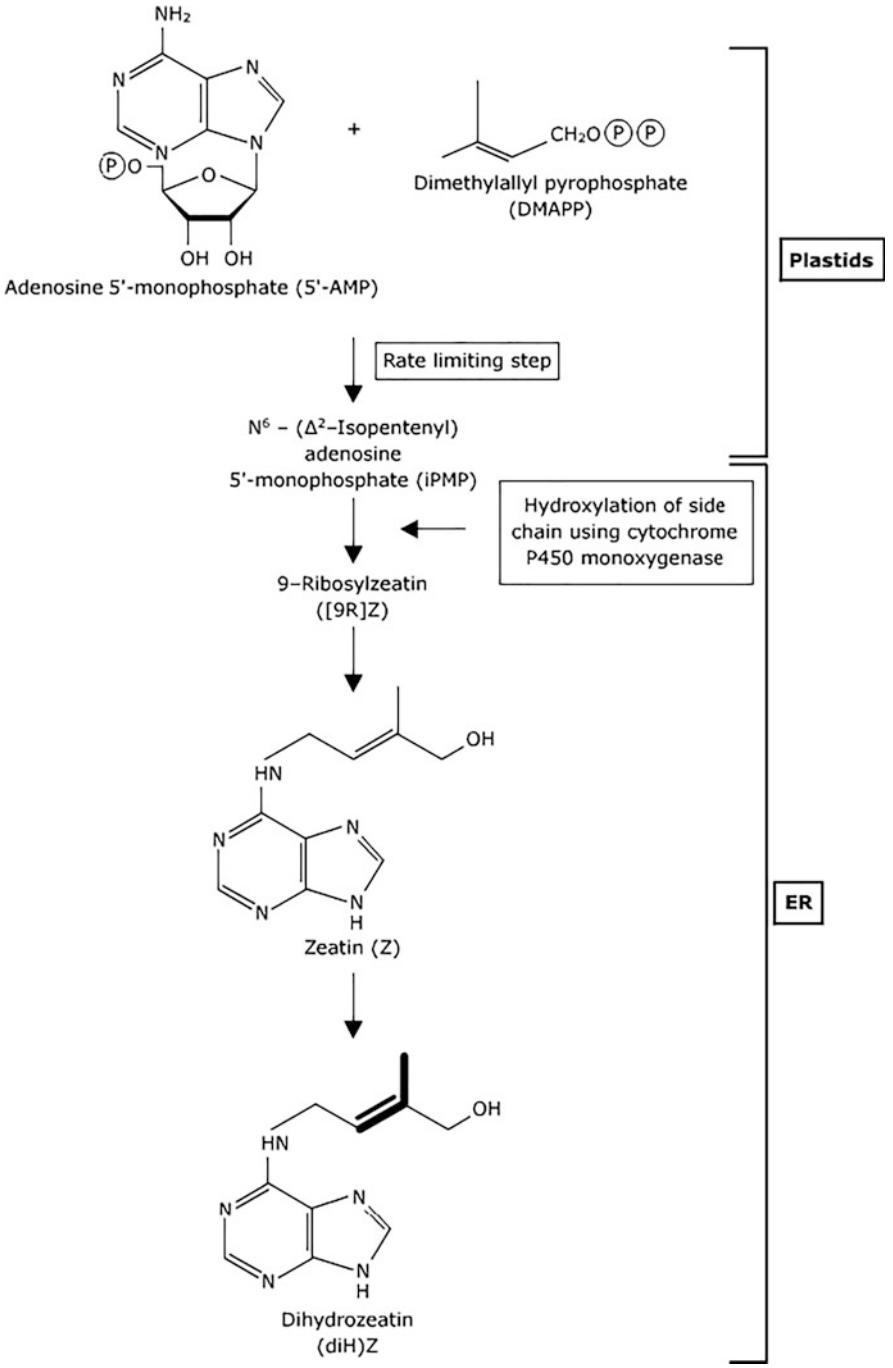

Fig. 16.3 Direct pathways of cytokinin biosynthesis from 5'-AMP and dimethylallyl pyrophosphate (DMAPP)

cytokinin upon degradation. Prenylation of these adenines is carried out by tRNA isopentenyltransferase. (*Prenylation refers to addition of hydrophobic molecule to a chemical compound.*)

16.3 Transport

Root apical meristem is a major site of cytokinin (CK) biosynthesis in plants. CKs move through the xylem into the shoot along with water and minerals. When shoot is separated from a rooted plant, xylem sap continues to flow from the root stock. Analysis of this root exudate shows the presence of cytokinins. Even if the flow of xylem exudate continues, the cytokinin content does not diminish. This shows that cytokinins are synthesized in the roots. Environmental factors like water stress reduce the cytokinin content of the xylem exudate.

16.4 Metabolism

Free cytokinins are readily converted to their respective nucleosides and nucleotides. Many plant tissues contain the enzyme cytokinin oxidase which cleaves the side chain from isopentenyladenine. Cytokinin oxidase irreversibly inactivates cytokinin and is thus important in regulating cytokinin effects. Cytokinin oxidase activity is induced by high cytokinin concentration. Cytokinin levels can also be regulated by conjugation of the hormone at various positions. Conjugated forms of cytokinin are inactive in bioassay. Their conjugation at the side chain can be removed by glucosidase enzyme to yield free cytokinin. Dormant seeds generally contain high levels of cytokinin glucosides but very low level of free cytokinins. As seed germination is initiated, levels of free cytokinin increase rapidly with simultaneous decrease in cytokinin glucoside (Fig. 16.4). Cytokinins can also be inactivated by linkage with sugars or amino acids. Glycosylation can occur either at N-7 or N-9 positions of the adenine moiety or on the OH group of the isopentenyl side chain. N-Glycosylation is irreversible and results in inactivation of CK, but O-glycosylation can be reversed. Alanine can be added to the N-9 position. This reaction is also reversible. Reversible modification of CKs by the addition of sugars or amino acids may be an important mechanism for storage since conjugated CKs are located in the vacuole.

16.5 Physiological Role of Cytokinins

16.5.1 Cell Division

When plants are cultured on auxin-containing medium without cytokinin, they increase in size but cells do not divide. Addition of cytokinin to the medium leads to cell division and differentiation. Presence of both auxin and cytokinin in equal concentration leads to formation of undifferentiated callus. Increased cytokinin

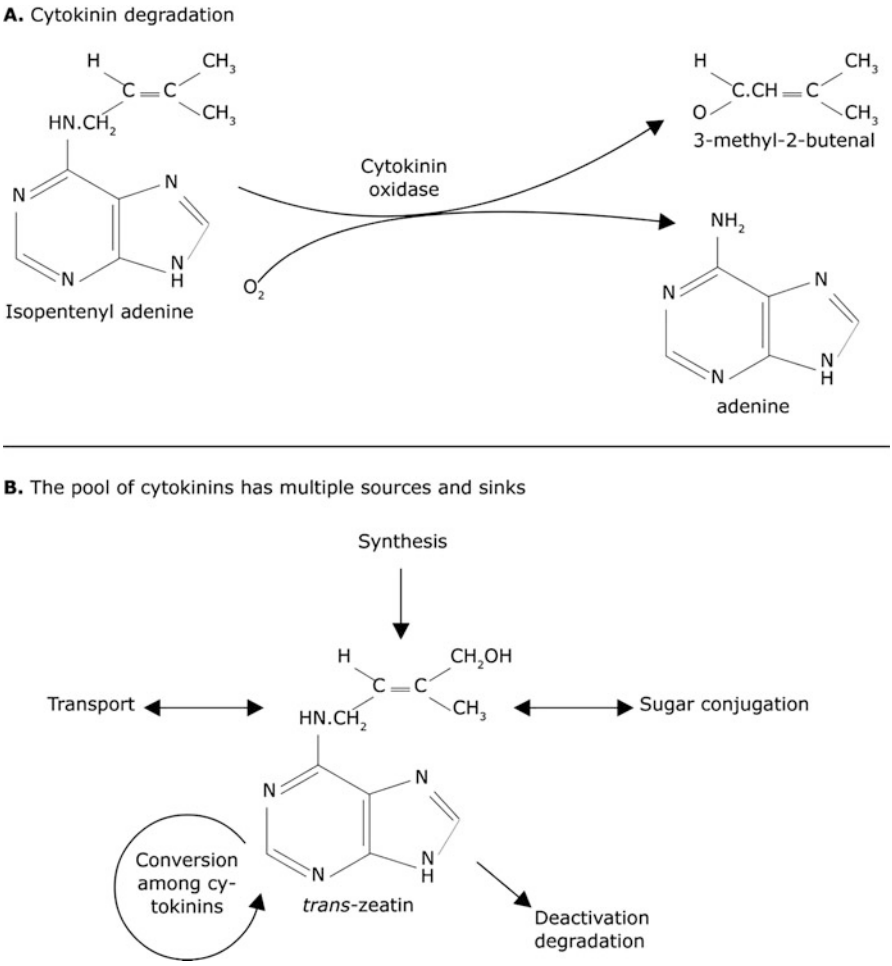

Fig. 16.4 Routes of cytokinin homeostasis via degradation, conjugation, synthesis, and transport

concentration induces growth of shoot buds, whereas increased auxin induces root formation. Evidence indicates that localized expression of *ipt* gene (responsible for overexpression of cytokinin) from *Agrobacterium* in the somatic cells of tobacco leaves causes the formation of ectopic (abnormally located) meristems. This indicates that elevated levels of cytokinins are sufficient to initiate cell divisions in these leaves. Overexpression of several Arabidopsis cytokinin oxidase genes in tobacco results in a reduction of endogenous cytokinin levels. This results in retardation of shoot development due to reduction in the rate of cell proliferation and shoot apical meristem. These findings strongly support the observation that endogenous cytokinins regulate cell division.

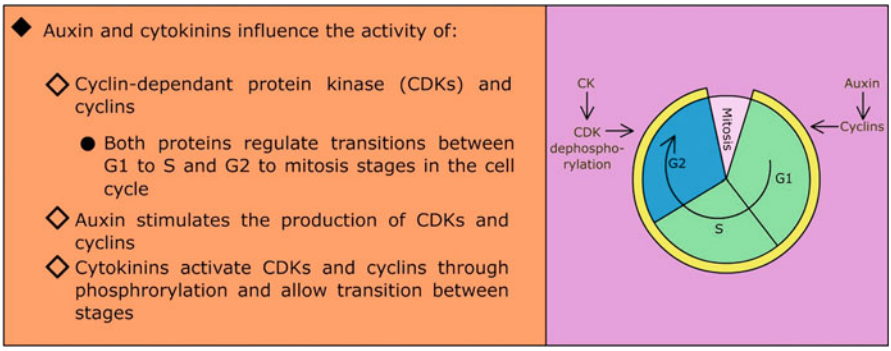

Fig. 16.5 Auxin-cytokinin interaction in regulating cell cycle through modulation of the activity of cyclin-dependent protein kinase and cyclins

16.5.2 Regulation of Cell Cycle

Cytokinins regulate cell proliferation, affecting mitosis and endoreplication. They control cell cycle both at G_1/S and G_2/M transition. There is evidence to suggest that both auxins and cytokinins participate in the regulation of cell cycle. This is accomplished by controlling the activity of cyclin-dependent kinases (CDKs). The expression of gene which encodes major CDKs is regulated by auxins. However, CDKs induced by auxins are enzymatically inactive. Cytokinins induce the removal of an inhibitory phosphate group from CDKs. This action of cytokinin provides a link between cytokinins and auxins in regulating the cell cycle (Fig. 16.5).

16.5.3 Morphogenesis

High auxin: cytokinin ratio stimulates the formation of roots whereas low ratio leads to the formation of shoots.

16.5.4 Lateral Bud Formation

Cytokinins are known to regulate axillary bud growth and apical dominance. It is postulated that auxin from apical buds travels through shoots to inhibit axillary bud growth. This promotes shoot growth and restricts lateral branching. On the other hand, cytokinins move from root to the shoot and stimulate lateral bud growth. Direct application of cytokinin to the axillary buds stimulates cell divisions and growth of buds. Removal of apical bud leads to uninhibited growth of axillary buds. Cytokinins also modify apical dominance. Cytokinin-overproducing plants generally exhibit a bushy appearance.

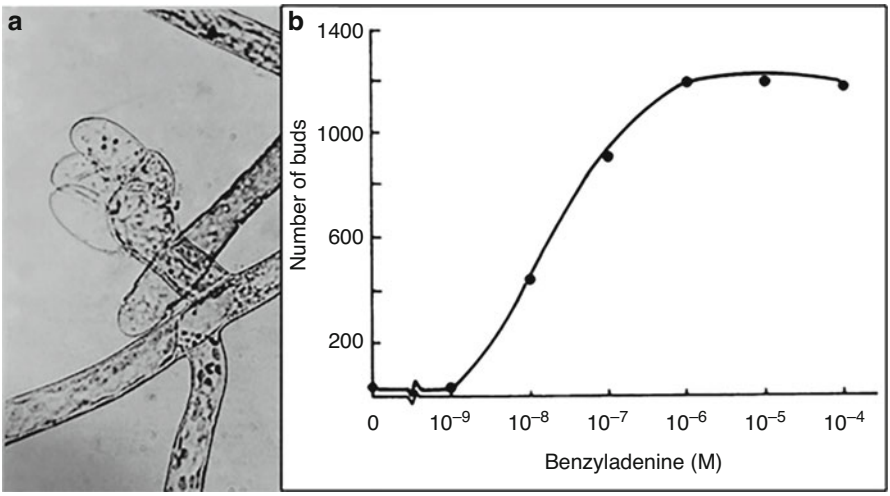

Fig. 16.6 (**a**) Bud induction in response to cytokinin on caulonema filaments in moss protonema. (**b**) Dose response curve of the effect of benzyladenine on bud formation in moss protonema

16.5.5 Bud Formation in Mosses

Life cycle of a moss includes the gametophytic protonemal phase followed by formation of leafy shoots from buds developing on the protonema. Cytokinins are known to stimulate bud development and also increase the total number of buds formed in a concentration-dependent manner (Fig. 16.6).

16.5.6 Delay of Leaf Senescence

Cytokinins have been shown to slow aging of plant organs by preventing protein breakdown, activating protein synthesis, and assembling nutrients from nearby tissues. Isolated leaves when treated with cytokinins exhibit delayed senescence. If the leaf of a plant is treated with cytokinin, it remains green for a long period, while untreated leaves of the same developmental age develop yellow color and eventually drop off. In soybean leaves, senescence is initiated by seed maturation. This phenomenon is known as *monocarpic senescence* and can be delayed by seed removal. Seed pods control the onset of senescence by controlling the transport of cytokinins derived from roots to the leaves. Role of cytokinins in senescence can be tested by transforming tobacco plants with chimeric gene in which senescence-specific promoter is used to drive the expression of *ipt* gene. At the time of senescence, elevated cytokinin levels block senescence and also limit further expression of *ipt* gene, hence preventing cytokinin overproduction (Box 16.1).

Box 16.1 Cytokinin Overproduction in Plants and Its Significance

Cytokinin overproduction is highly beneficial for agriculture. Since CKs promote plant cell division and growth, they are used by farmers to increase crop productivity. *Ipt* gene from *Agrobacterium* Ti plasmid is introduced into many plant species resulting in overproduction of cytokinin. These plants exhibit all characteristics which point to the role of cytokinins in plant growth and development, viz., (i) more leaf production from shoot apical meristems, (ii) higher chlorophyll levels in leaves, (iii) adventitious shoots development from unwounded leaf veins and petioles, (iv) retardation of leaf senescence, (v) reduced apical dominance, and (vi) reduced rooting on stem cutting.

Application of cytokinin to cotton seedlings has been reported to increase the yield by 5–10% under drought conditions. If leaf senescence could be delayed in plants, it is also possible to extend their photosynthetic productivity. Cytokinin production is also linked to the damage caused by predators, and it plays role in plant pathogenesis. For example, cytokinins have been known to induce resistance against *Pseudomonas syringe* in *Arabidopsis thaliana* and *Nicotiana tabacum.* Tobacco plants transformed with *ipt* gene under the control of promoter from a wound-inducible protease inhibitor II gene are more resistant to insect damage. Also in context of biological control of plant diseases, cytokinins seem to have potential functions.

16.5.7 Movement of Nutrients

Cytokinins promote movement of nutrients by creating a new source-sink relationship. Cytokinin-treated tissue acts as strong sink and thereby nutrients are channelized toward it. If a plant requires excessive nutrients, cytokinins accumulate in the root zone to stimulate growth.

16.5.8 Chloroplast Development

Etiolated leaves, when treated with cytokinin, develop chloroplasts with more extensive grana, and photosynthetic enzymes are synthesized at a greater rate upon illumination. This suggests that cytokinins along with other factors, like light and nutrition, regulate the biosynthesis of photosynthetic pigments and proteins.

16.5.9 Mechanical Extensibility of Cell Wall

Cotyledons of dicots, like mustard and sunflower, expand on treatment with cytokinin.

16.6 Mode of Cytokinin Action

Cytokinin receptor is related to bacterial two-component receptors. First clue to the nature of cytokinin receptor came from the discovery of CKI1gene. Plants require cytokinins in order to divide in culture. However, a cell line of *Arabidopsis* which overexpresses CKI1 gene is capable of growing in culture in the absence of added cytokinin. Phenotype resulting from CKI1 overexpression has suggested that histidine kinases are cytokinin receptors. Support for this model has come from the identification of the CRE1 gene. *cre1*, a loss-of-function mutant, exhibits the absence of shoot development from the undifferentiated tissue culture cells in response to cytokinin. CKI1 encodes a protein similar in sequence to bacterial two-component sensor histidine kinase. CK receptor is composed of two functional elements, a histidine kinase to which CK binds and a downstream response regulator whose activity is regulated via phosphorylation by histidine kinase. Histidine kinase is a membrane-bound protein that contains two distinct domains called input and transmitter domains. Detection of signal (CK) by the input domain alters the activity of the histidine kinase domain. Active sensor kinases are dimers which transphosphorylate a conserved histidine residue. The phosphate group is then transferred to a conserved aspartate residue in the receiver domain of a response regulator, and this phosphorylation alters the activity of the kinases. Most response regulators also contain an output domain which acts as a transcription factor.

Cytokinins also cause a rapid increase in the expression of response regulator genes. The first set of genes reported to be upregulated in response to cytokinin are the ARR (*Arabidopsis* response regulator) gene. Response regulators in *Arabidopsis* are encoded by a multigene family. These genes are grouped into two basic classes, namely, type-A ARR genes and type-B ARR genes. Type A gene is solely made up of a receiver domain, whereas type B contains a transcription factor domain in addition to the receiver domain. Rate of transcription of type A gene is enhanced within 10 min in response to applied cytokinin. This rapid induction is specific for cytokinin and does not require new protein synthesis. The rapid induction of type A gene suggests that these elements act downstream of the CRE 1 cytokinin receptor to mediate primary cytokinin response. Also, type A gene, ARR5, is expressed primarily in the apical meristem of shoot. This is consistent with its role in regulating cell proliferation.

The complete model for cytokinin signal transduction cascade is as follows (Fig. 16.7):

Cytokinin binds to an extracellular portion of CRE 1 (a dimer) called the CHASE domain of the CK receptor. Two other hybrid sensor kinases (AHK2 and AHK3) containing a CHASE domain are also likely to act as cytokinin receptors in *Arabidopsis.* Cytokinin binding to these receptors activates their histidine kinase activity. The phosphate group is transferred to an aspartate residue (D) on the fused receiver domain. The phosphate is then transferred to a conserved histidine present in an AHP protein. Phosphorylation causes the AHP protein to move into the nucleus where it transfers the phosphate group to an aspartate residue located within the receiver domain of a type-B ARR. The phosphorylation of the type-B ARR activates

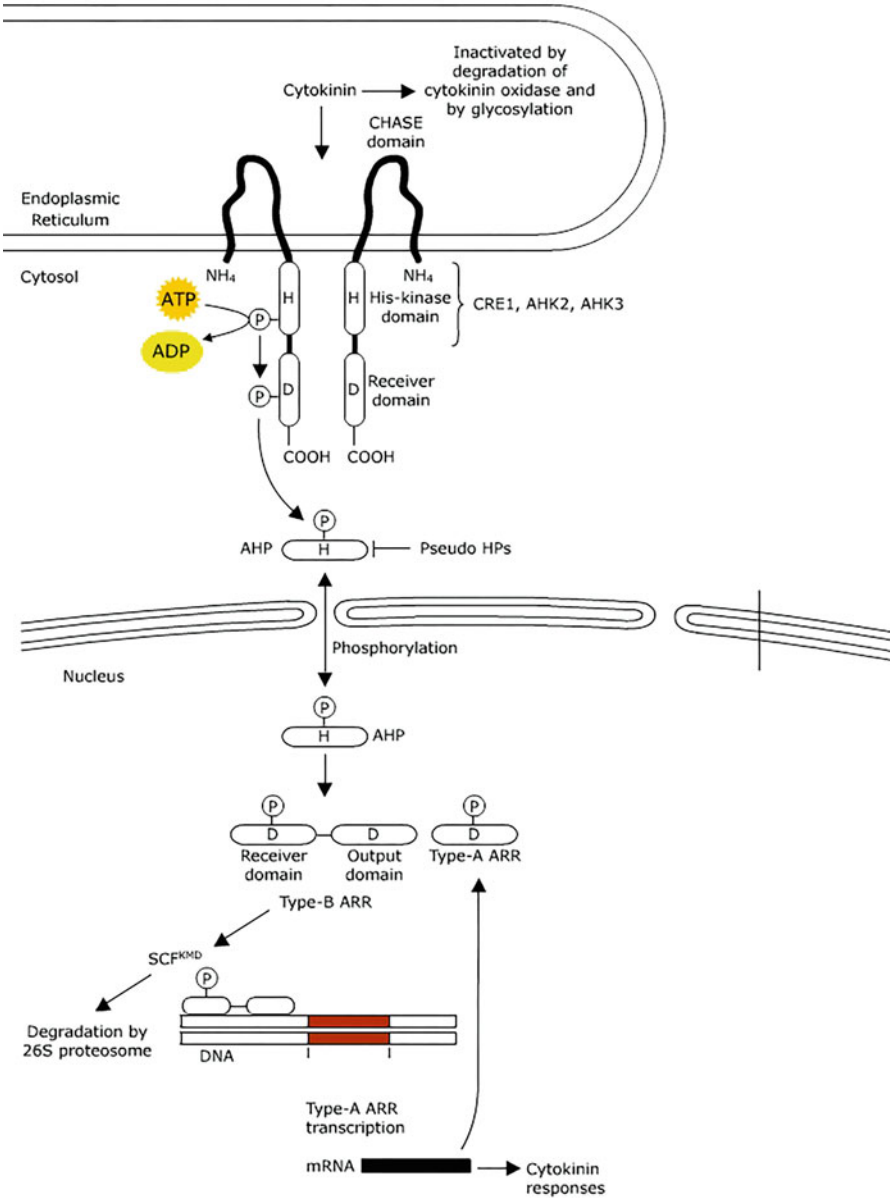

Fig. 16.7 Signaling events triggered by cytokinin

the output domain to induce transcription of genes encoding type-A ARRs. These are, in turn, phosphorylated by the AHP proteins. The phosphorylated type A ARRs interact with various effectors to bring about cytokinin response.

Summary

- Cytokinins (CK) are a class of plant growth substances which promote cell division. Zeatin was the first natural cytokinin reported from unripe maize kernels by Miller and Letham in 1963.It exists in both *cis* and *trans* configurations, where *trans* form is biologically more active.
- Two pathways have been proposed for CK biosynthesis. The *direct pathway* involves formation of N^6-isopentyladenosine monophosphate (iPMP) from 5′-AMP and dimethylallyl pyrophosphate (DMAPP), followed by hydroxylation of the side chain to form zeatin-type compounds. The *indirect pathway* involves turnover of tRNA containing *cis*-zeatin.
- Major site of cytokinin biosynthesis in plants is root apical meristem.
- Enzyme cytokinin oxidase inactivates cytokinin and is thus responsible for maintaining cytokinin homeostasis.
- Cytokinins regulate cell proliferation, affecting mitosis and endoreplication by controlling the activity of cyclin-dependent kinases (CDKs). They retard aging of plant organs by preventing protein breakdown, activating protein synthesis, and assembling nutrients from nearby tissues.
- They also promote movement of nutrients by creating a new source-sink relationship and also regulate the biosynthesis of photosynthetic pigments and proteins.
- CK receptor is composed of two functional elements, a histidine kinase to which the hormone binds and a downstream response regulator whose activity is regulated via phosphorylation by histidine kinase.

Multiple-Choice Questions

1. A plant hormone which is known to regulate cell division and delays leaf senescence is:
   (a) Auxins
   (b) Cytokinins
   (c) Ethylene
   (d) Abscisic acid
2. Which of the following is a natural cytokinin?
   (a) Benzylaminopurine
   (b) Thidiazuron
   (c) Benzyladenine
   (d) Kinetin
3. _____ is the major site of cytokinin biosynthesis.
   (a) Shoot apical meristem
   (b) Root apical meristem
   (c) Floral tips
   (d) Leaf tips

4. Which of the following hormones resemble nucleic acids with reference to their structures?
   (a) Auxins
   (b) Gibberellins
   (c) Cytokinins
   (d) Ethylene
5. Which hormone is responsible for a disease called "witch's broom" where lateral buds are stimulated to grow abnormally?
   (a) Cytokinins
   (b) ABA
   (c) Gibberellins
   (d) Ethylene

Answers

1. b 2. d 3. b 4. c 5. a

Suggested Further Readings

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