Laboratoř růstových regulátorů

Miroslav Strnad

Gibereliny
[kap. 20]

- Univerzita Palackého & Ústav experimentální botaniky AV CR

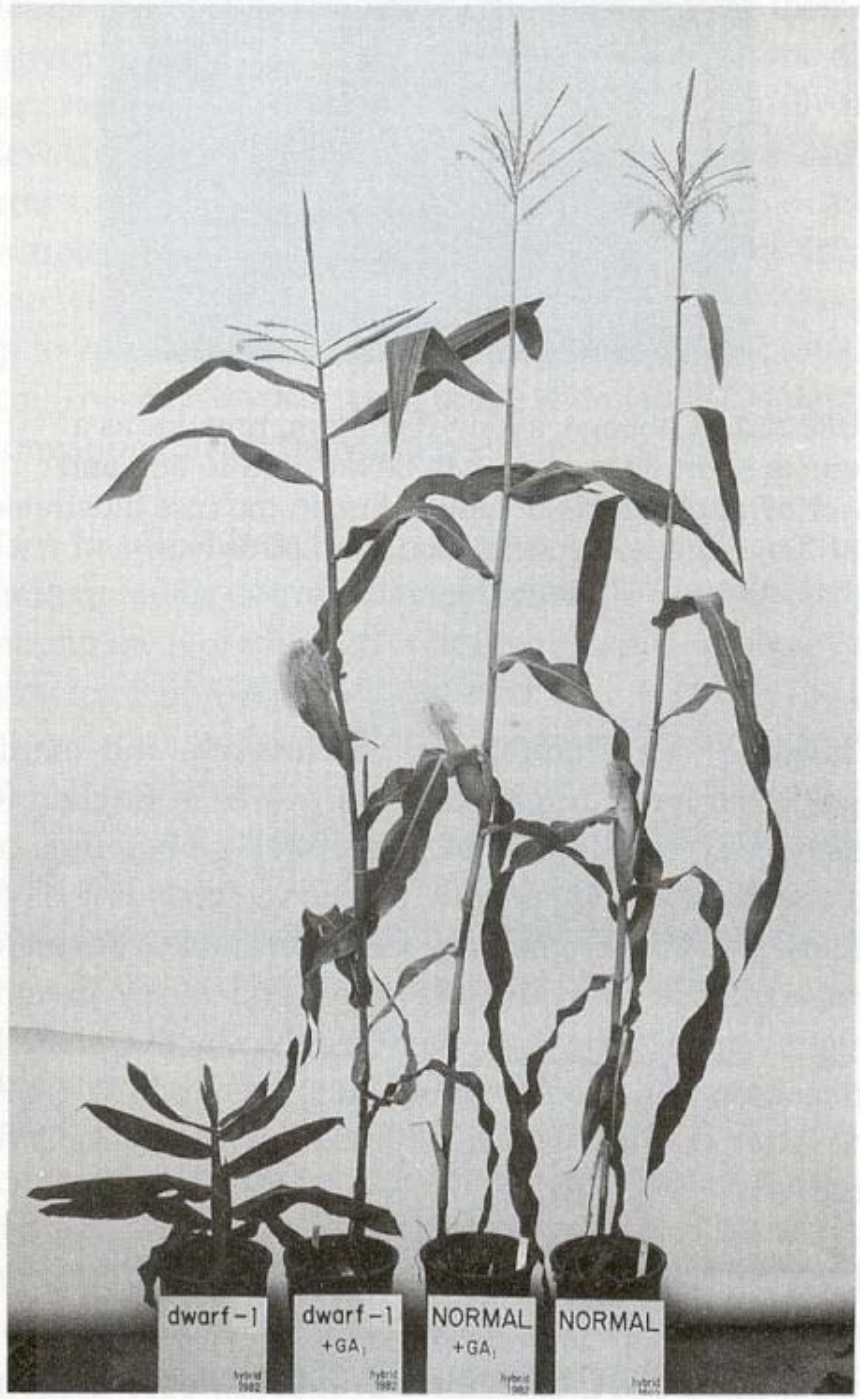
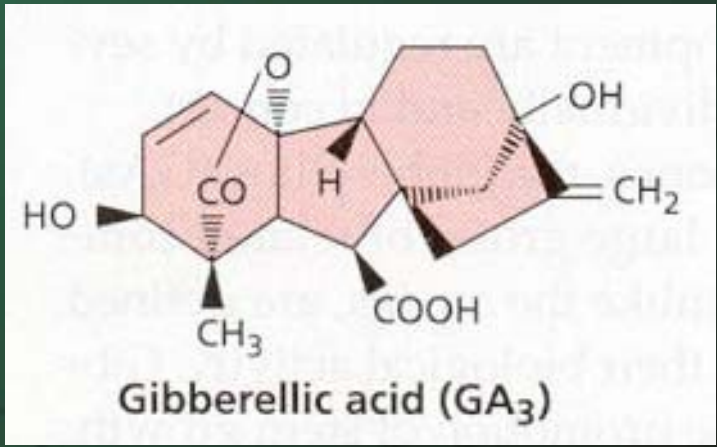
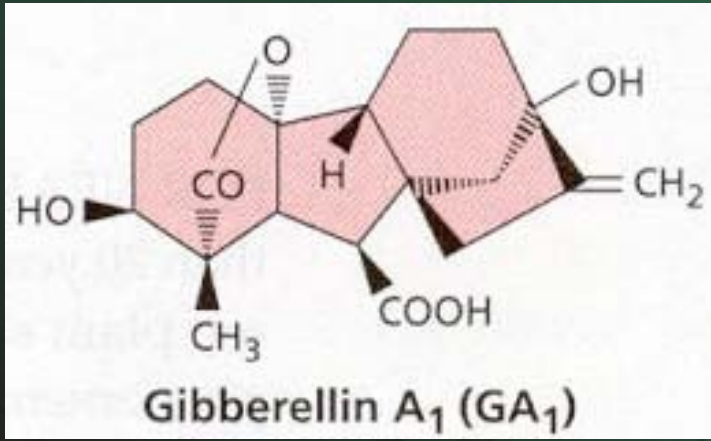


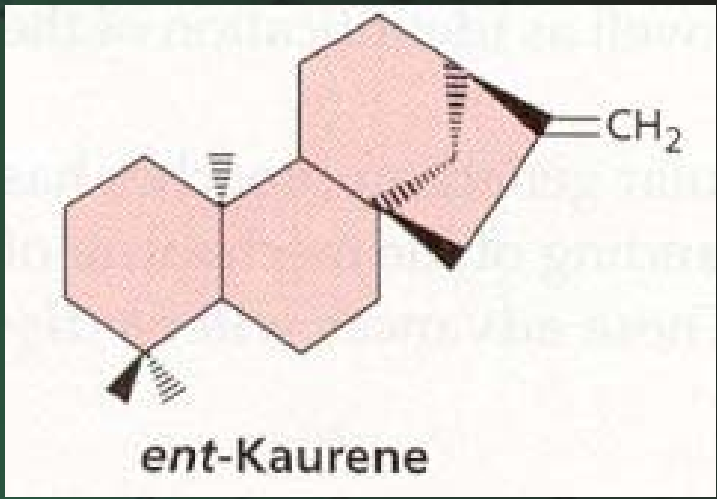
FIGURE 20.1 The effect of exogenous GA_1 on normal and dwarf (*d1*) corn. Gibberellin stimulates dramatic stem elongation in the dwarf mutant but has little or no effect on the tall wild-type plant. (Courtesy of B. Phinney.)



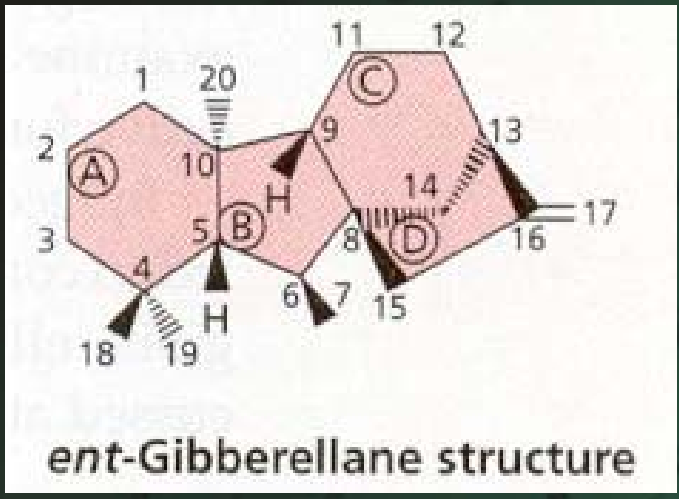
Gibberellic acid (GA_3)



Gibberellin A_1 (GA_1)



ent-Kaurene



ent-Gibberellane structure

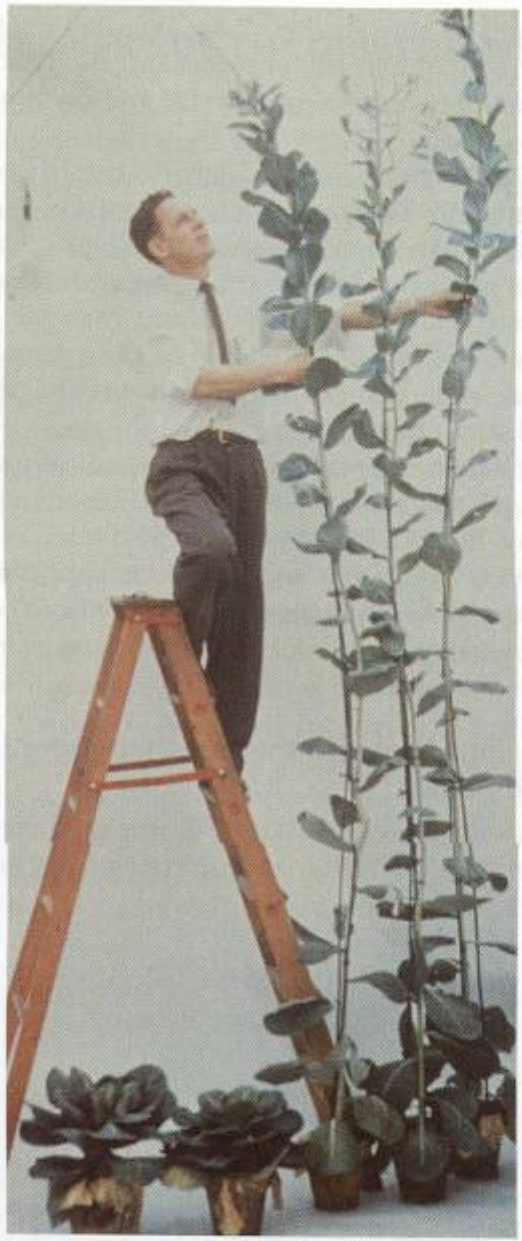


FIGURE 20.2 Cabbage, a long-day plant, remains as a rosette in short days, but it can be induced to bolt and flower by applications of gibberellin. In the case illustrated, giant flowering stalks were produced. (© Sylvan Wittwer/Visuals Unlimited.)

FIGURE 20.3 Anthers develop in the ears of a gibberellin-deficient dwarf mutant of corn (*Zea mays*). (Bottom) Unfertilized ear of the dwarf mutant *an1*, showing conspicuous anthers. (Top) Ear from a plant that has been treated with gibberellin. (Courtesy of M. G. Neuffer.)

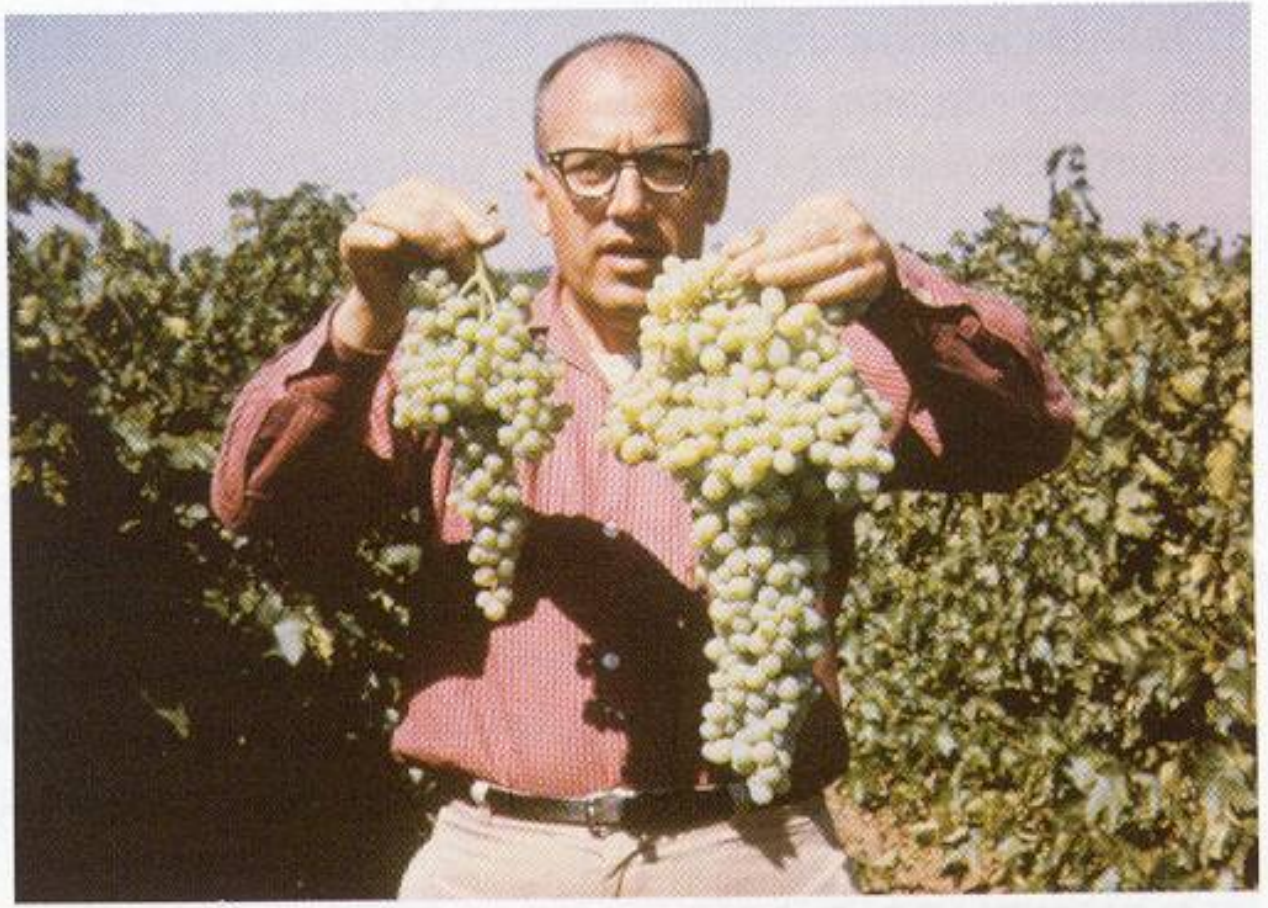


FIGURE 20.4 Gibberellin induces growth in Thompson's seedless grapes. The bunch on the left is an untreated control. The bunch on the right was sprayed with gibberellin during fruit development. (© Sylvan Wittwer/Visuals Unlimited.)

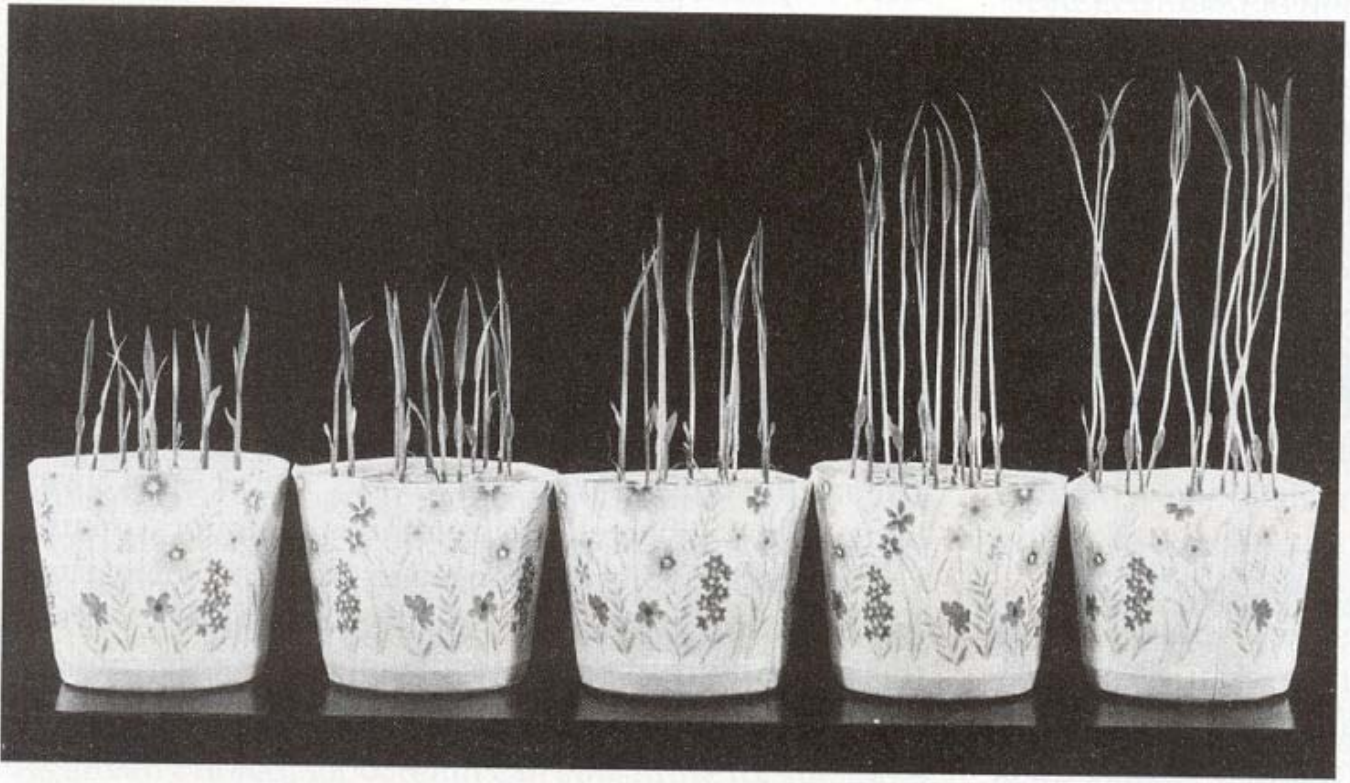


FIGURE 20.5 Gibberellin causes elongation of the leaf sheath of rice seedlings, and this response is used in the dwarf rice leaf sheath bioassay. Here 4-day-old seedlings were treated with different amounts of GA and allowed to grow for another 5 days. (Courtesy of P. Davies.)

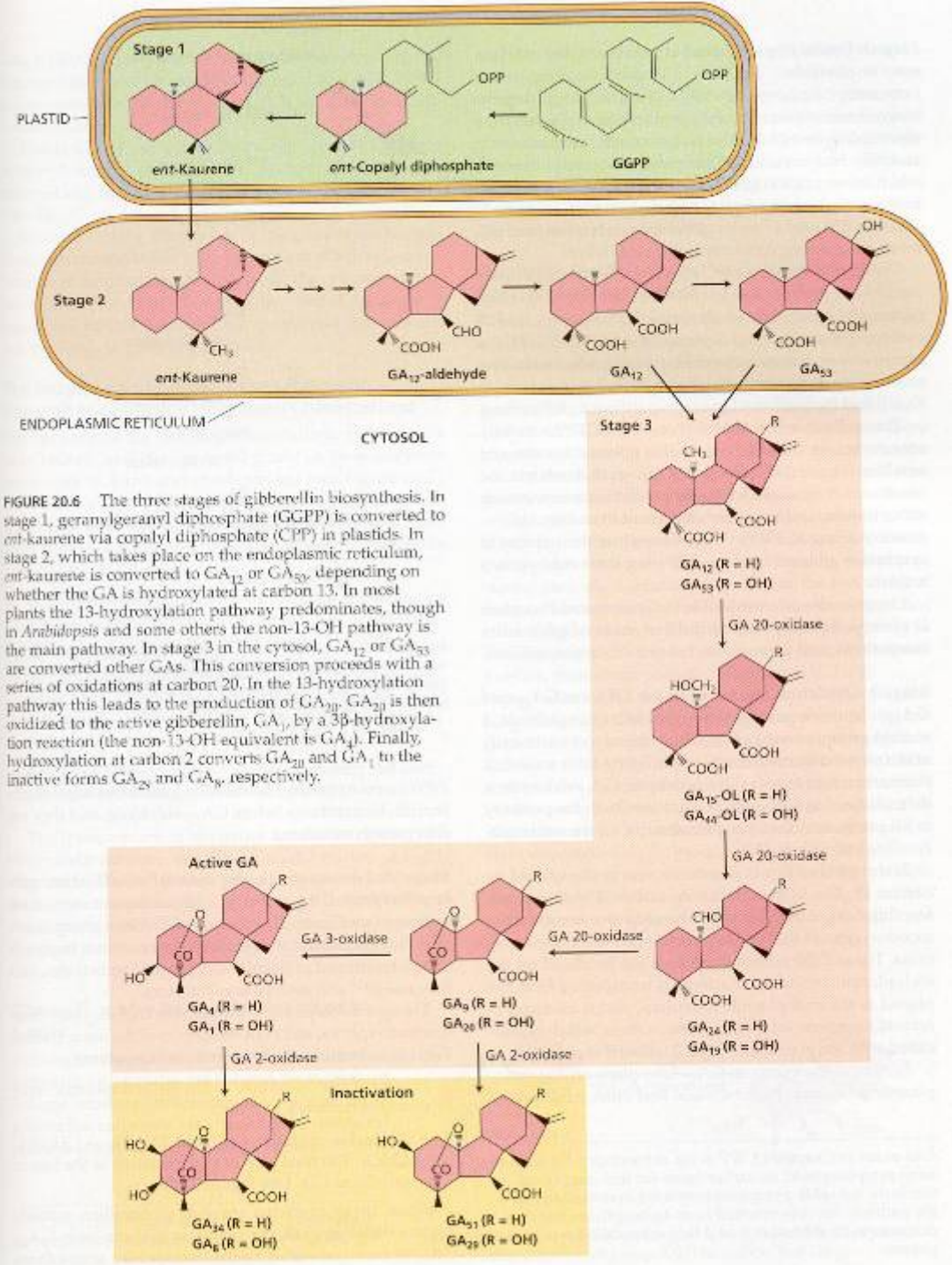


FIGURE 20.6 The three stages of gibberellin biosynthesis. In stage 1, geranylgeranyl diphosphate (GGPP) is converted to *ent*-kaurene via copalyl diphosphate (CPP) in plastids. In stage 2, which takes place on the endoplasmic reticulum, *ent*-kaurene is converted to GA_{12} or GA_{53}, depending on whether the GA is hydroxylated at carbon 13. In most plants the 13-hydroxylation pathway predominates, though in *Arabidopsis* and some others the non-13-OH pathway is the main pathway. In stage 3 in the cytosol, GA_{12} or GA_{53} are converted other GAs. This conversion proceeds with a series of oxidations at carbon 20. In the 13-hydroxylation pathway this leads to the production of GA_{20}. GA_{20} is then oxidized to the active gibberellin, GA_1, by a 3β-hydroxylation reaction (the non-13-OH equivalent is GA_4). Finally, hydroxylation at carbon 2 converts GA_{20} and GA_1 to the inactive forms GA_{29} and GA_8, respectively.

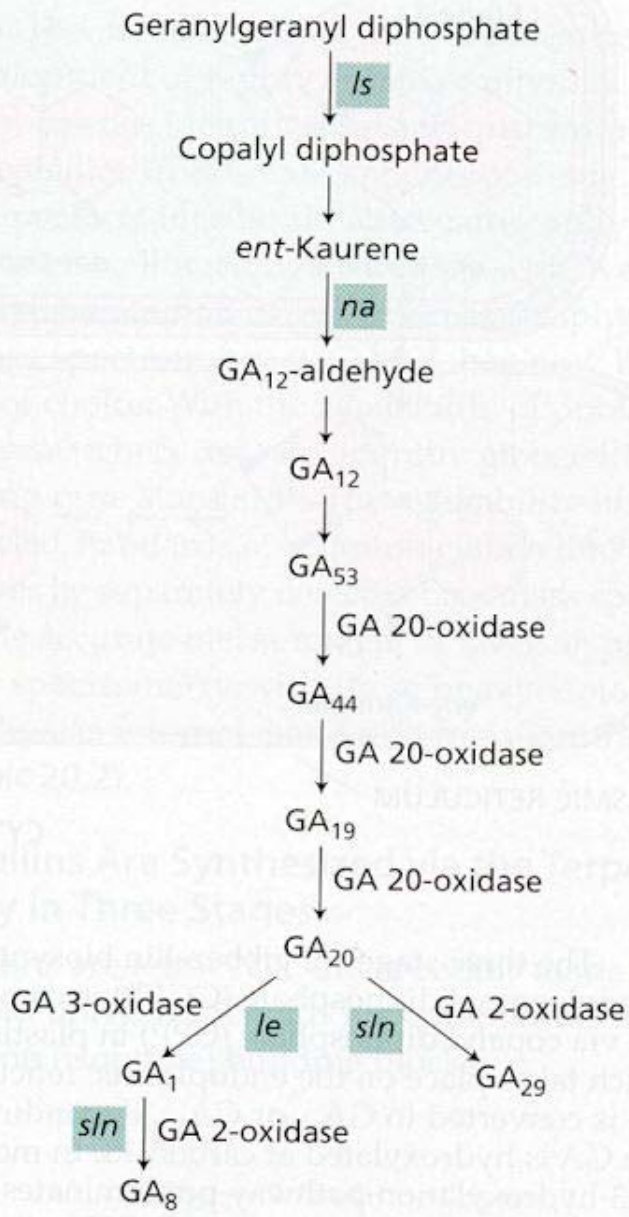
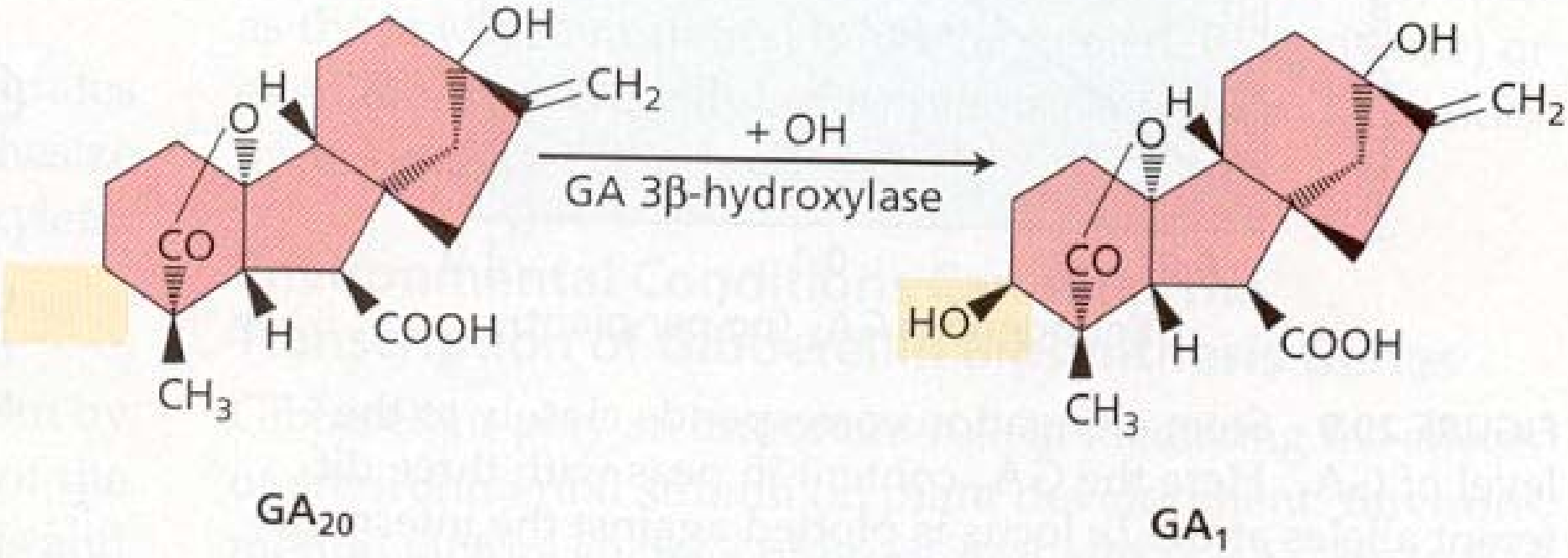


FIGURE 20.7 A portion of the gibberellin biosynthetic pathway showing the abbreviations and location of the mutant genes that block the pathway in pea and the enzymes involved in the metabolic steps after GA_{53}.



GA_{20} GA_1

FIGURE 20.8 Conversion of GA_{20} to GA_1 by GA 3β-hydroxylase, which adds a hydroxyl group (OH) to carbon 3 of GA_{20}.

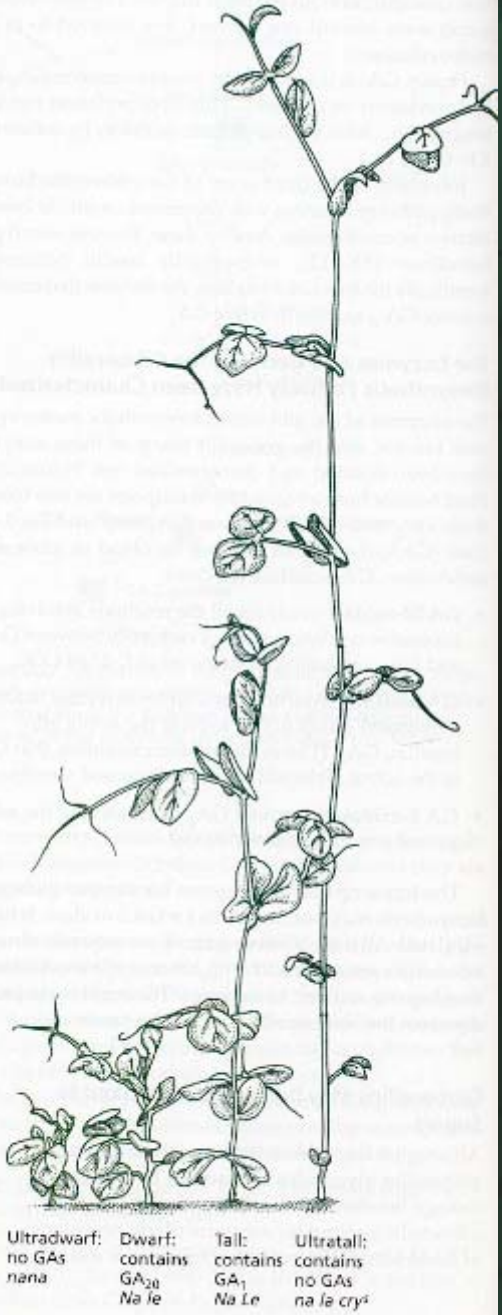


FIGURE 20.10 Phenotypes and genotypes of peas that differ in gibberellin content of their vegetative tissue. (All alleles are homozygous.) (After Davies 1995.)

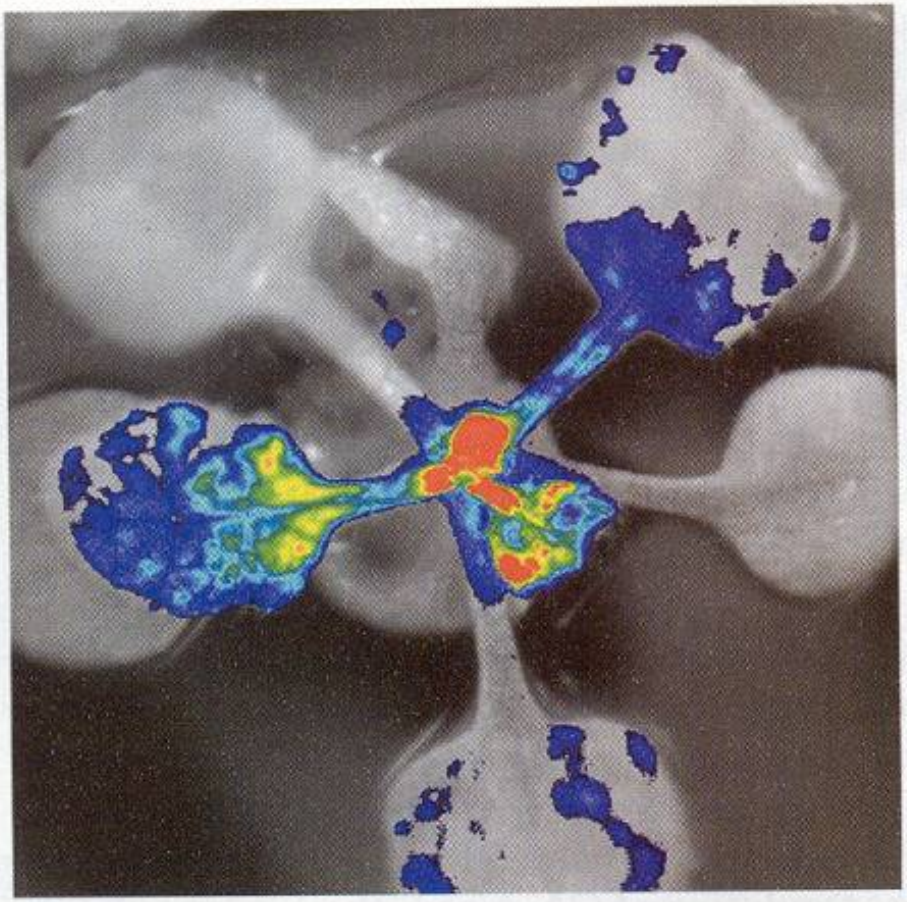
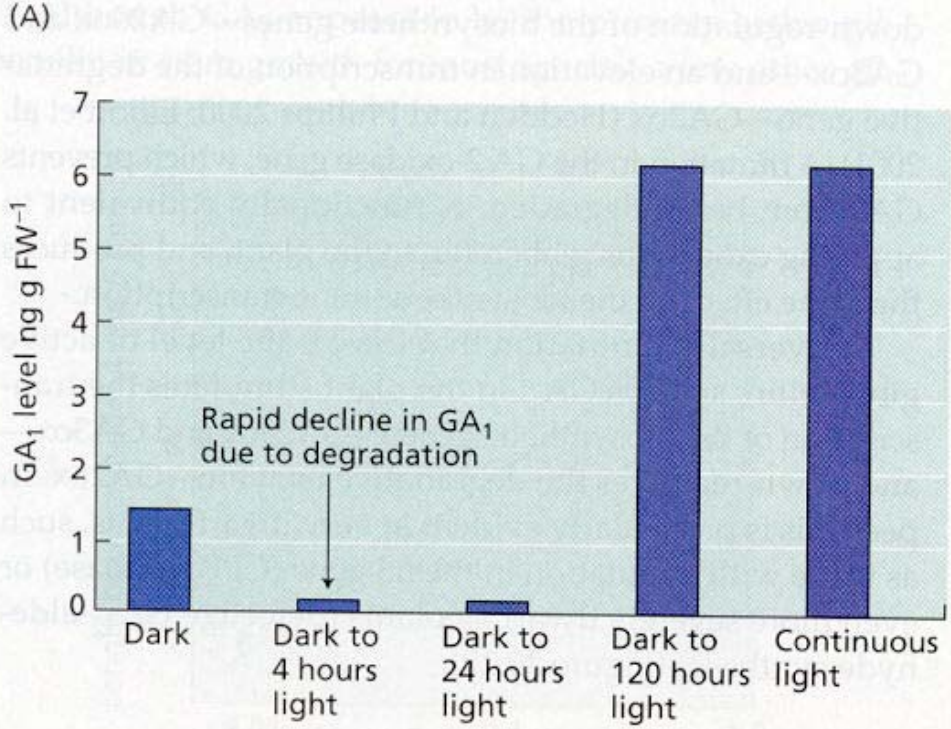


FIGURE 20.11 Gibberellin is synthesized mainly in the shoot apex and in young developing leaves. This false color image shows light emitted by transgenic *Arabidopsis* plants expressing the firefly luciferase coding sequence coupled to the GA20ox gene promoter. The emitted light was recorded by a CCD camera after the rosette was sprayed with the substrate luciferin. The image was then color-coded for intensity and superimposed on a photograph of the same plant. The red and yellow regions correspond to the highest light intensity. (Courtesy of Jeremy P. Coles, Andrew L. Phillips, and Peter Hedden, IACR-Long Ashton Research Station.)



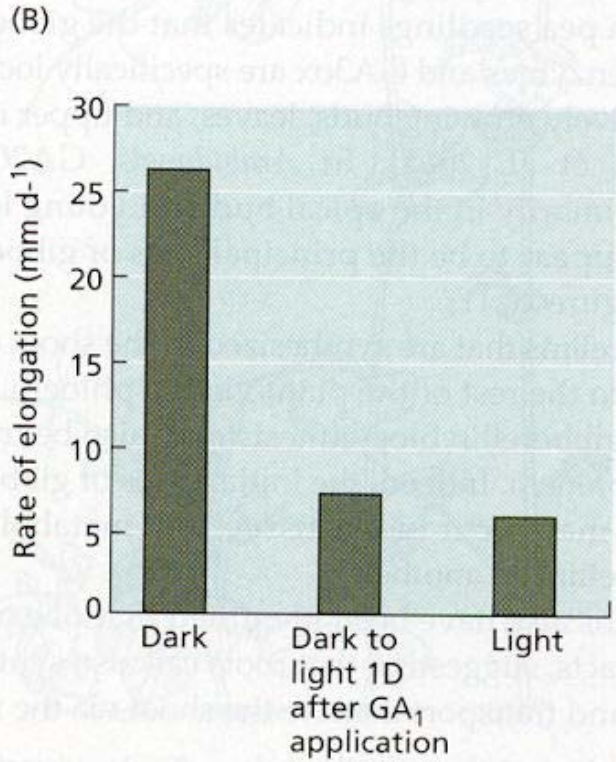
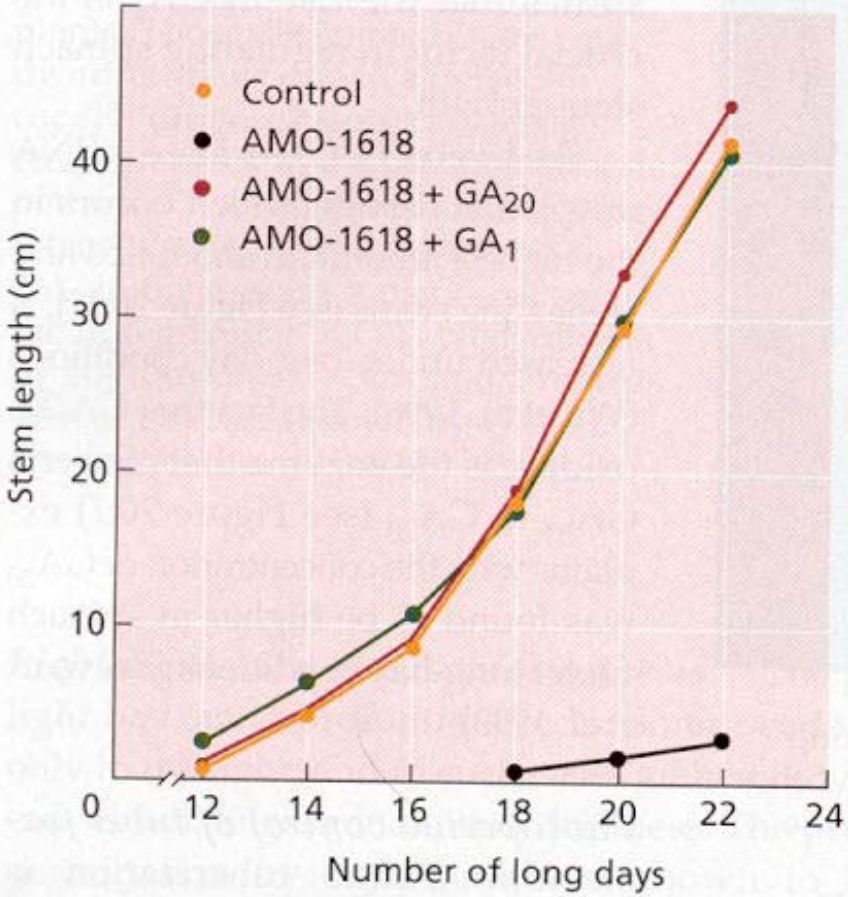


FIGURE 20.13 When a plant grows in the light, the rate of extension slows down through regulation by changes in hormone levels and sensitivity. (A) When dark-grown pea seedlings are transferred to light, GA_1 level drops rapidly because of metabolism of GA_1, but then increases to a higher level, similar to that of light-grown plants, over the next 4 days. (B) To investigate the GA_1 response in various light regimes, 10 mg of GA_1 was applied to the internode of GA-deficient *na* plants in darkness, 1 day after the start of the light, or 6 days of continuous light, and growth in the next 24 hours was measured. The results show that the gibberellin sensitivity of pea seedlings falls rapidly upon transfer from darkness to light, so the elongation rate of plants in the light is lower than in the dark, even though their total GA_1 content is higher. (After O'Neill et al. 2000.)

FIGURE 20.14 Spinach plants undergo stem and petiole elongation only in long days, remaining in a rosette form in short days. Treatment with the GA biosynthesis inhibitor AMO-1618 prevents stem and petiole elongation and maintains the rosette growth habit even under long days. Gibberellic acid can reverse the inhibitory effect of AMO-1618 on stem and petiole elongation. As shown in Figure 20.16, long days cause changes in the gibberellin content of the plant. (Courtesy of J. A. D. Zeevaart.)

(A) AMO-1618

AMO-1618, which blocks GA biosynthesis at the cyclization step, does not inhibit growth in the presence of either GA_{20} or GA_1.



(B) BX-112

In contrast, BX-112, which blocks the conversion of GA_{20} to GA_1, inhibits growth even in the presence of GA_{20}.

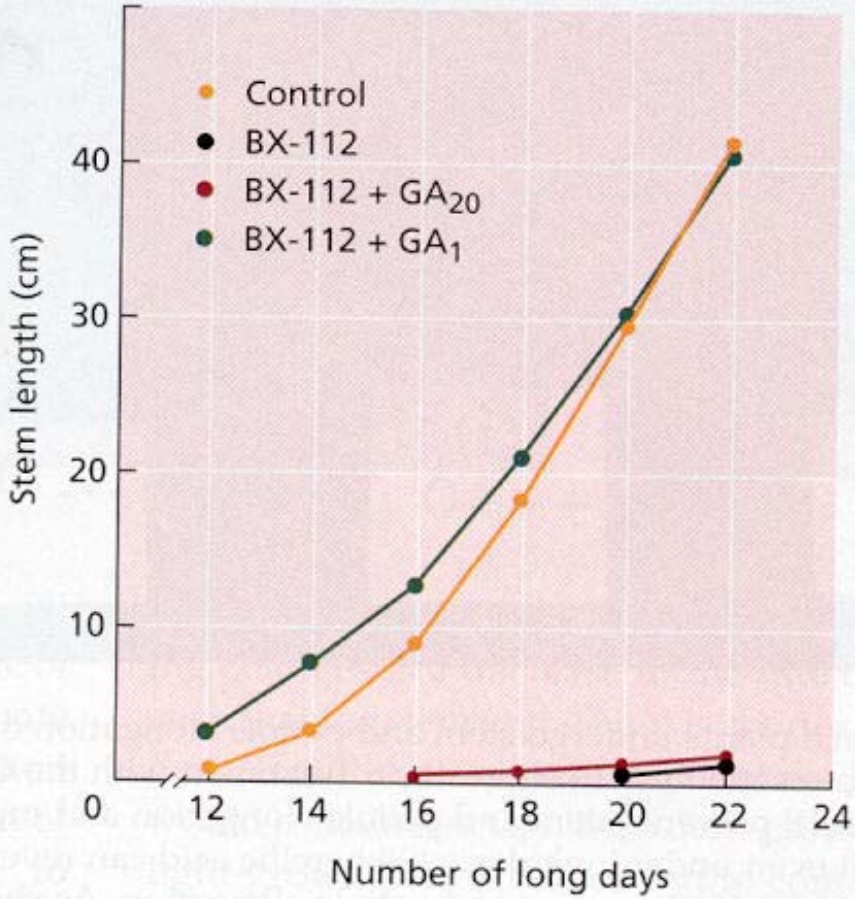


FIGURE 20.16 The use of specific growth retardants (GA biosynthesis inhibitors) and the reversal of the effects of the growth retardants by different GAs can show which steps in GA biosynthesis are regulated by environmental change, in this case the effect of long days on stem growth in spinach. The control lacks inhibitors or added GA. (After Zeevaart et al. 1993.)

FIGURE 20.17 Tuberization of potatoes is promoted by short days. Potato (*Solanum tuberosum* spp. *Andigena*) plants were grown under either long days or short days. The formation of tubers in short days is associated with a decline in GA_1 levels (see Chapter 24). (Courtesy of S. Jackson.)

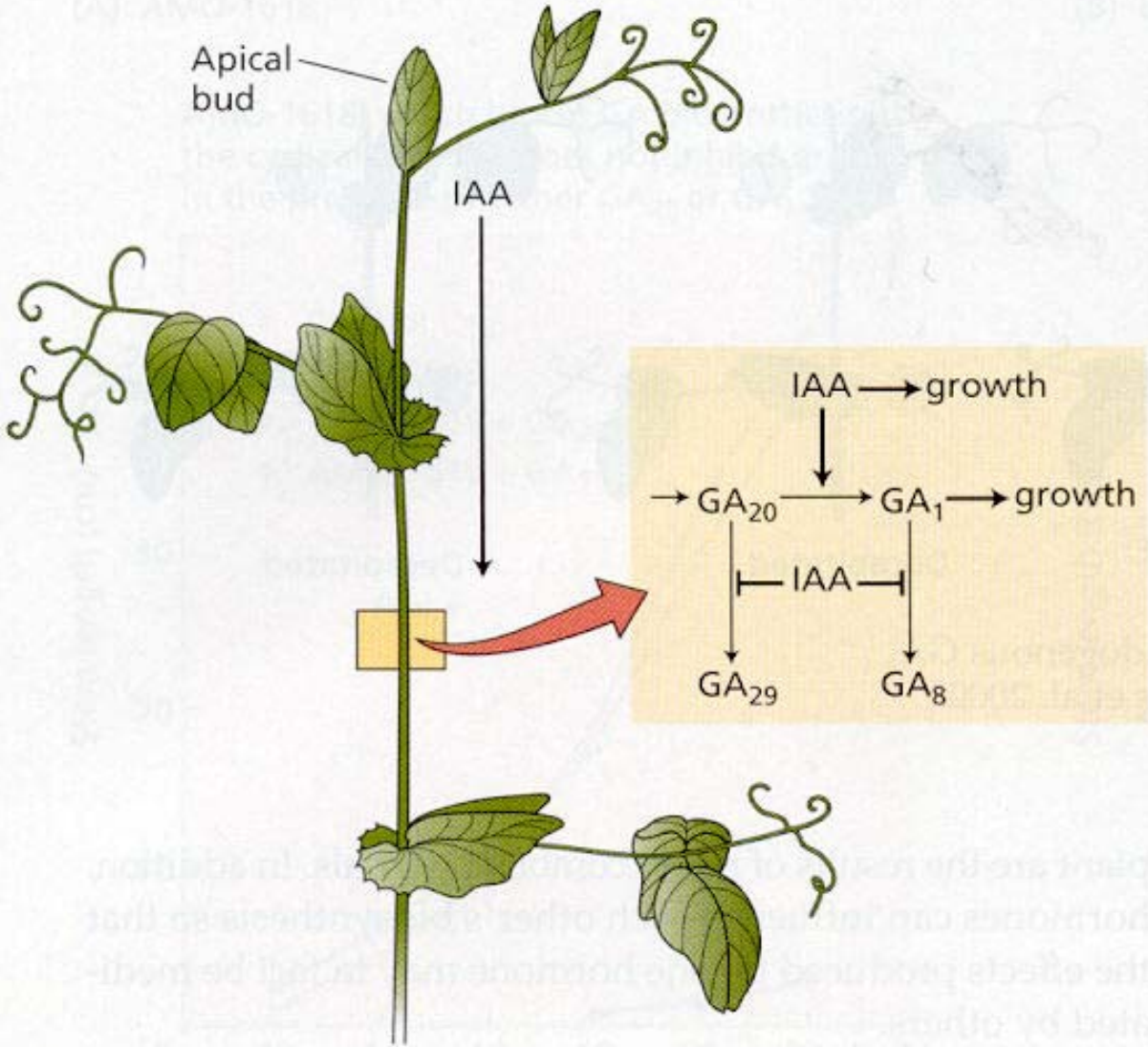


FIGURE 20.20 IAA (from the apical bud) promotes and is required for GA_1 biosynthesis in subtending internodes. IAA also inhibits GA_1 breakdown. (From Ross and O'Neill 2001.)

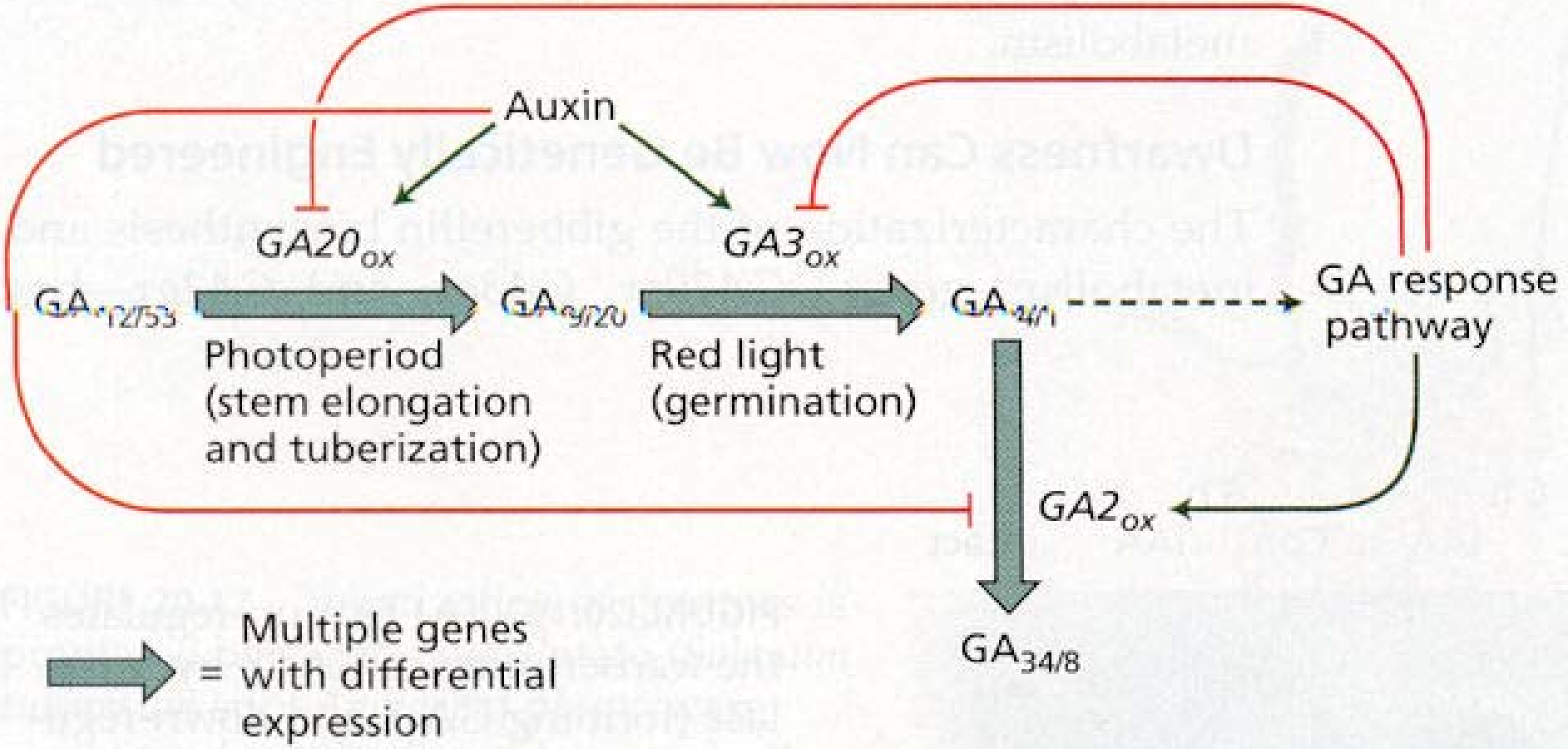


FIGURE 20.21 The pathway of gibberellin biosynthesis showing the identities of the genes for the metabolic enzymes and the way that their transcription is regulated by feedback, environment, and other endogenous hormones.

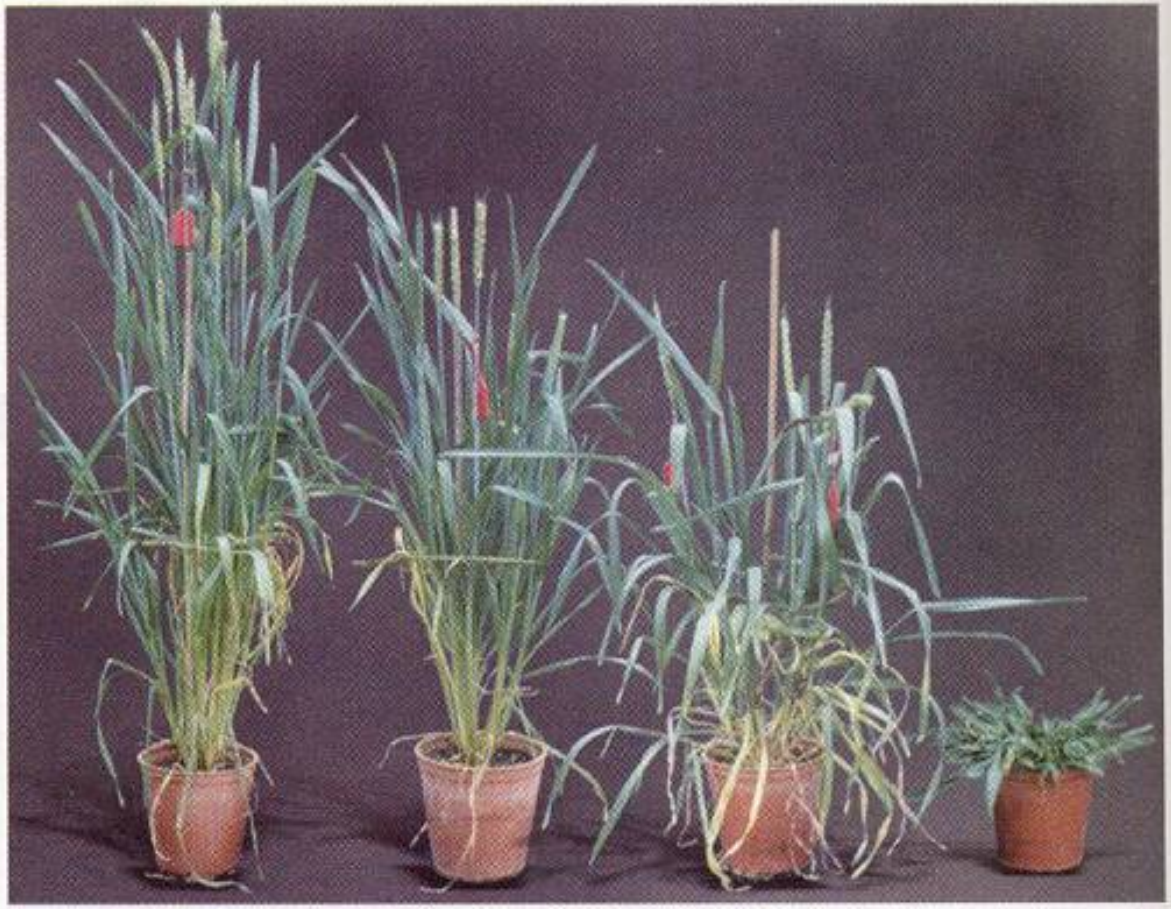


FIGURE 20.22 Genetically engineered dwarf wheat plants. The untransformed wheat is shown on the extreme left. The three plants on the right were transformed with a gibberellin 2-oxidase cDNA from bean under the control of a constitutive promoter, so that the endogenous active GA_1 was degraded. The varying degrees of dwarfing reflects varying degrees of overexpression of the foreign gene. (Photo from Hedden and Phillips 2000, courtesy of Andy Phillips.)

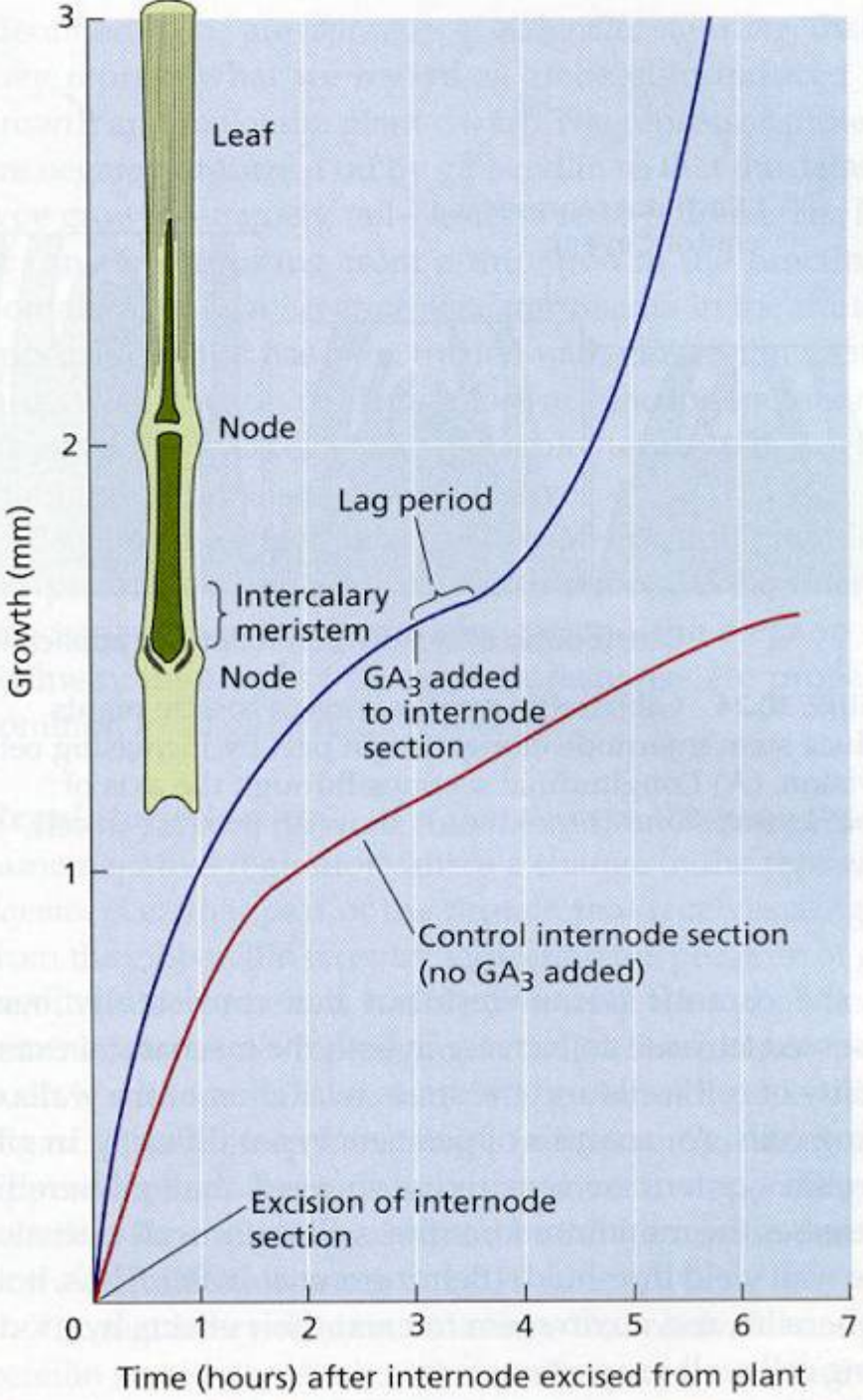


FIGURE 20.23 Continuous recording of the growth of the upper internode of deep-water rice in the presence or absence of exogenous GA_3. The control internode elongates at a constant rate after an initial growth burst during the first 2 hours after excision of the section. Addition of GA after 3 hours induced a sharp increase in the growth rate after a 40-minute lag period (upper curve). The difference in the initial growth rates of the two treatments is not significant here, but reflects slight variation in experimental materials. The inset shows the internode section of the rice stem used in the experiment. The intercalary meristem just above the node responds to GA. (After Sauter and Kende 1992.)

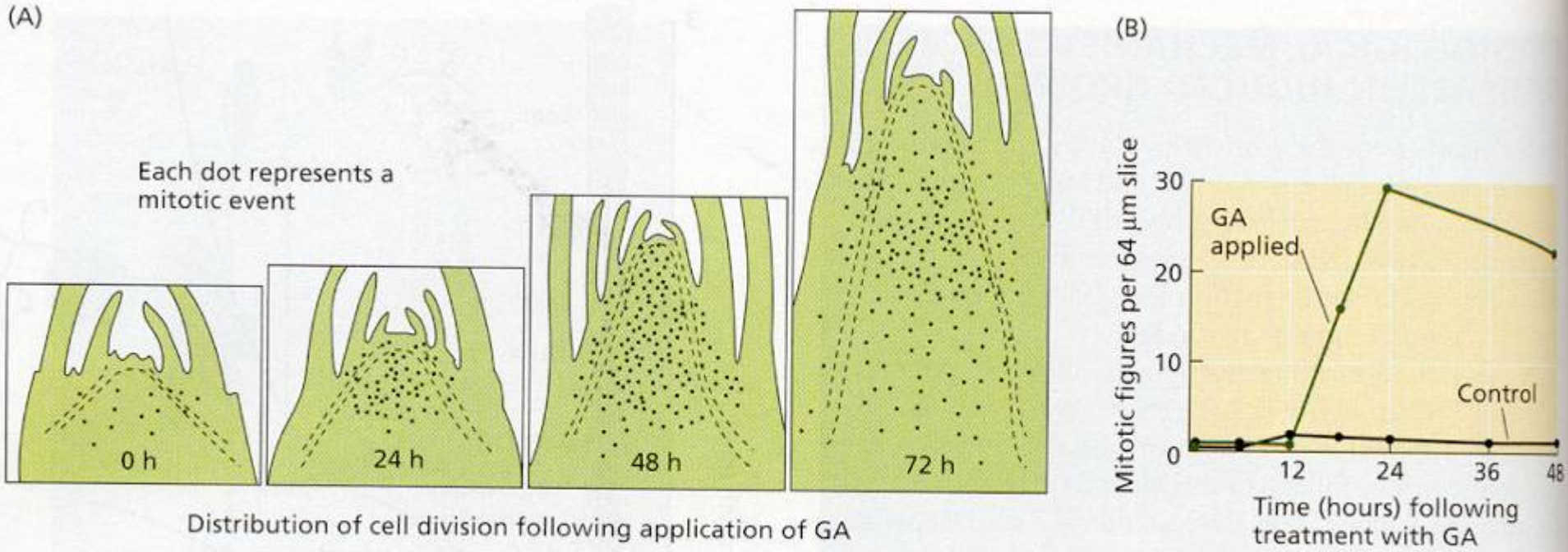


FIGURE 20.24 Gibberellin applications to rosette plants induce stem internode elongation in part by increasing cell division. (A) Longitudinal sections through the axis of *Samolus parviflorus* (brookweed) show an increase in cell division after application of GA. (Each dot represents one mitotic figure in a section 64 µm thick.) (B) The number of such mitotic figures with and without GA in stem apices of *Hyoscyamus niger* (black henbane). (After Sachs 1965.)

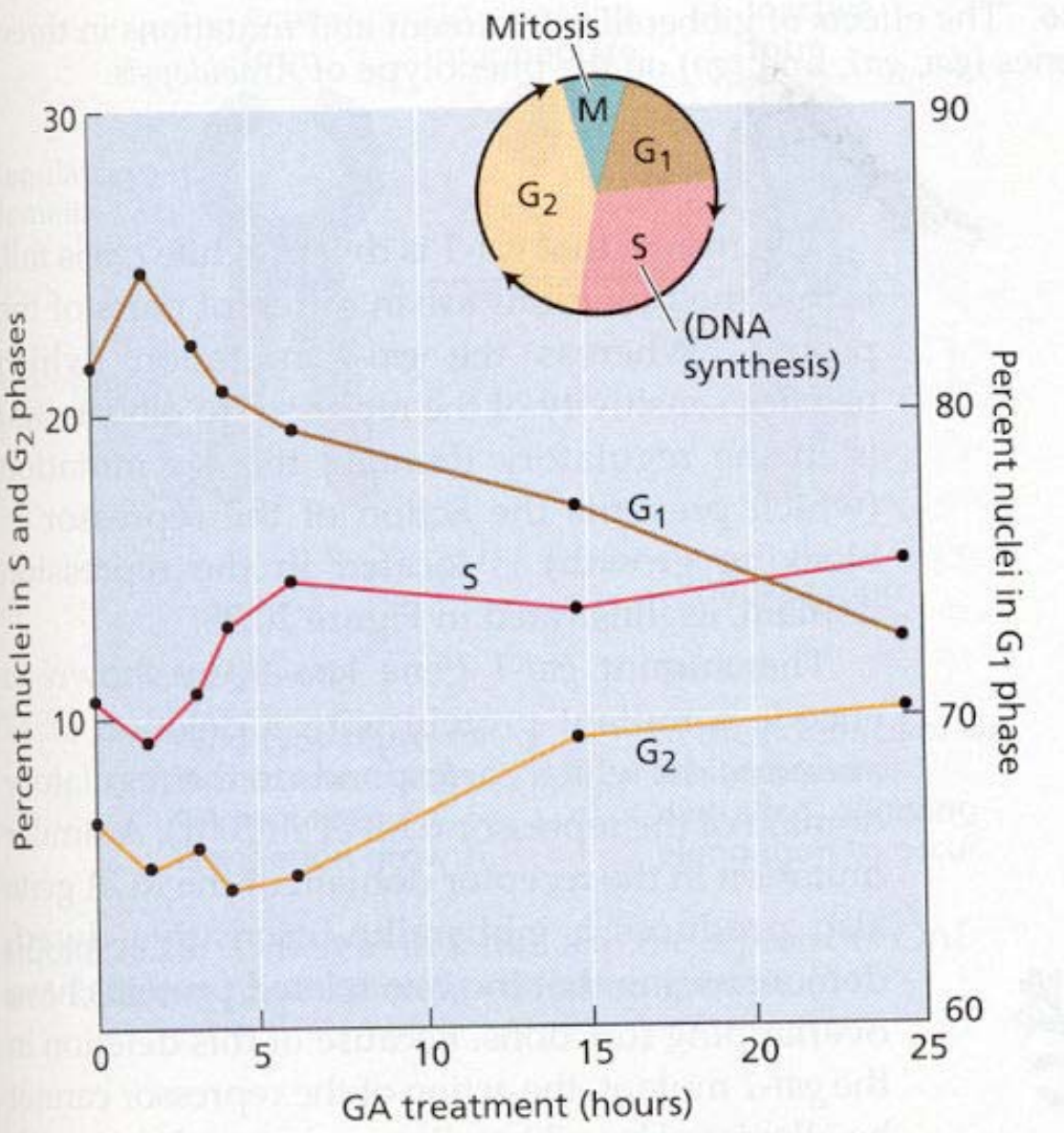
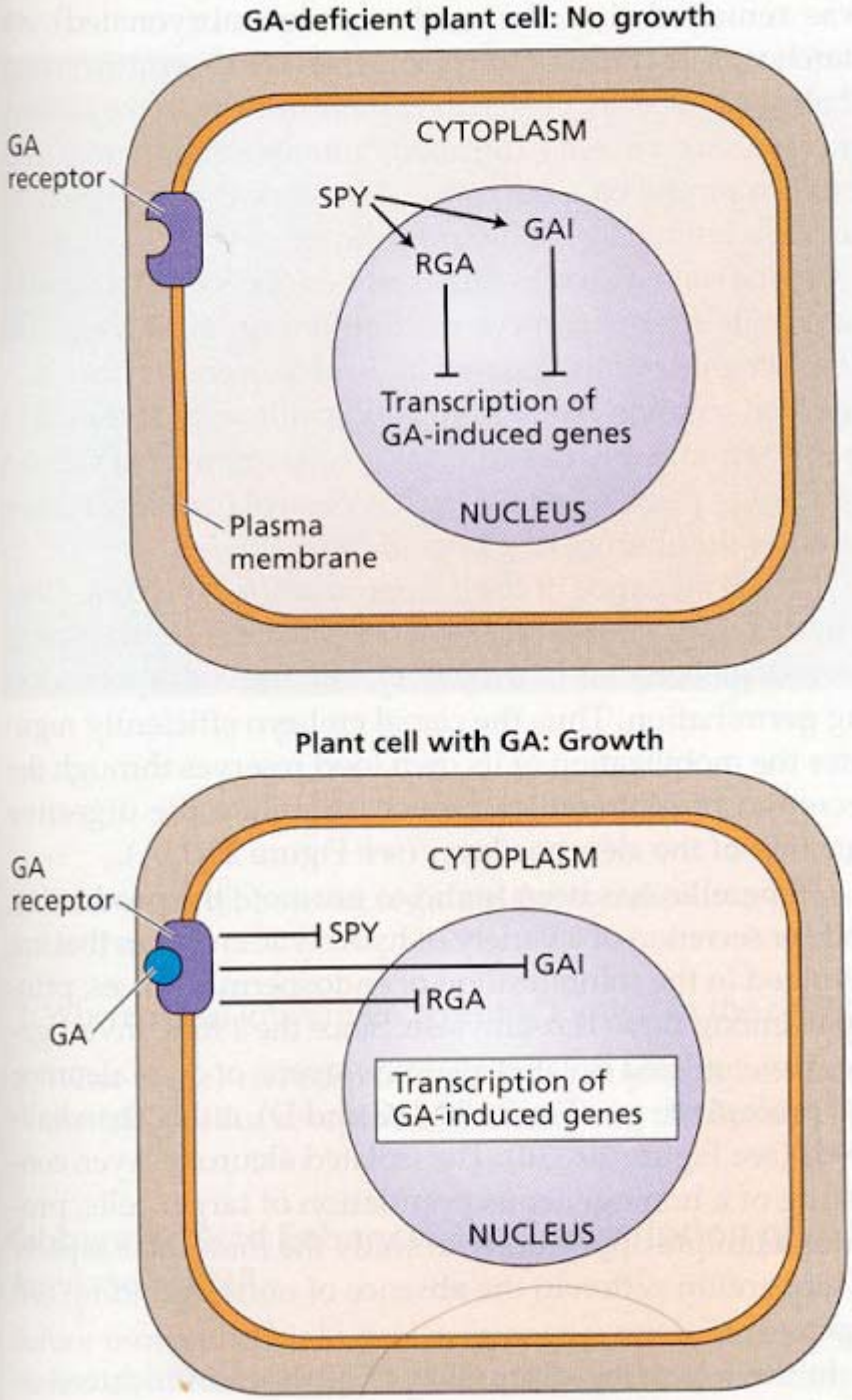


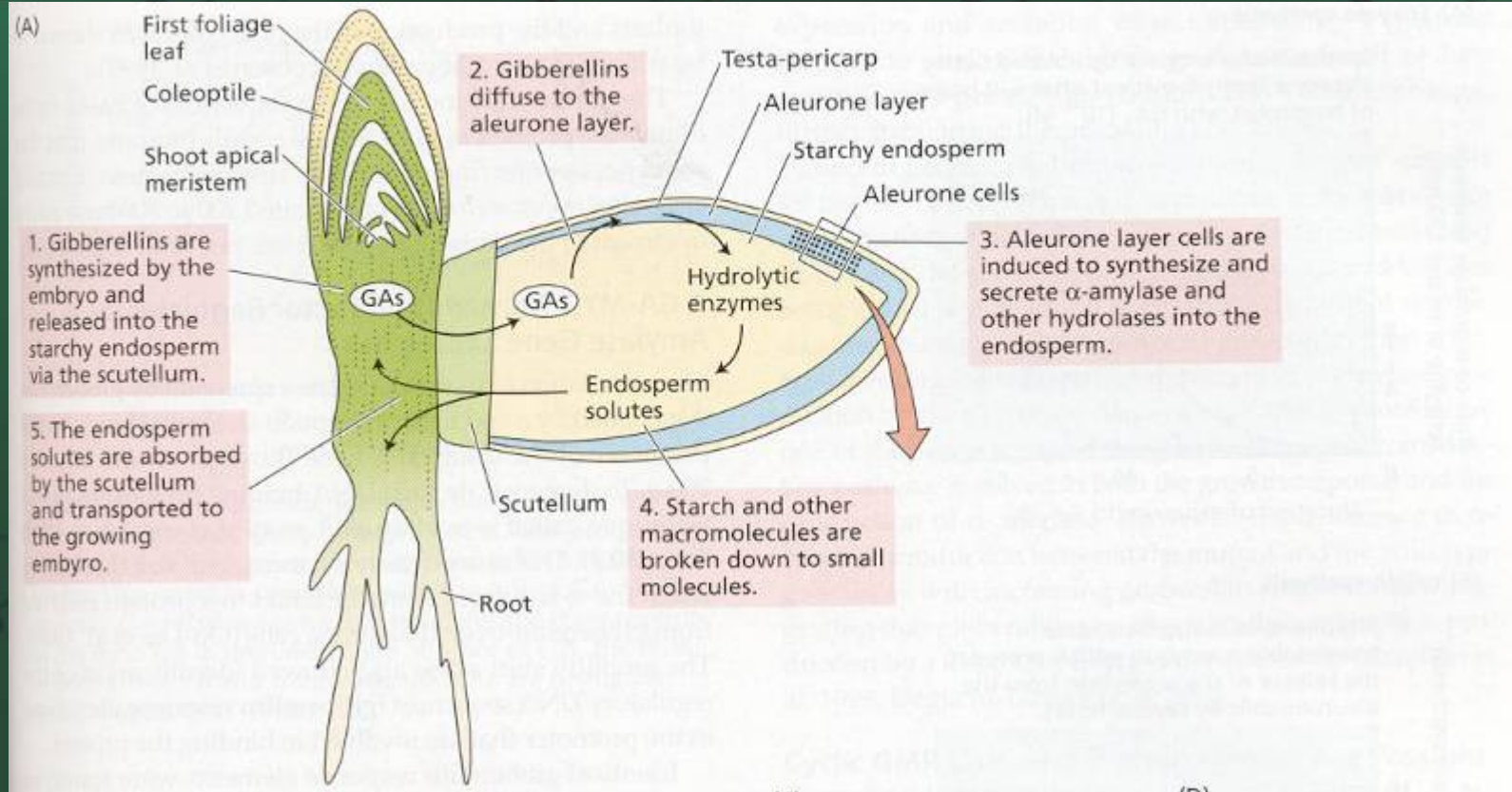
FIGURE 20.25 Changes in the cell cycle status of nuclei from the intercalary meristems of deep-water rice internodes treated with GA_3. Note that the scale for the G_1 nuclei is on the right side of the graph. (After Sauter and Kende 1992.)

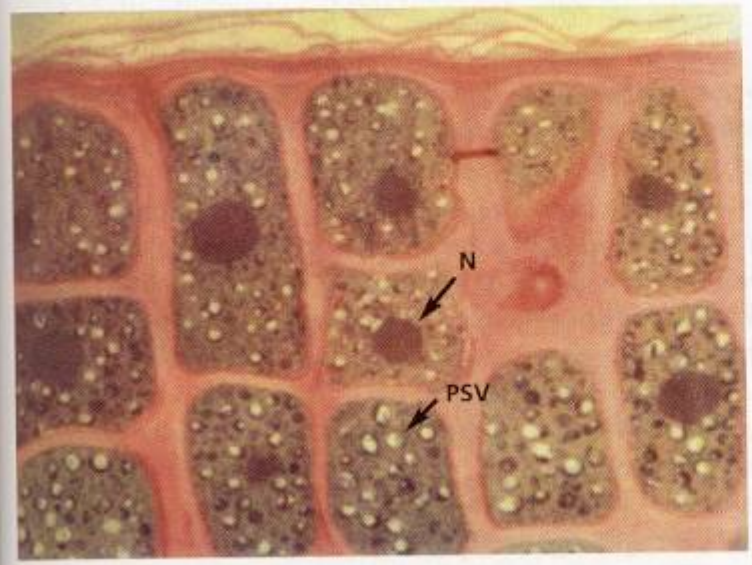


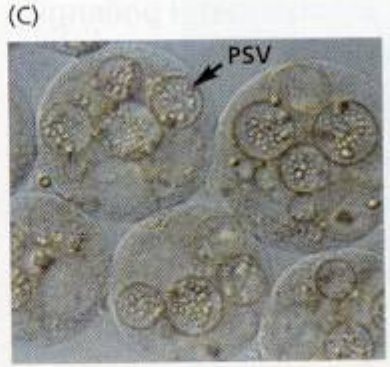
In a GA-deficient cell in a GA biosynthesis mutant, or a wild-type cell without the GA signal, the transmembrane GA receptor is inactive in the absence of GA signal. In this situation, SPY is an active O-GlcNAc transferase that catalyzes the addition of a signal GlcNAc residue (from UDP-GlcNAc) via an O linkage to specific serine and/or threonine residues of target proteins, possibly RGA and GAI. Active RGA and GAI function as repressors of transcription, and they indirectly or directly inhibit the expression of GA-induced genes.

In the presence of GA the GA receptor is activated by binding of bioactive GA. The GA signal inhibits RGA and GAI repressors both directly and by deactivating SPY. In the absence of repression by RGA and GAI, GA-induced genes are transcribed.

FIGURE 20.32 Proposed roles of the active SPY, GAI, and RGA proteins in the GA signaling pathway within a plant cell.







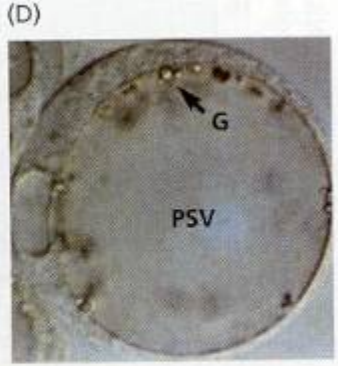


FIGURE 20.33 Structure of a barley grain and the functions of various tissues during germination (A). Microscope photos of the barley aleurone layer (B) and barley aleurone protoplasts at an early (C) and late stage (D) of amylase production. Protein storage vesicles (PSV) can be seen in each cell. G = phytin globoid; N = nucleus. (Photos from Bethke et al. 1997, courtesy of P. Bethke.)

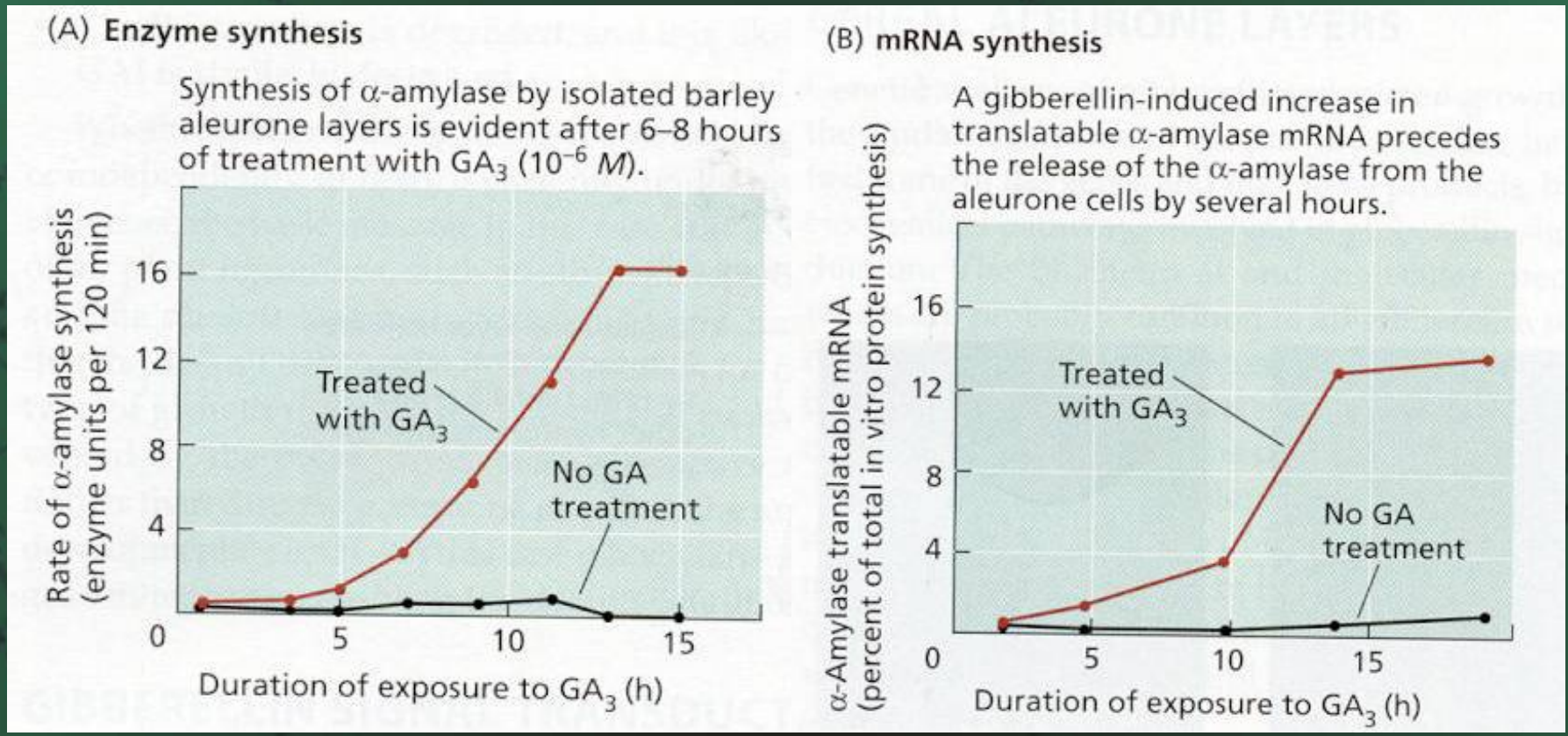


FIGURE 20.34 Gibberellin effects on enzyme synthesis and mRNA synthesis. The α-amylase mRNA in this case was measured by the in vitro production of α-amylase as a percentage of the protein produced by the translation of the bulk mRNA. (From Higgins et al. 1976.)

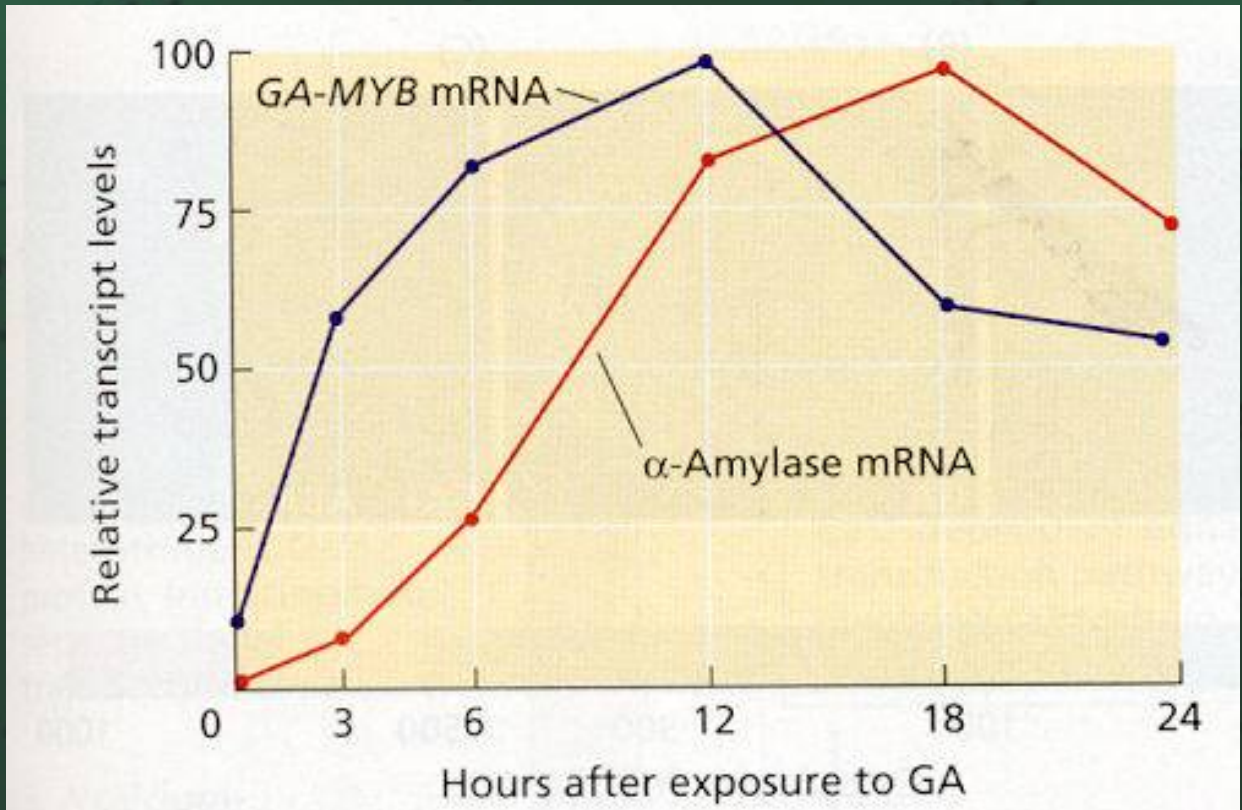


FIGURE 20.35 Time course for the induction of *GA-MYB* and α-amylase mRNA by gibberellic acid. The production of *GA-MYB* mRNA precedes α-amylase mRNA by about 5 hours. This result is consistent with the role of *GA-MYB* as an early *GA* response gene that regulates the transcription of the gene for α-amylase. In the absence of GA, the levels of both *GA-MYB* and α-amylase mRNAs are negligible. (After Gubler et al. 1995.)

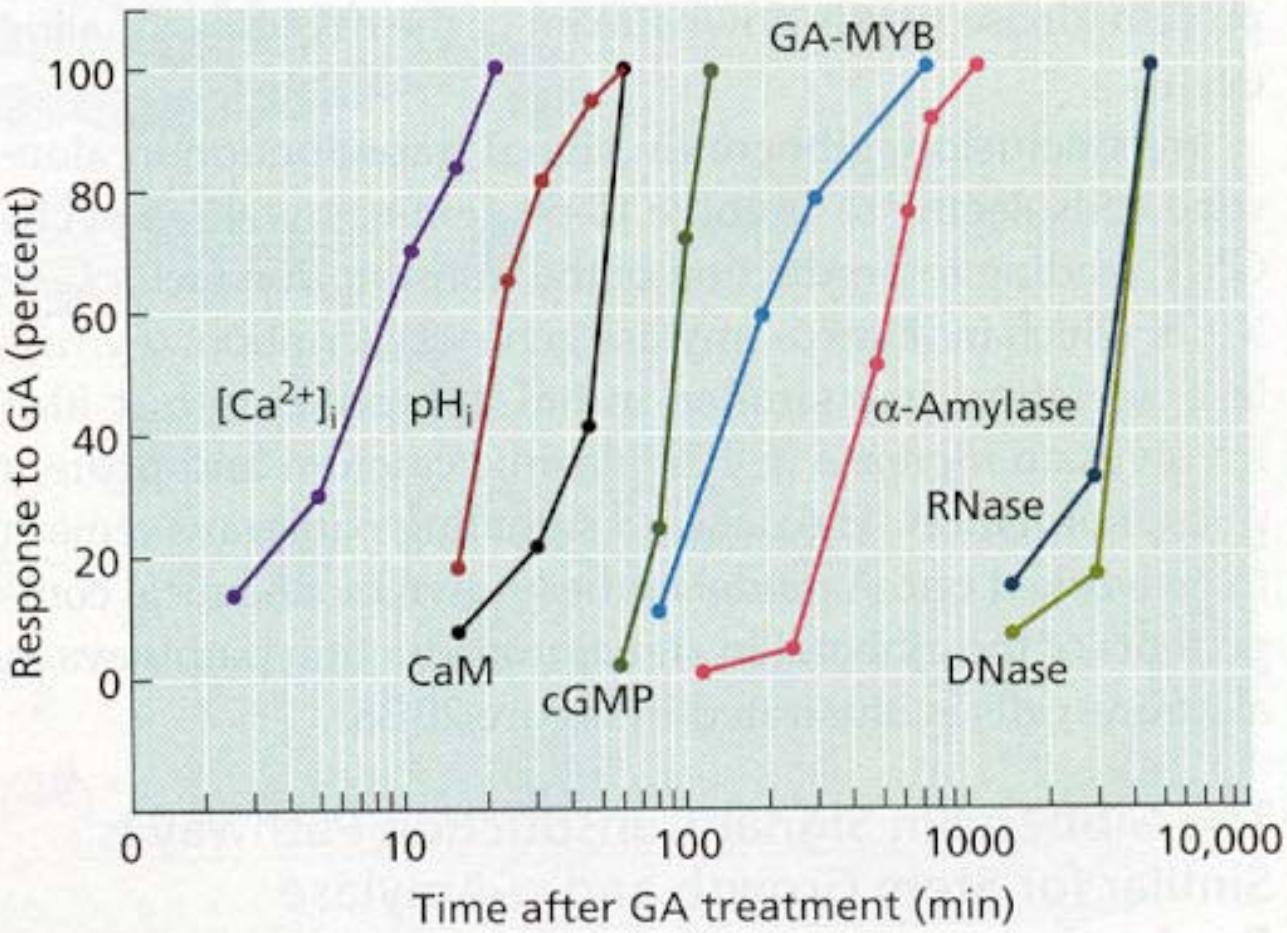


FIGURE 20.36 Following the addition of GA to barley aleurone protoplasts, a multiple signal transduction pathway is initiated. The timing of some of these events is shown. (From Bethke et al. 1997.)

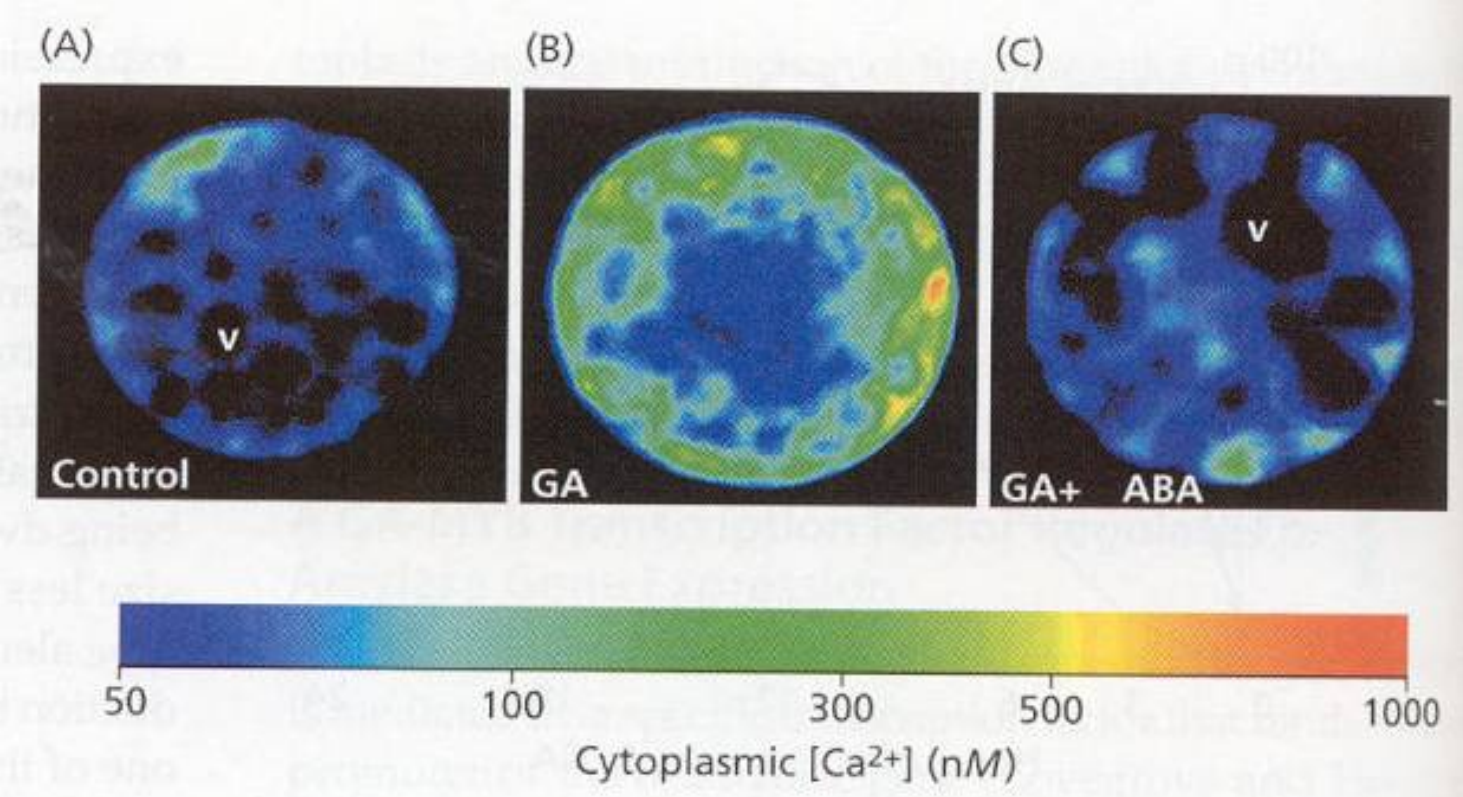
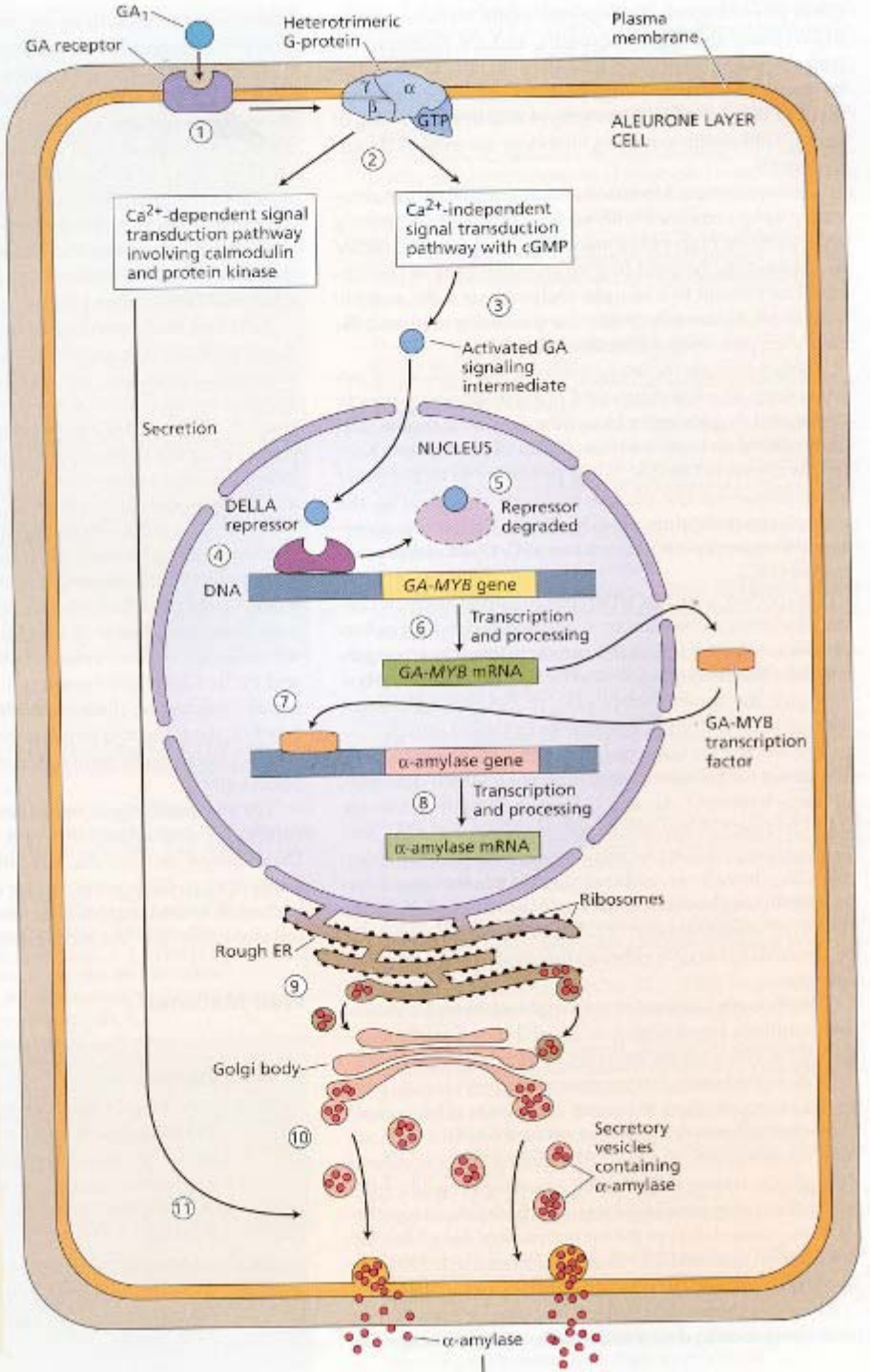


FIGURE 20.37 Increase in the calcium in barley aleurone protoplasts following GA addition can be seen from this false color image. The level of calcium corresponding to the colors is in the lower scale. (A) Untreated protoplast. (B) GA-treated protoplast. (C) Protoplast treated with both abscisic acid (AB) and GA. Abscisic acid opposes the effects of GA in aleurone cells. (From Ritchie and Gilroy 1998b.)



1. GA_1 from the embryo first binds to a cell surface receptor.

2. The cell surface GA receptor complex interacts with a heterotrimeric G-protein, initiating two separate signal transduction chains.

3. A calcium-independent pathway, involving cGMP, results in the activation of a signaling intermediate.

4. The activated signaling intermediate binds to DELLA repressor proteins in the nucleus.

5. The DELLA repressors are degraded when bound to the GA signal.

6. The inactivation of the DELLA repressors allows the expression of the *MYB* gene, as well as other genes, to proceed through transcription, processing, and translation.

7. The newly synthesized MYB protein then enters the nucleus and binds to the promoter genes for α-amylase and other hydrolytic enzymes.

8. Transcription of α-amylase and other hydrolytic genes is activated.

9. α-Amylase and other hydrolases are synthesized on the rough ER.

10. Proteins are secreted via the Golgi.

11. The secretory pathway requires GA stimulation via a calcium–calmodulin-dependent signal transduction pathway.