Laboratoř růstových regulátorů

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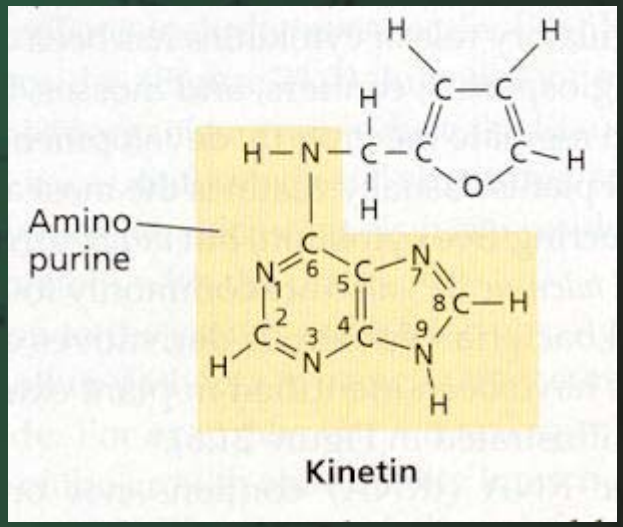
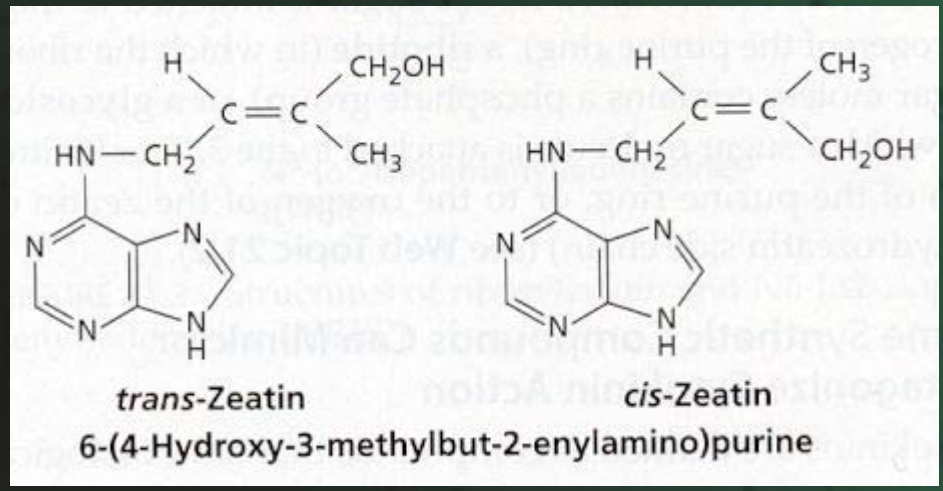
Cytokininy
[kap. 21]

- Univerzita Palackého & Ústav experimentální botaniky AV CR

FIGURE 21.1 Tumor that formed on a tomato stem infected with the crown gall bacterium, *Agrobacterium tumefaciens*. Two months before this photo was taken the stem was wounded and inoculated with a virulent strain of the crown gall bacterium. (From Aloni et al. 1998, courtesy of R. Aloni.)



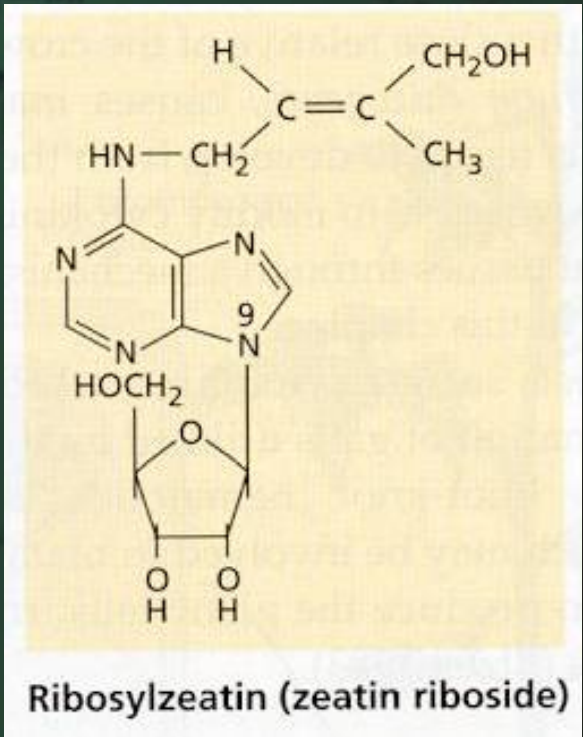
Kinetin



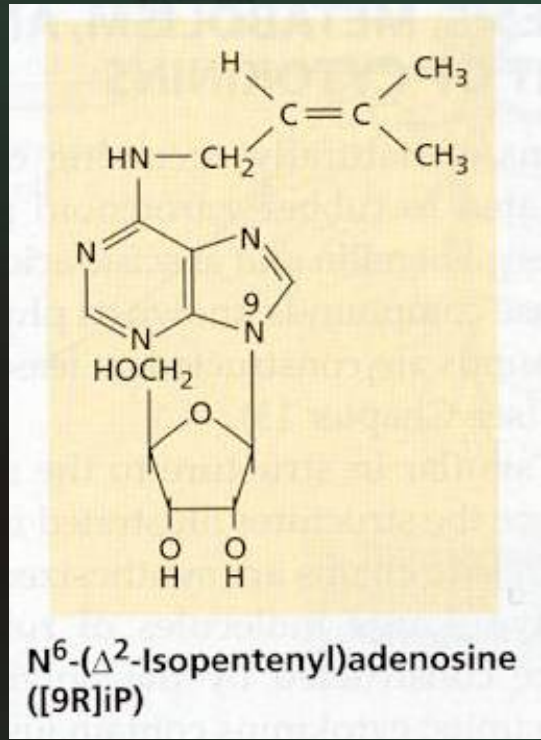
trans-Zeatin

cis-Zeatin

6-(4-Hydroxy-3-methylbut-2-enylamino)purine



Ribosylzeatin (zeatin riboside)



N^6-(Δ^2-Isopentenyl)adenosine ([9R]iP)

FIGURE 21.2 Structures of ribosylzeatin and N6-(Δ2-isopentenyl)adenosine ([9R]iP).

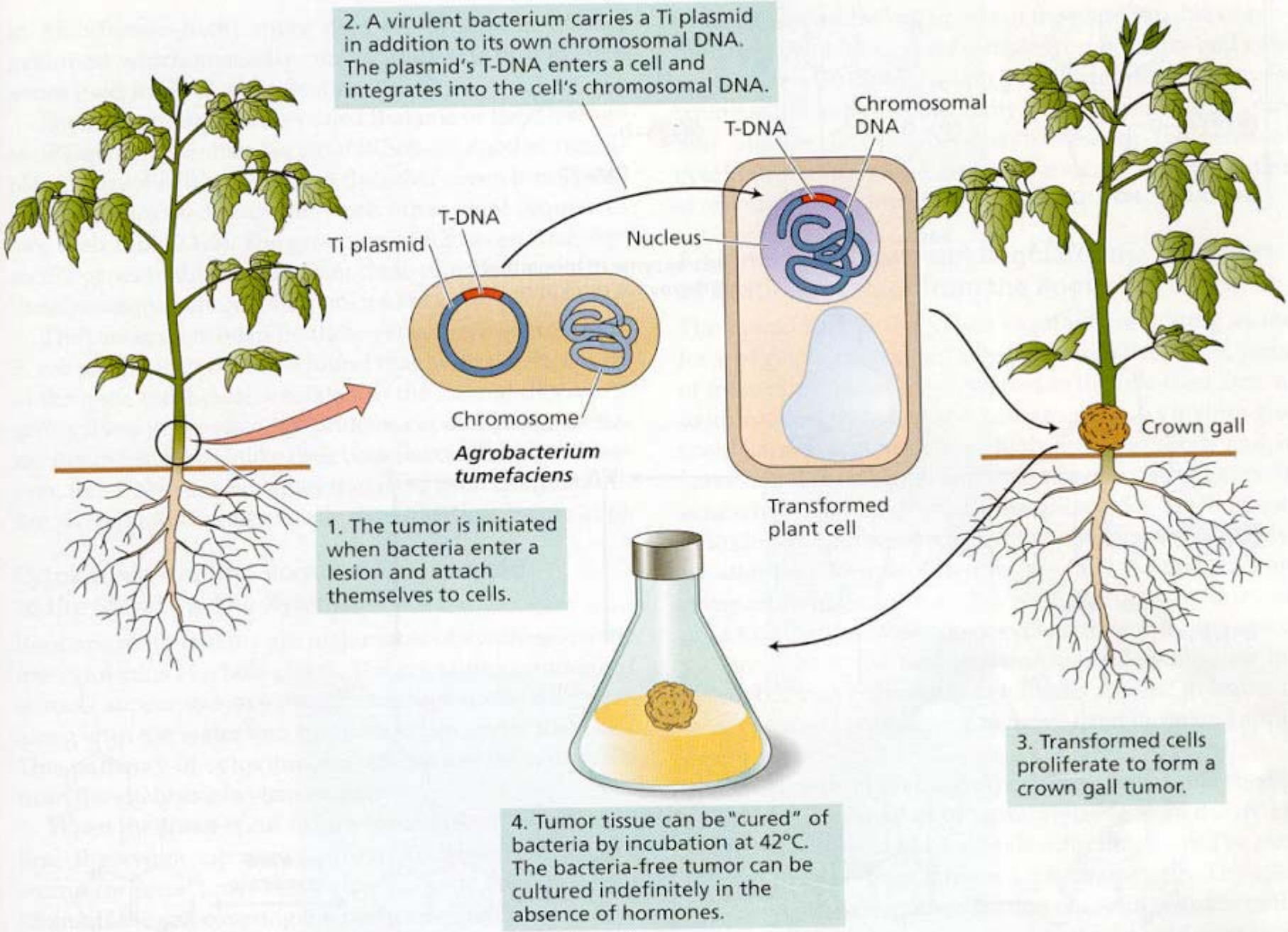
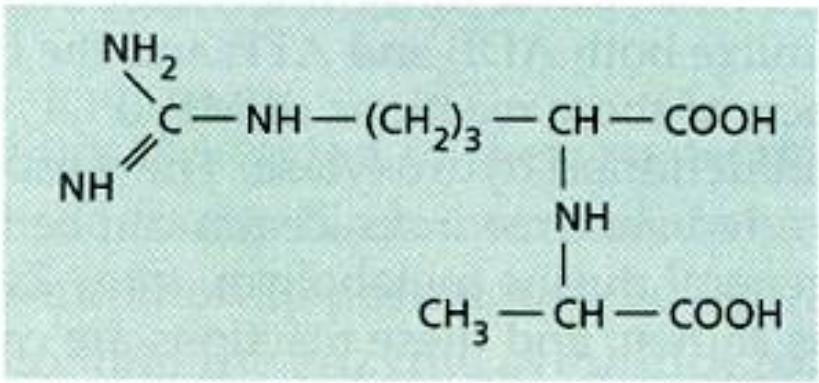
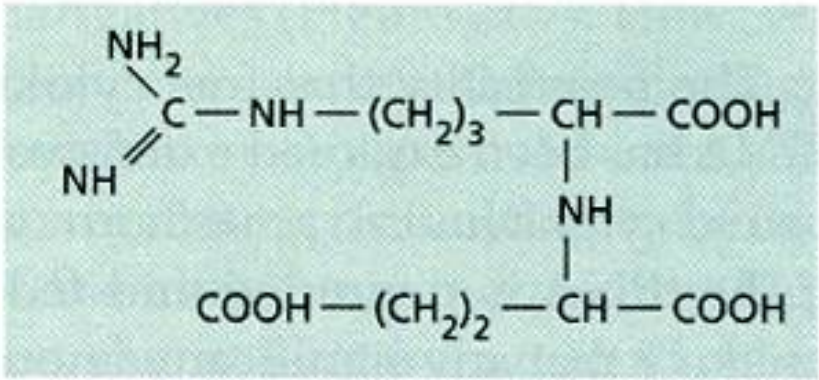


FIGURE 21.4 Tumor induction by *Agrobacterium tumefaciens*. (After Chilton 1983.)



Octopine



Nopaline

FIGURE 21.5 The two major opines, octopine and nopaline, are found only in crown gall tumors. The genes required for their synthesis are present in the T-DNA from *Agrobacterium tumefaciens*. The bacterium, but not the plant, can utilize the opines as a nitrogen source.

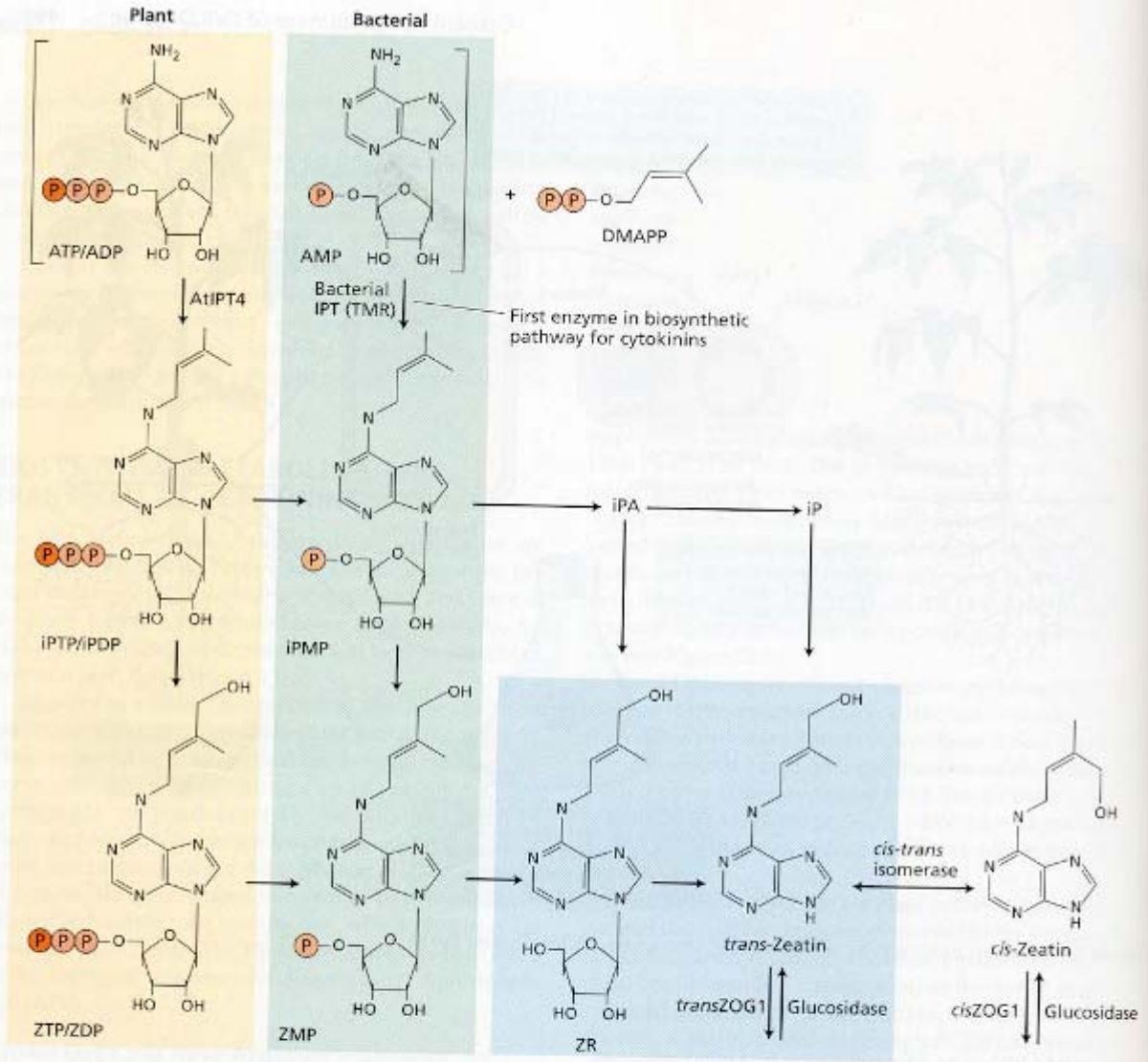
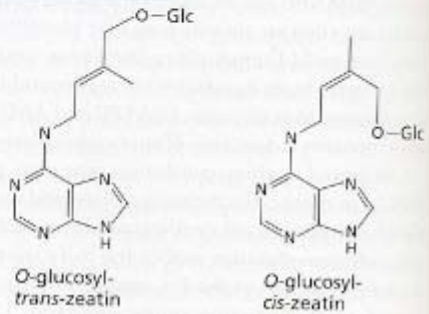
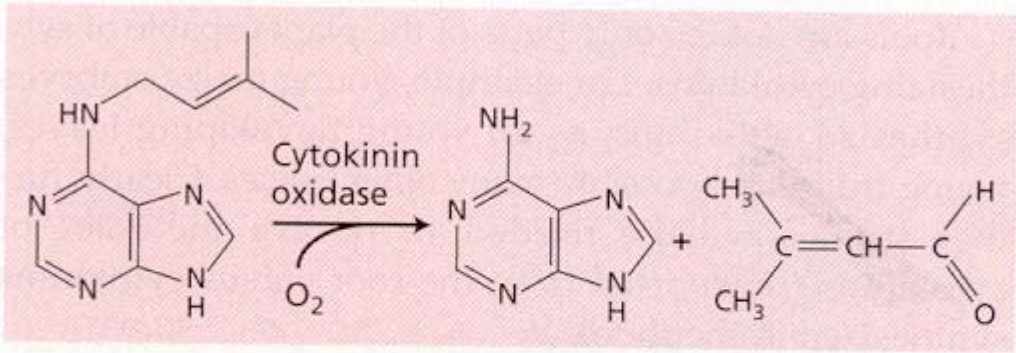


FIGURE 21.6 Biosynthetic pathway for cytokinin biosynthesis. The first committed step in cytokinin biosynthesis is the addition of the isopentenyl side chain from DMAPP to an adenosine moiety. The plant and bacterial IPT enzymes differ in the adenosine substrate used; the plant enzyme appears to utilize both ADP and ATP, and the bacterial enzyme utilizes AMP. The products of these reactions (iPMP, iPDP, or iPTP) are converted to zeatin by an unidentified hydroxylase. The various phosphorylated forms can be interconverted and free *trans*-Zeatin can be formed from the riboside by enzymes of general purine metabolism. *trans*-Zeatin can be metabolized in various ways as shown, and these reactions are catalyzed by the indicated enzymes.





iP Adenine 3-Methyl-2-butenal

FIGURE 21.7 Cytokinin oxidase irreversibly degrades some cytokinins.

FIGURE 21.8 Tobacco plants overexpressing the gene for cytokinin oxidase. The plant on the left is wild type. The two plants on the right are overexpressing two different constructs of the *Arabidopsis* gene for cytokinin oxidase: *AtCKX1* and *AtCKX2*. Shoot growth is strongly inhibited in the transgenic plants. (From Werner et al. 2001.)

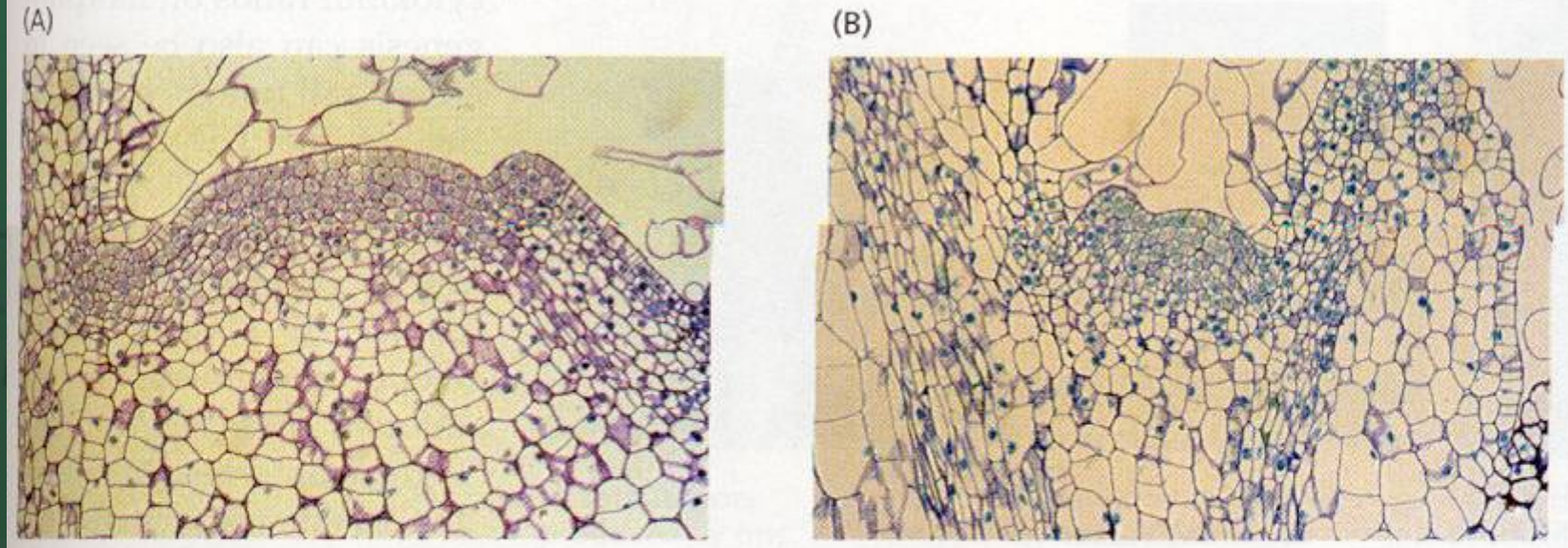


FIGURE 21.9 Cytokinin is required for normal growth of the shoot apical meristem. (A) Longitudinal section through the shoot apical meristem of a wild-type tobacco plant. (B) Longitudinal section through the shoot apical meristem of a transgenic tobacco over-expressing the gene that encodes cytokinin oxidase (*AtCKX1*). Note the reduction in the size of the apical meristem in the cytokinin-deficient plant. (From Werner et al. 2001.)

FIGURE 21.10 Cytokinin suppresses the growth of roots. The cytokinin-deficient *AtCKX1* roots (right) are larger than those of the wild-type tobacco plant (left). (From Werner et al. 2001.)

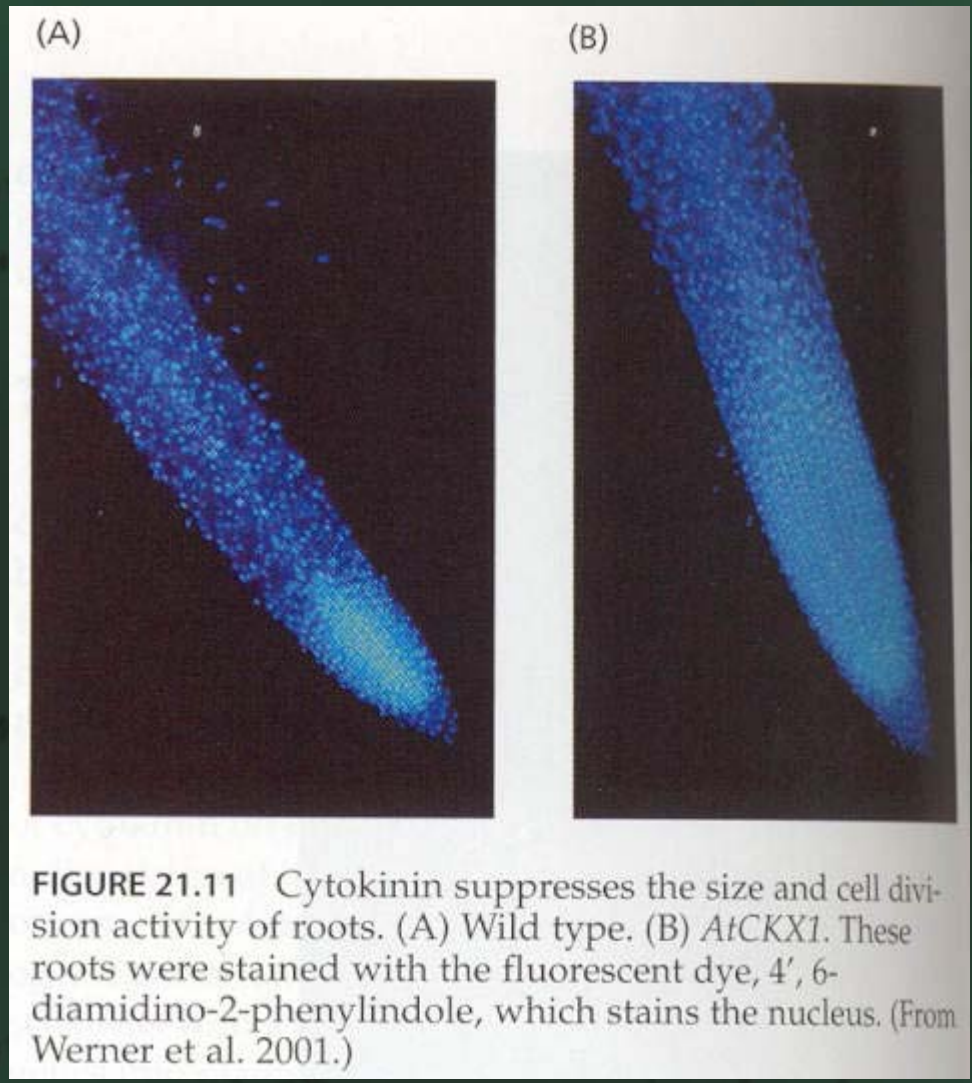


FIGURE 21.11 Cytokinin suppresses the size and cell division activity of roots. (A) Wild type. (B) *AtCKX1*. These roots were stained with the fluorescent dye, 4′, 6-diamidino-2-phenylindole, which stains the nucleus. (From Werner et al. 2001.)

FIGURE 21.12 *CYCD3*-expressing callus cells can divide in the absence of cytokinin. Leaf explants from transgenic Arabidopsis plants expressing *CYCD3* under a cauliflower mosaic virus 35S promoter were induced to form calluses through culturing in the presence of auxin plus cytokinin or auxin alone. The wild-type control calluses required cytokinin to grow. The *CYCD3*-expressing calluses grew well on medium containing auxin alone. The photographs were taken after 29 days. (From Riou-Khamlichi et al. 1999.)

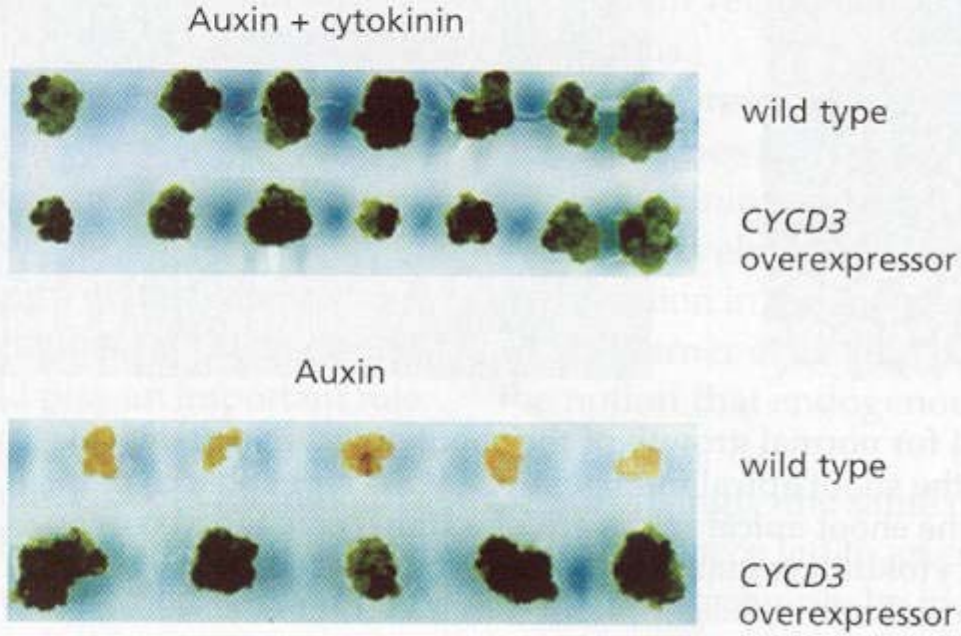


FIGURE 21.13 The regulation of growth and organ formation in cultured tobacco callus at different concentrations of auxin and kinetin. At low auxin and high kinetin concentrations (lower left) buds developed. At high auxin and low kinetin concentrations (upper right) roots developed. At intermediate or high concentrations of both hormones (middle and lower right) undifferentiated callus developed. (Courtesy of Donald Armstrong.)

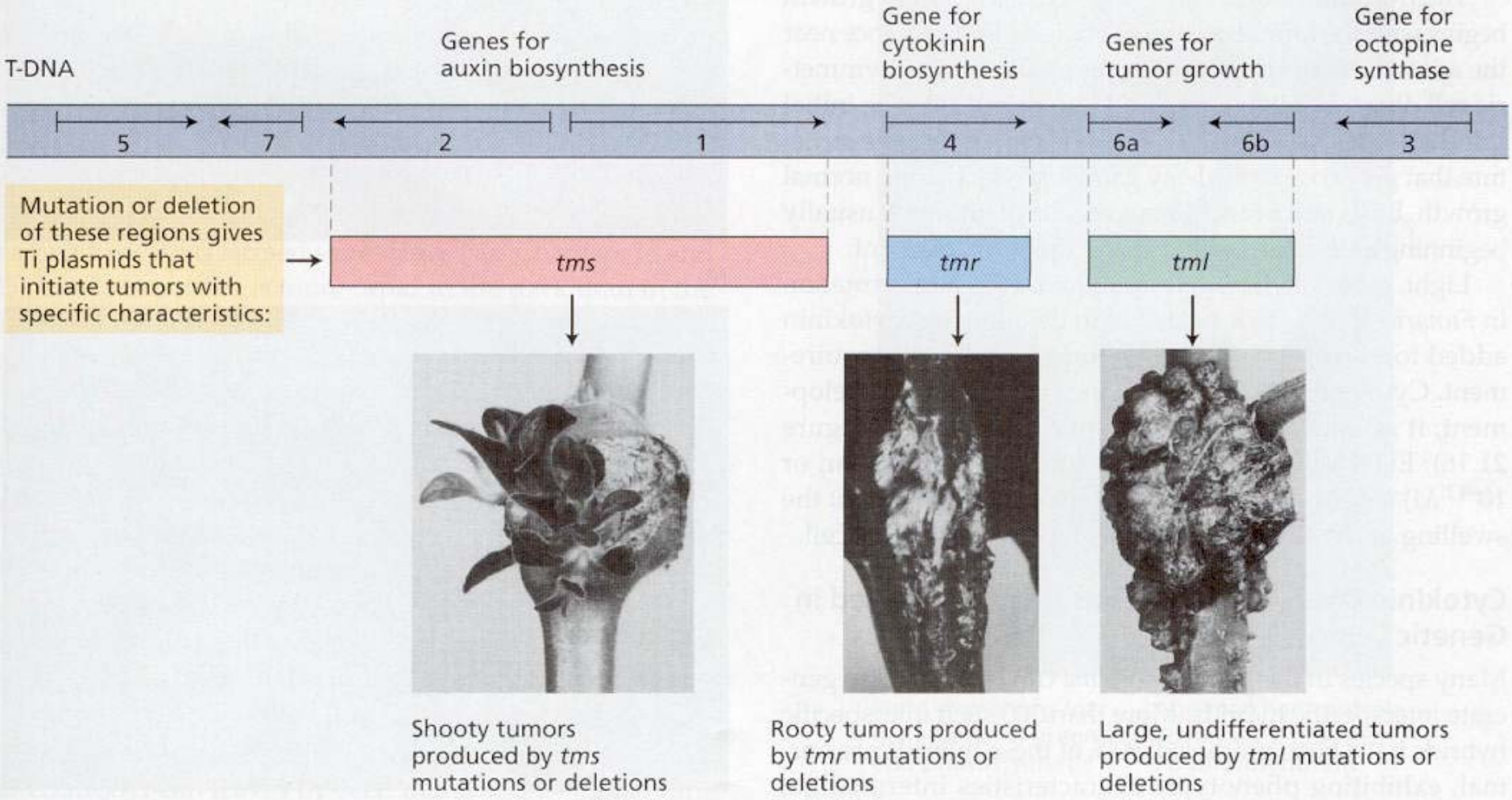


FIGURE 21.14 Map of the T-DNA from an *Agrobacterium* Ti plasmid, showing the effects of T-DNA mutations on crown gall tumor morphology. Genes 1 and 2 encode the two enzymes involved in auxin biosynthesis; gene 4 encodes a cytokinin biosynthesis enzyme. Mutations in these genes produce the phenotypes illustrated. (From Morris 1986, courtesy of R. Morris.)

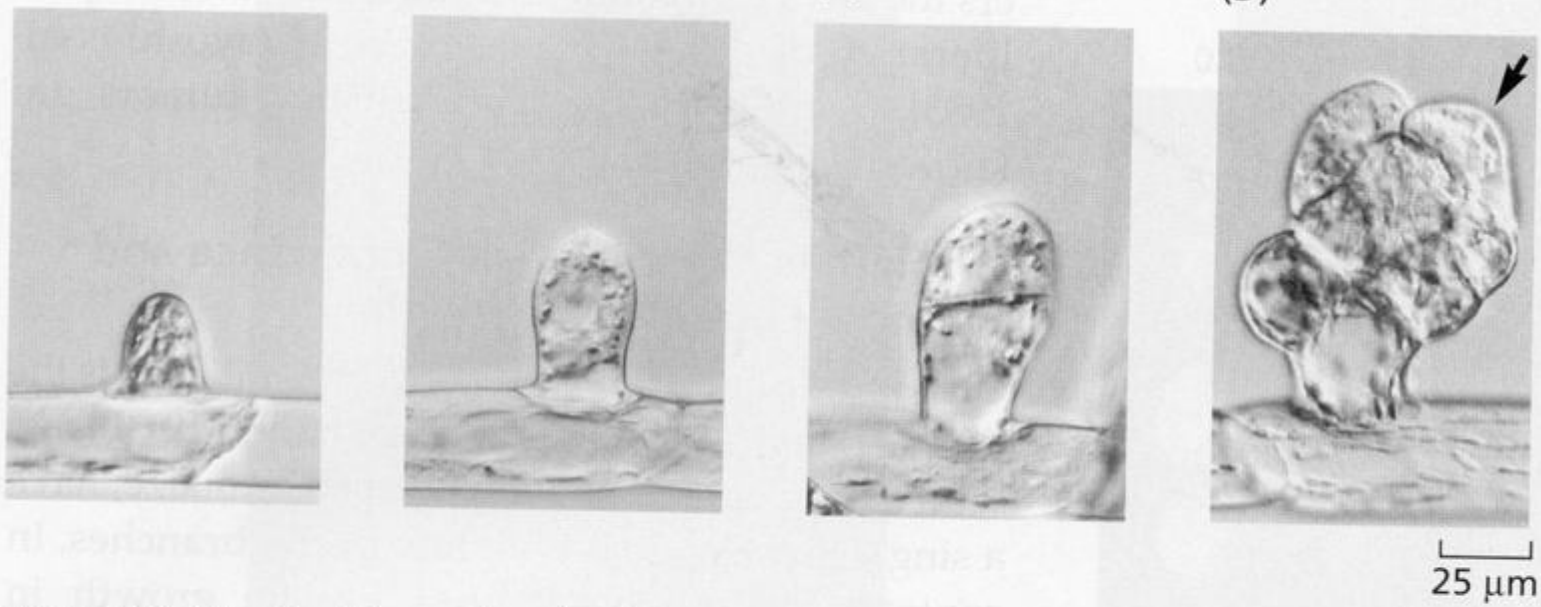
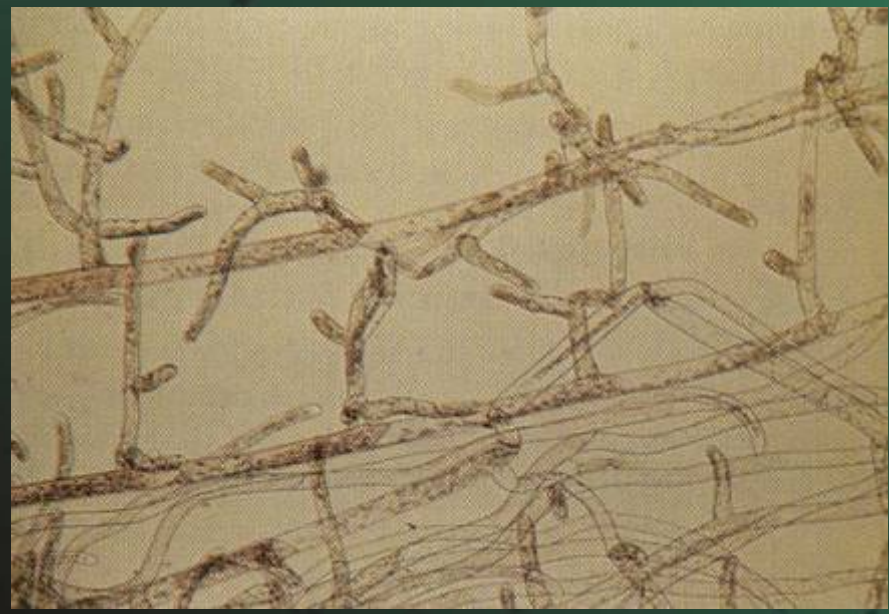


FIGURE 21.15 Bud formation in the moss *Funaria* begins with the formation of a protuberance at the apical ends of certain cells in the protonema filament. A–D show various stages of bud development. Once formed, the bud goes on to produce the leafy gametophyte stage of the moss. (Courtesy of K. S. Schumaker.)



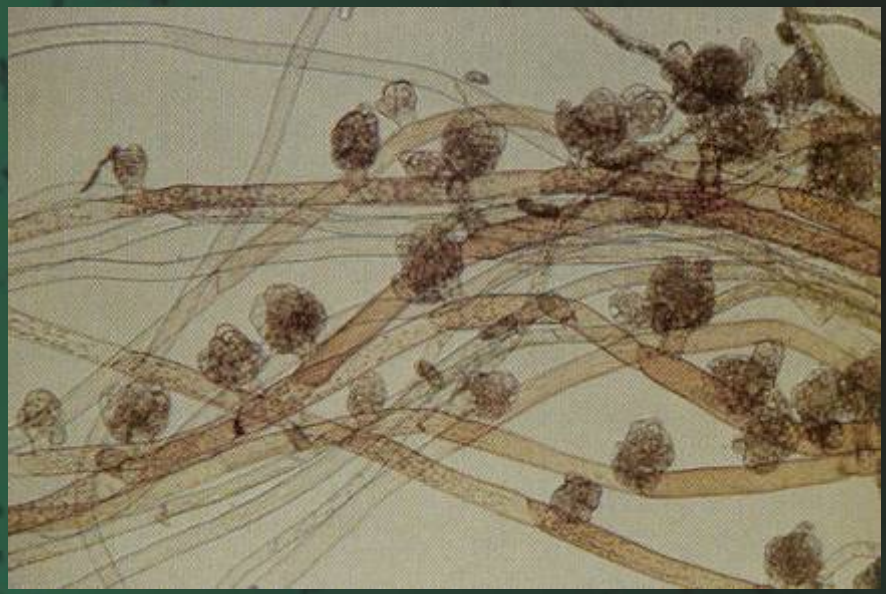


FIGURE 21.16 Cytokinin stimulates bud development in *Funaria*. (A) Control protonemal filaments. (B) Protonemal filaments treated with benzyladenine. (Courtesy of H. Kende.)

FIGURE 21.17 Expression of genetic tumors in the hybrid *Nicotiana langsdorffii* × *N. glauca*. (From Smith 1988.)

FIGURE 21.18 Leaf senescence is retarded in a transgenic tobacco plant containing a cytokinin biosynthesis gene, *ipt*. The *ipt* gene is expressed in response to signals that induce senescence. (From Gan and Amasino 1995, courtesy of R. Amasino.)

In seedling A, the left cotyledon was sprayed with water as a control. The left cotyledon of seedling B, and the right cotyledon of seedling C, were each sprayed with a solution containing 50m*M* kinetin.

The dark stippling represents the distribution of the radioactive amino acid as revealed by autoradiography.

The results show that the cytokinin-treated cotyledon has become a nutrient sink. However, radioactivity is retained in the cotyledon to which the amino acid was applied when the labeled cotyledon is treated with kinetin (seedling C).

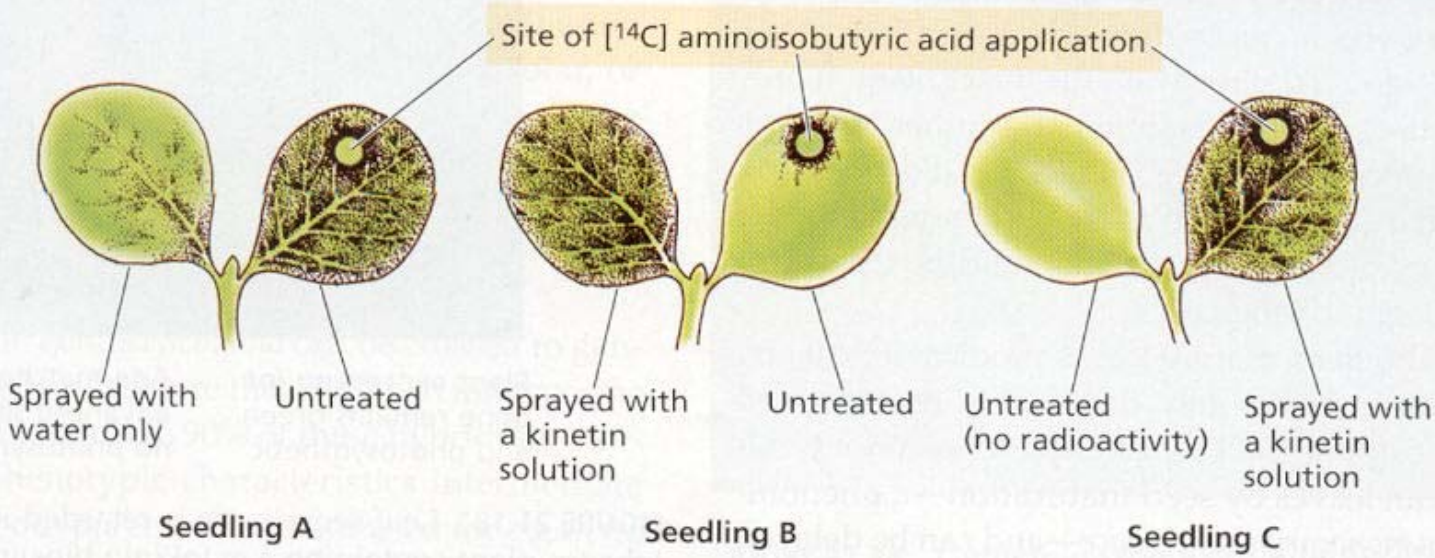


FIGURE 21.19 The effect of cytokinin on the movement of an amino acid in cucumber seedlings. A radioactively labeled amino acid that cannot be metabolized, such as aminoisobutyric acid, was applied as a discrete spot on the right cotyledon of each of these seedlings. (Drawn from data obtained by K. Mothes.)

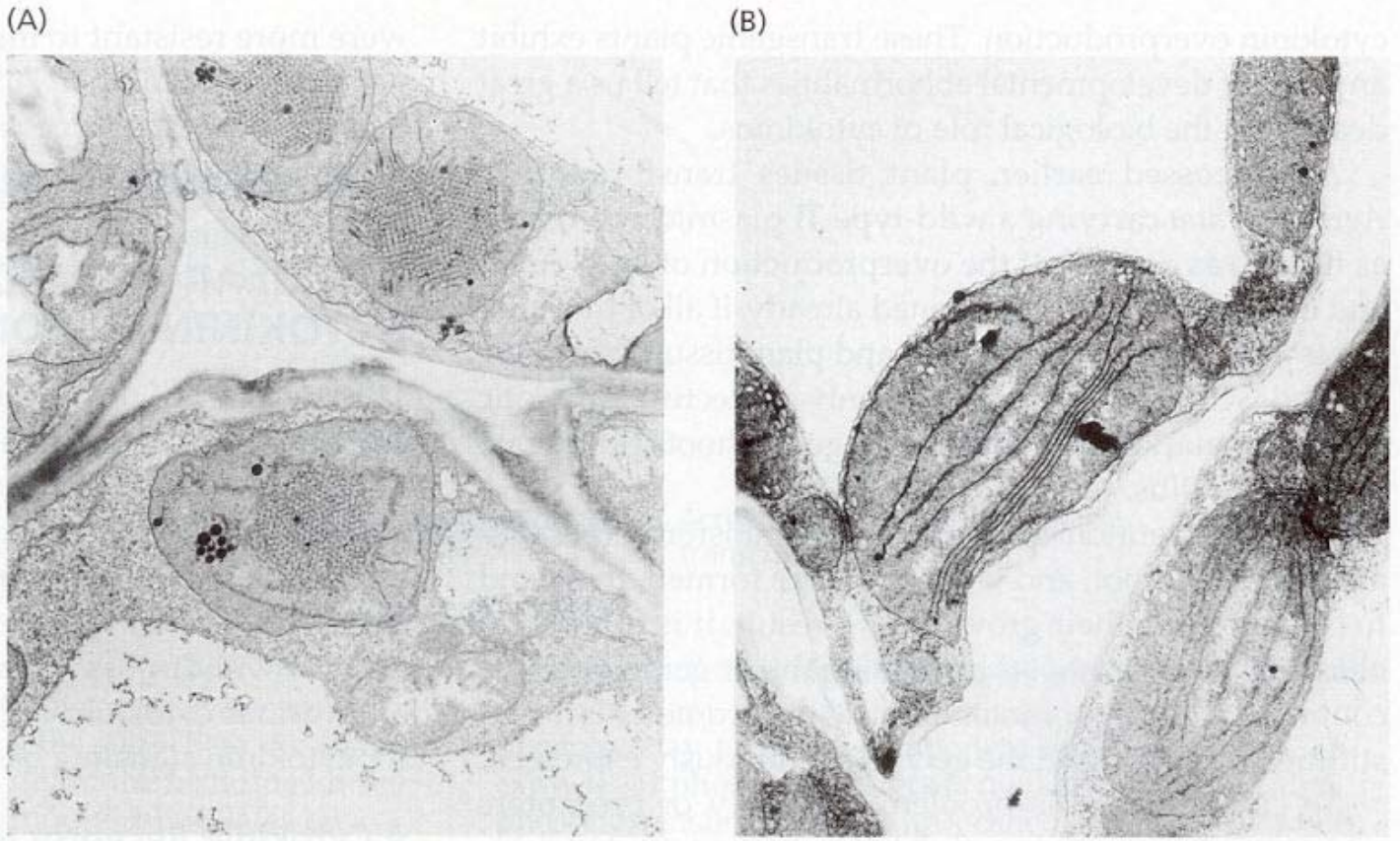


FIGURE 21.20 Cytokinin influence on the development of wild-type *Arabidopsis* seedlings grown in darkness. (A) Plastids develop as etioplasts in the untreated, dark grown control. (B) Cytokinin treatment resulted in thylakoid formation in the plastids of dark-grown seedlings. (From Chory et al. 1994, courtesy of J. Chory, © American Society of Plant Biologists, reprinted with permission.)

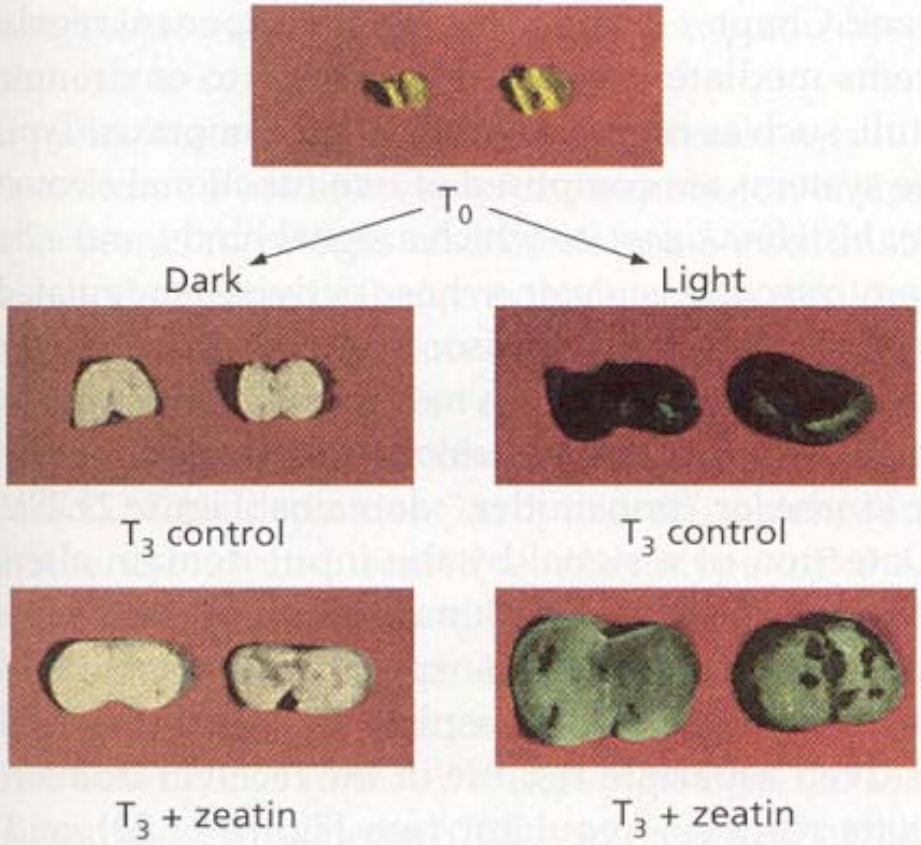


FIGURE 21.21 The effect of cytokinin on the expansion of radish cotyledons. The experiment described here shows that the effects of light and cytokinin are additive. T_0 represents germinating radish seedlings before the experiment began. The detached cotyledons were incubated for 3 days (T_3) in either darkness or light with or without 2.5 m*M* zeatin. In both the light and the dark, zeatin-treated cotyledons expanded more than in the control. (From Huff and Ross 1975.)

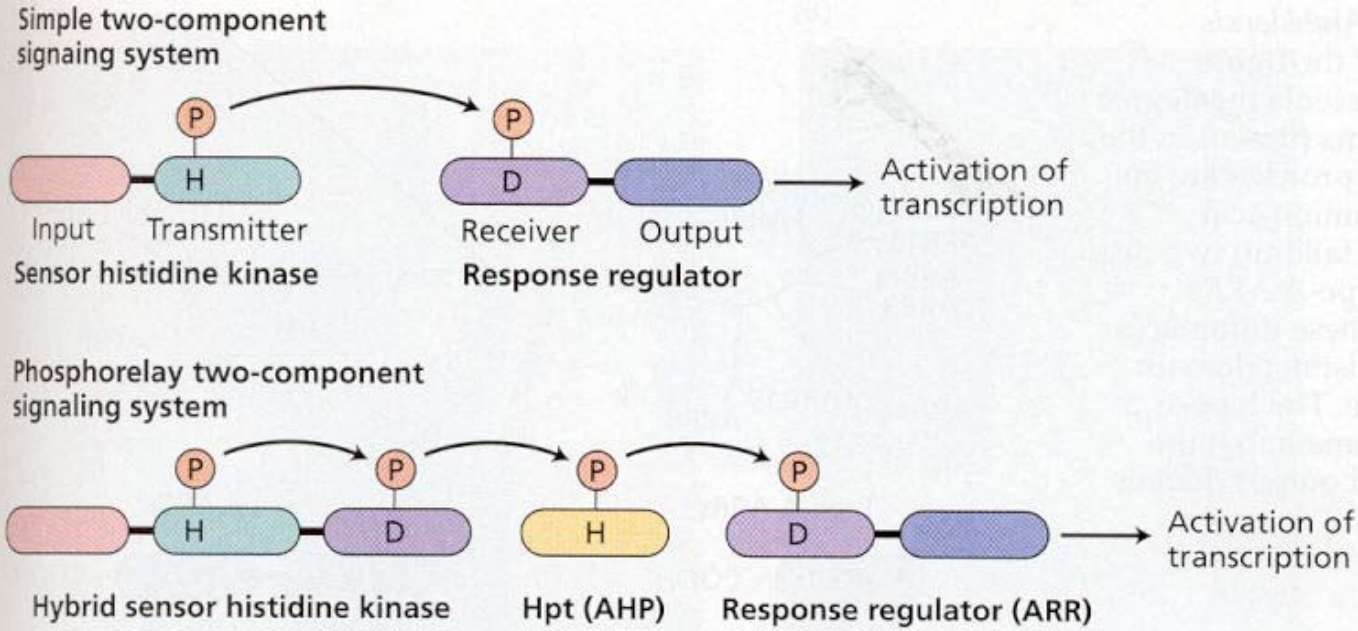
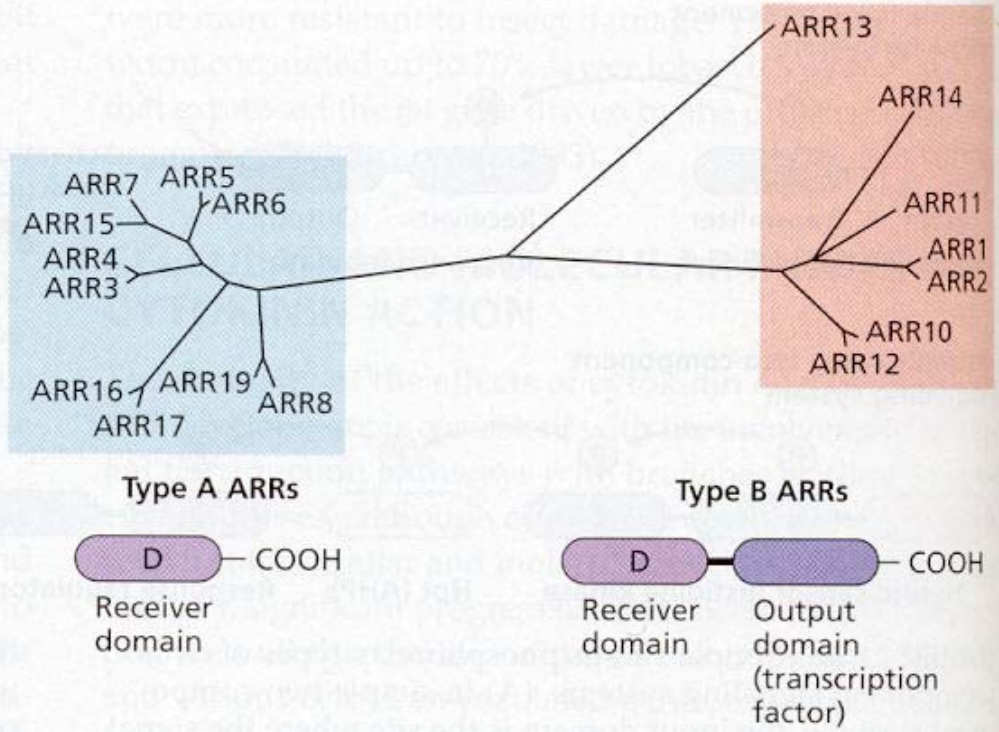


FIGURE 21.22 Simple versus phosphorelay types of two-component signaling systems. (A) In simple two-component systems, the input domain is the site where the signal is sensed. This regulates the activity of the histidine kinase domain, which when activated autophosphorylates on a conserved His residue. The phosphate is then transferred to an Asp residue that resides within the receiver domain of a response regulator. Phosphorylation of this Asp regulates the activity of the output domain of the response regulator, which in many cases is a transcription factor. (B) In the phosphorelay-type two-component signaling system, an extra set of phosphotransfers is mediated by a histidine phosphotransfer protein (Hpt), called AHP in *Arabidopsis*. The *Arabidopsis* response regulators are called ARRs. H = histidine, D = aspartate.

FIGURE 21.23 Phylogenetic tree of *Arabidopsis* response regulators. The top part of the figure shows a phylogenetic tree that represents the degree of relatedness of the receiver domains present in the *Arabidopsis* genome. The closer two proteins are on the tree, the more similar are their amino acid sequences. Note that these proteins fall into two distinct groups, or clades, called the type-A ARRs (blue) and the type-B ARRs (red). These differences in sequence are also reflected in a distinct domain structure, as depicted below the tree. The type-A ARRs consist solely of a receiver domain, but the type-A proteins also contain a fused output domain at the carboxy-terminus.



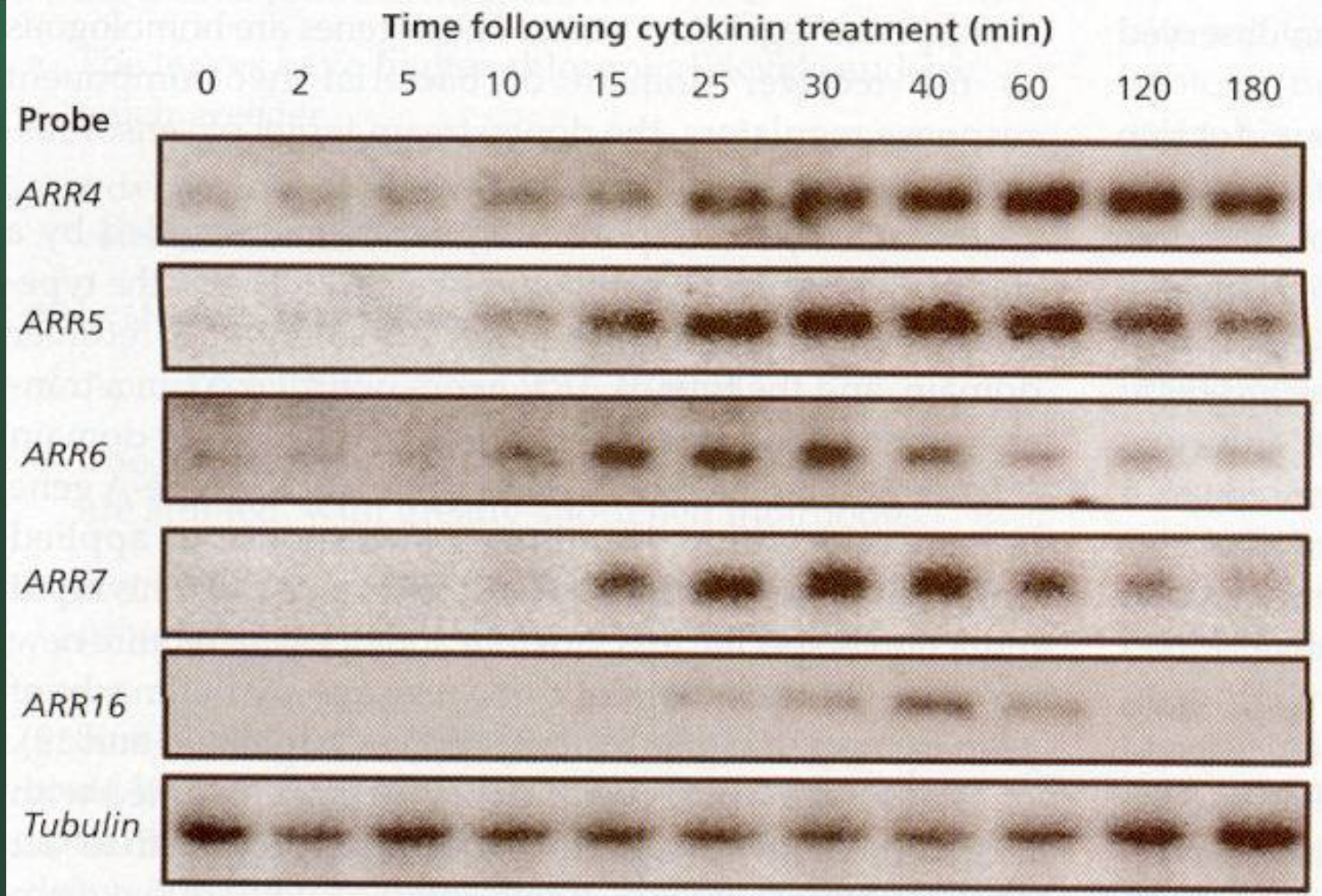


FIGURE 21.24 Induction of type-A *ARR* genes in response to cytokinin. RNA from *Arabidopsis* seedlings treated for the indicated time with cytokinin was isolated and analyzed by Northern blotting. Each row shows the result of probing the Northern blot with an individual type-A gene, and each lane contains RNA derived from *Arabidopsis* seedlings treated for the indicated time with cytokinin. The darker the band, the higher the level of *ARR* mRNA in that sample. (From D'Agostino et al. 2000.)

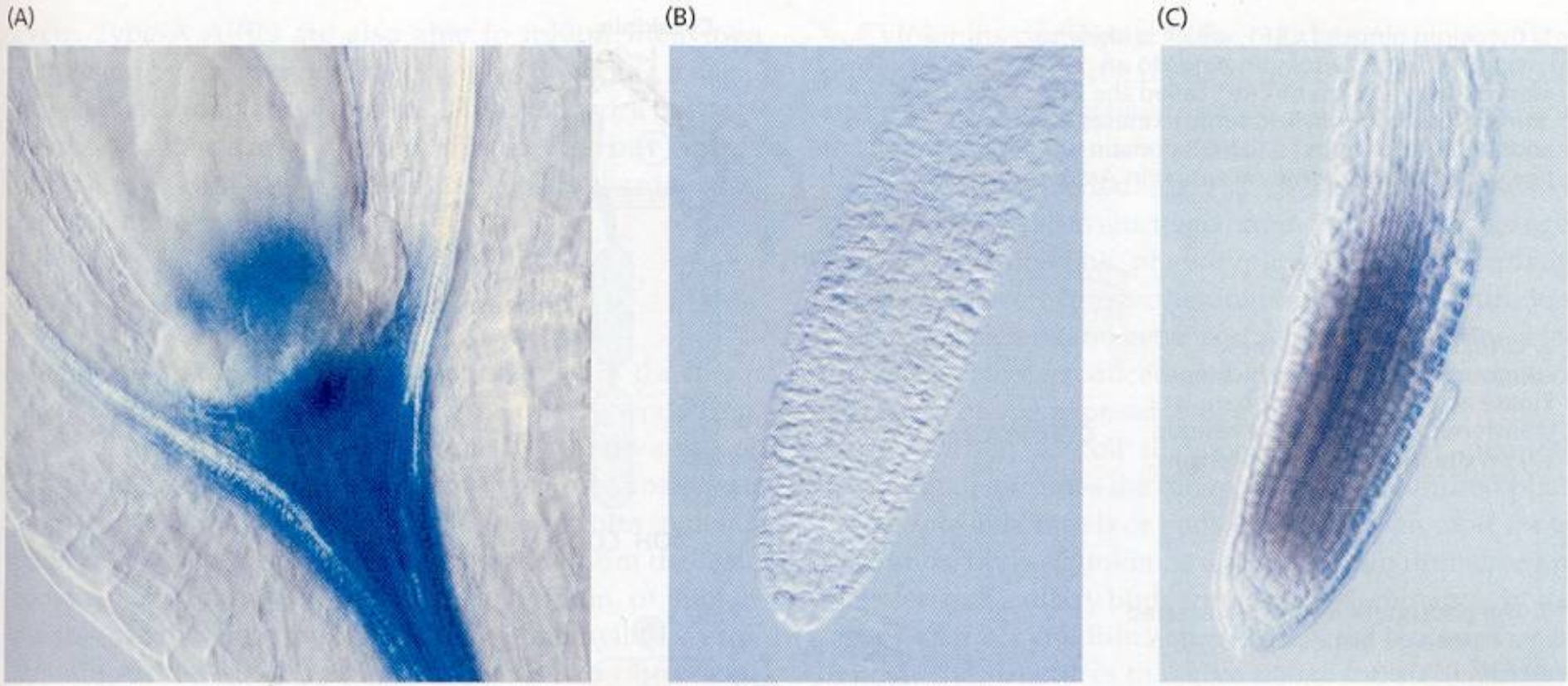


FIGURE 21.25 Expression of *ARR5*. The pattern of *ARR5* expression was examined by fusion of the promoter to a *GUS* reporter gene (A) or by whole-mount in situ hybridization (B and C). For the latter, the tissue is hybridized with labeled single-stranded *ARR5* RNA in either the sense orientation (B) or the antisense (C). The sense RNA is a negative control and reveals background, nonspecific staining. The antisense probe specifically hybridizes with the *ARR5* mRNA present in the tissue, thereby revealing its spatial distribution. With both methods, *ARR5* expression is observed primarily in the apical meristems. (From D'Agostino et al. 2000.)

1. Cytokinin binds to CRE1, which is likely to occur as a dimer. Cytokinin binds to an extracellular portion of CRE1 called the CHASE domain.Two other hybrid sensor kinases (AHK2 and AHK3) containing a CHASE domain are also likely to act as cytokinin receptors in *Arabidopsis*.

2. Cytokinin binding to these receptors activates their histidine kinase activity. The phosphate is transferred to an asparate residue (D) on the fused receiver domains.

3. The phosphate is then transferred to a conserved histidine present in an AHP protein.

4. Phosphorylation causes the AHP protein to move into the nucleus, where it transfers the phosphate to an asparate residue located within the receiver domain of a type-B ARR.

5. The phosphorylation of the type-B ARR activates the output domain to induce transcription of genes encoding type-A ARRs.

6. The type-A ARRs are likely also to be phosphorylated by the AHP proteins.

7. The phosphorylated type-A ARRs interact with various effectors to mediate the changes in cell function appropriate to cytokinin (indicated in the model as "cytokinin responses").

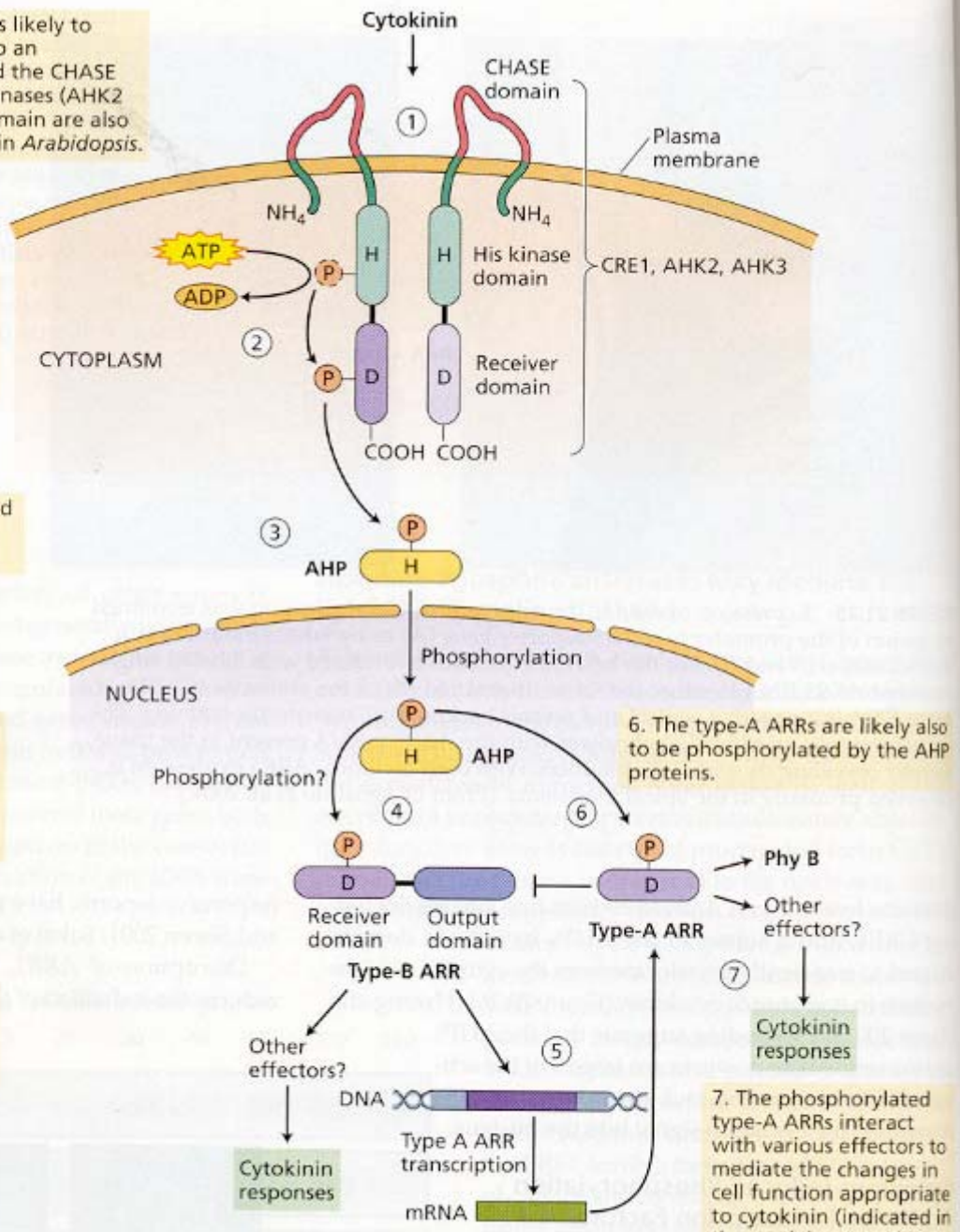


FIGURE 21.27 Model of cytokinin signaling. The near future should see significant refinement of this model, the tools are now in hand to analyze the interactions among these elements.