

# *Gibberellins: Regulators of Plant Height*

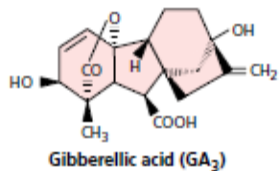
# Discovery:



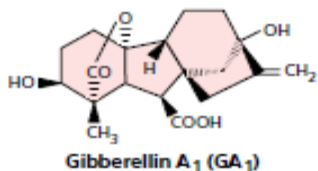
Foolish seedling disease was known in Japan since 1800.

1930 Yabuta and Hayashi purified the active compound.

## Bakanae Disease of Rice



In mid-1950s did two groups—one at the Imperial Chemical Industries (ICI) research station at Welwyn in Britain, the other at the U.S. Department of Agriculture (USDA) in Peoria, Illinois—succeed in elucidating the structure of the material that they had purified from fungal culture filtrates, which they named *gibberellic acid*.



In 1958 a gibberellin (gibberellin  $A_1$ ) was conclusively identified from a higher plant (runner bean seeds, *Phaseolus coccineus*) by MacMillan and Sutler

# EFFECTS OF GIBBERELLIN ON GROWTH AND DEVELOPMENT

## Gibberellins Stimulate Stem Growth in Dwarf and Rosette Plants



*Many long-day rosette plants have a cold requirement for stem elongation and flowering, and this requirement is overcome by applied gibberellin. GA also promotes internodal elongation in members of the grass family. The target of gibberellin action is the **intercalary meristem—a meristem near the base of the internode** that produces derivatives above and below.*



## **Gibberellins Regulate the Transition from Juvenile to Adult Phases**

*In English ivy GA3 can cause a reversion from a mature to a juvenile state, and many juvenile conifers can be induced to enter the reproductive phase by applications of nonpolar gibberellins such as GA4 + GA7.*

## **Gibberellins Influence Floral Initiation and Sex Determination**

In maize, for example, the staminate flowers (male) are restricted to the tassel, and the pistillate flowers (female) are contained in the ear. Exposure to short days and cool nights increases the endogenous gibberellin levels in the tassels 100-fold and simultaneously causes feminization of the tassel flowers. Application of exogenous gibberellic acid to the tassels can also induce pistillate flowers. The primary role of gibberellin in sex determination in maize seems to be to suppress stamen development.

In dicots such as cucumber, hemp, and spinach, gibberellin seems to have the opposite effect. In these species, application of gibberellin promotes the formation of staminate flowers, and inhibitors of gibberellin biosynthesis promote the formation of pistillate flowers.

## **Gibberellins Promote Fruit Set**

Applications of gibberellins can cause *fruit set* (*the initiation* of fruit growth following pollination) and growth of some fruits, in cases where auxin may have no effect.

For example, stimulation of fruit set by gibberellin has been observed in apple .

## **Gibberellins Promote Seed Germination**

- Promotion of vegetative growth of embryo

- Mobilization of storage material

- Substitute cold and light.

## Gibberellins Have Commercial Applications

*Fruit production*

*Malting of barley*

*Increasing sugarcane yields*

*Uses in plant breeding*

*Gibberellin biosynthesis inhibitors.*

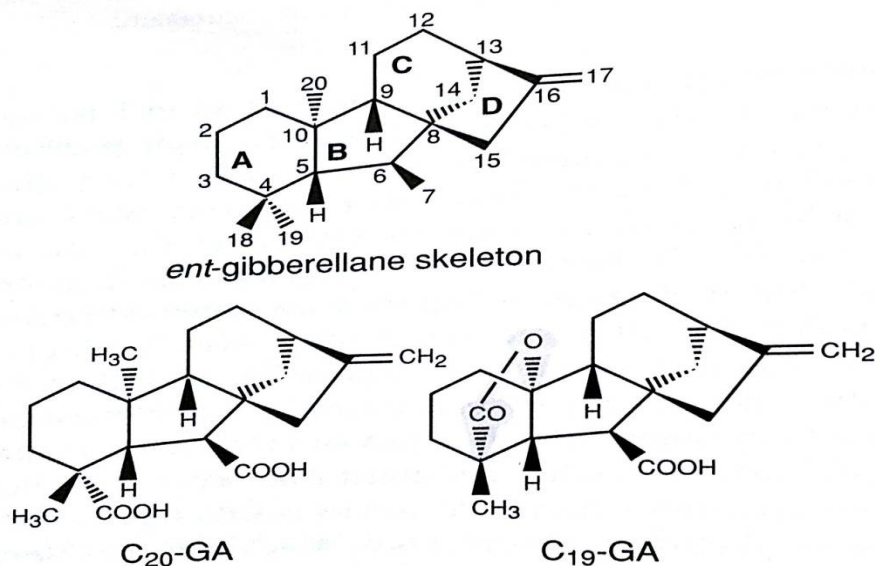


# GIBBERELLINS ARE A FAMILY OF COMPOUNDS

Gibberellins are defined by their structure rather than their biological activity. They are all cyclic diterpenes with an ent-gibberellane ring.

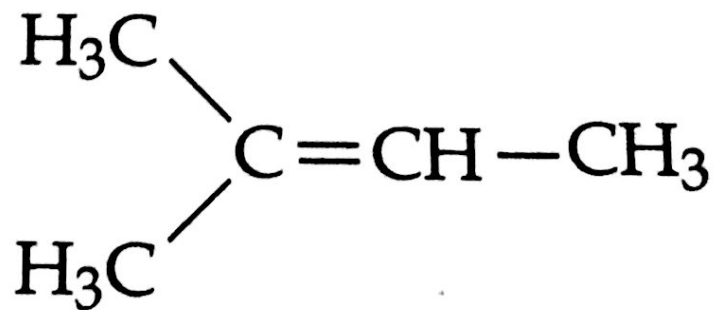
GAs occurs widely in the plant kingdom- angiosperms, gymnosperms, ferns fungi.

125 GAs are known and numbers are still increasing.



**FIGURE 7-2** *ent*-Gibberellane skeleton and structures of C<sub>20</sub>- and

GA 19 compounds are biological active in higher plants.



*Terpenes are characterized by their basic structural unit, the five carbon isoprene unit.*

*Monoterpenes*

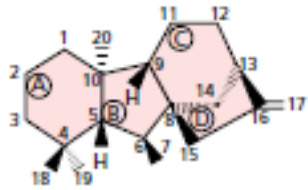
*Sesquiterpenes*

*Diterpenes*

*Triterpenes*

*Tetraterpenes and Polyterpenes.*

Terpenes or terpenoids have major role in plant defence against insect and herbivores.  
(Secondary metabolites)



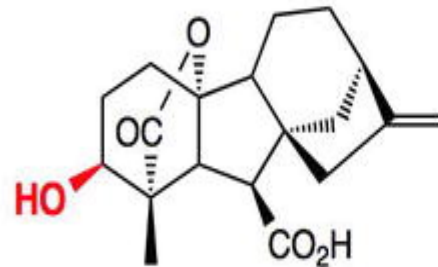
**ent-Gibberellane structure**

All gibberellins are based on the *ent-gibberellane skeleton*:

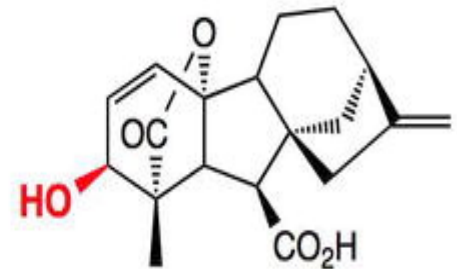
As more and more gibberellins from fungal and plant sources were characterized, they were numbered as gibberellin AX (or GAX), where *X is a number, in the order of their discovery*. This scheme was adopted for all gibberellins in 1968. The scheme was established by MacMillan and Takahashi.

However, the number of a gibberellin is simply a cataloging convenience, designed to prevent chaos in the naming of the gibberellins. The system implies no close chemical similarity or metabolic relationship between gibberellins with adjacent numbers.

GA<sub>4</sub> is the major active  
GA in Arabidopsis

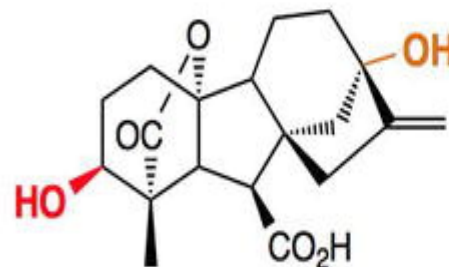


GA<sub>4</sub>

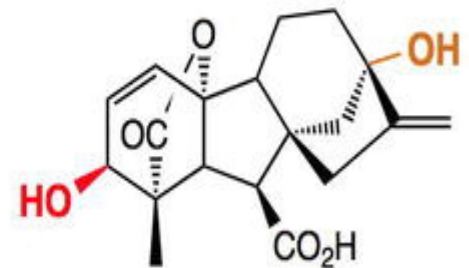


GA<sub>7</sub>

Only some GAs are  
biologically active. The  
major bioactive gibberellins  
are shown here.



GA<sub>1</sub>



GA<sub>3</sub>

## WHY THERE ARE SO MANY GAS?

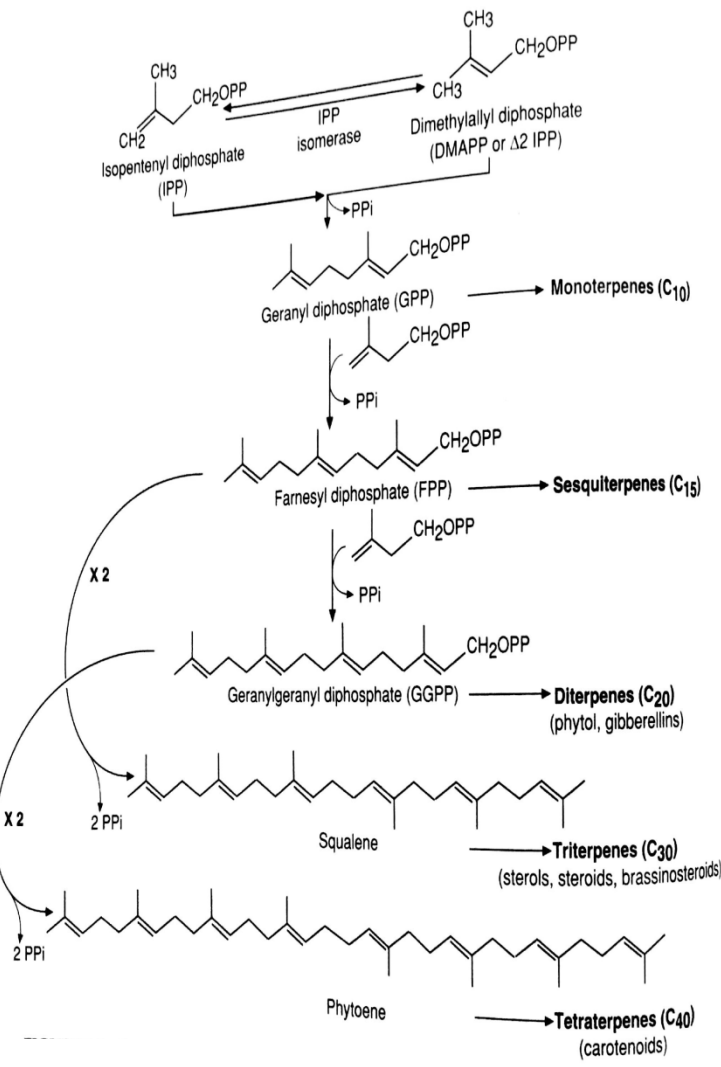
- Only a few GAs are biologically active; others are either intermediates in biosynthesis or inactive metabolites.
- Allows many regulatory points in biosynthesis.
- Taxonomic preference.
- Different GAs regulate different processes.

**Biological Activities of 3,13-Hydroxylated GAs ( $GA_1$ ,  $GA_3$ ) vs 3-Hydroxylated GAs ( $GA_4$ ,  $GA_7$ ) in Four Bioassays<sup>a</sup>**

GA	Barley aleurone induction of $\alpha$ -amylase	Elongation response		
		Dwarf pea epicotyl	Cucumber hypocotyl	Arabidopsis hypocotyl
$GA_1$	****	***	**	***
$GA_3$	****	****	**	—
$GA_4$	***	***	***	****
$GA_7$	***	***	****	—
$GA_8$	*	*	0	0

<sup>a</sup>Comparative data for  $GA_8$ , a 2 $\beta$ -hydroxylated GA, are given also. The greater the number of asterisks, the greater the activity; — not determined. Data from Crozier and Durley (1983), except for *Arabidopsis*, which are from Zee-vart and Talon (1992).

# Biosynthesis of GAs



Isopentenyl diphosphate (IPP) is the five carbon activated building block of all terpenes.

IPP synthesized by Melvonic acid pathways or independent from it.

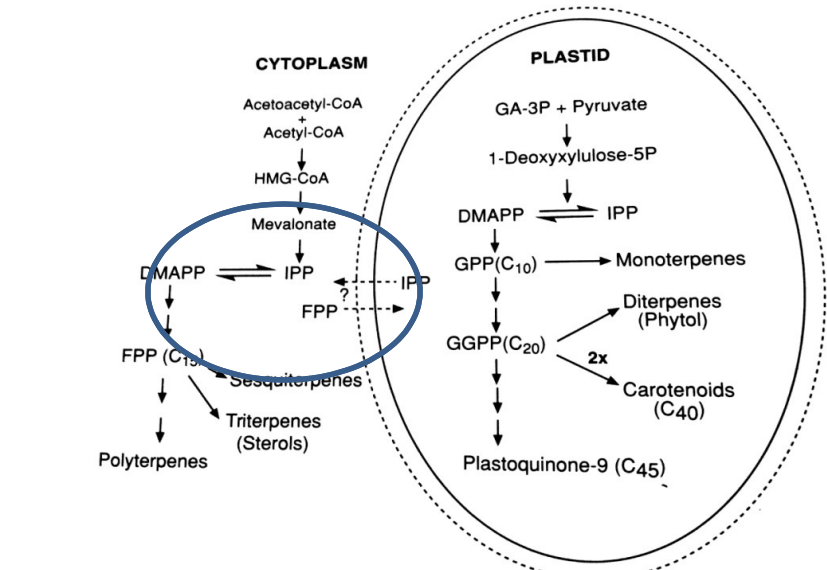
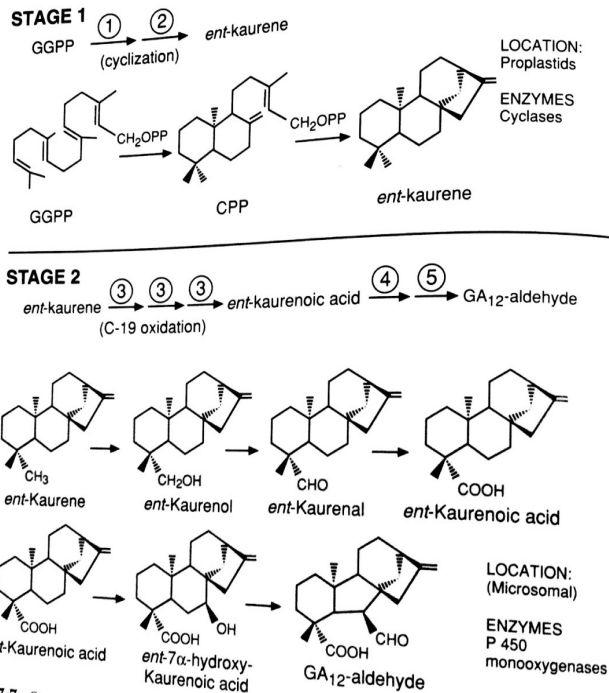


FIGURE 7.6 Two independent pathways for the synthesis of isopentenyl diphosphate (IPP). The cytoplasmic pathway starts with the condensation of acetoacetyl-CoA and acetyl-CoA (HMG-CoA). HMG-CoA reductase is a highly conserved enzyme.

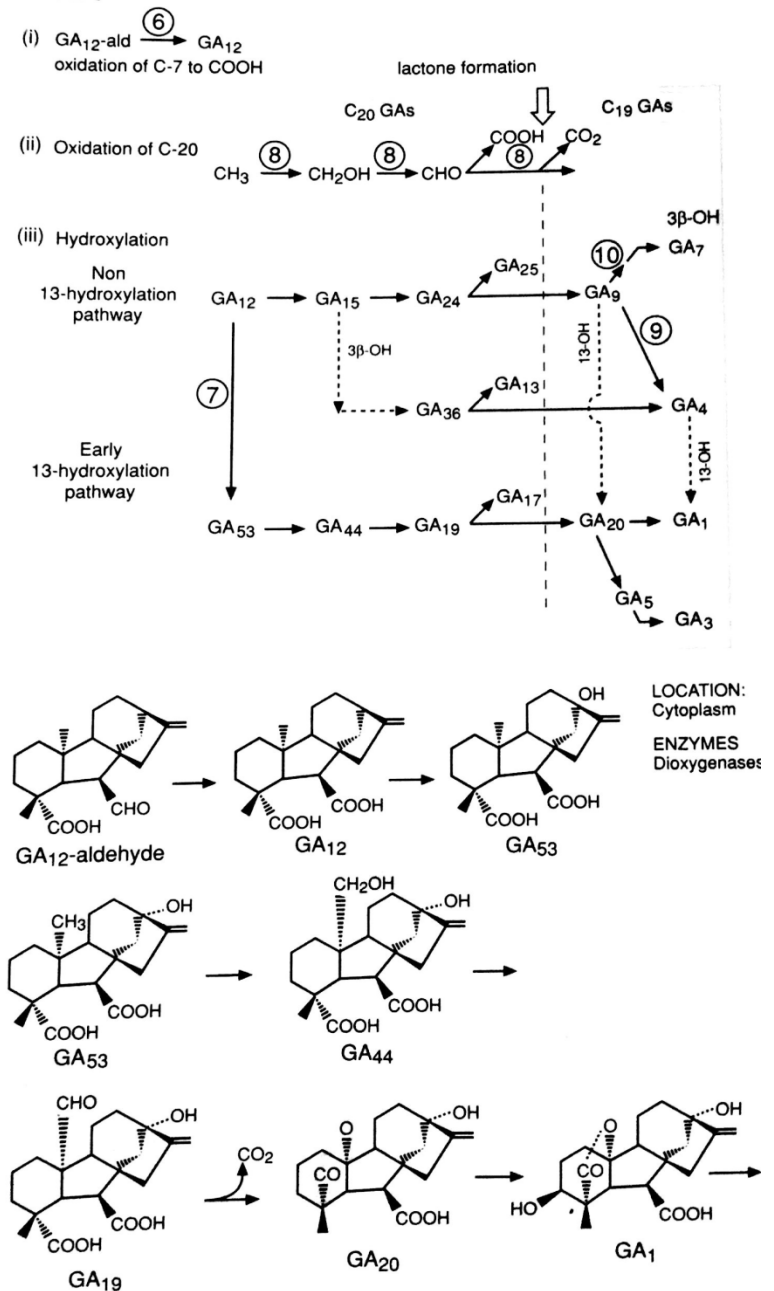
## GA Biosynthesis



**FIGURE 7-7** Stages in GA biosynthesis. Stage 1: Cyclization of GGPP via a series of oxidations converting

First and second stage from GGPP to GA12 formation are common in all plants investigated, After GA12 aldehyde there are variation, some of which are species or taxon specific.

### STAGE 3



## Enzymes of GA biosynthesis:

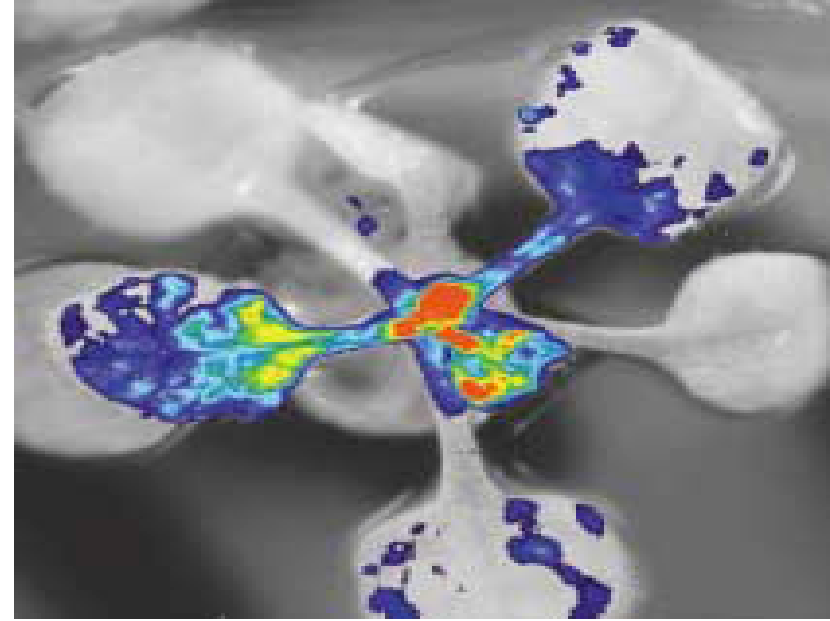
1. Copalyl diphosphate synthase
2. ent-kaurene synthase
3. ent-kaurene oxidase
4. ent kaurenoic acid hydroxylase
5. GA12- aldehyde synthase
6. C-7 oxidases
7. 13-hydroxylases
8. GA20 oxidases
9. 3 $\beta$ -hydroxylase
10.  $\Delta$ 2,3 desaturases

## Gibberellins Are Biosynthesized in Apical Tissues

In *Arabidopsis*, *GA20ox* is expressed primarily in the apical bud and young leaves, which thus appear to be the principal sites of gibberellin Synthesis.

The gibberellins that are synthesized in the shoot can be transported to the rest of the plant via the phloem. Intermediates of gibberellin biosynthesis may also be translocated in the phloem. Indeed, the initial steps of gibberellin biosynthesis may occur in one tissue, and metabolism to active gibberellins in another tissue.

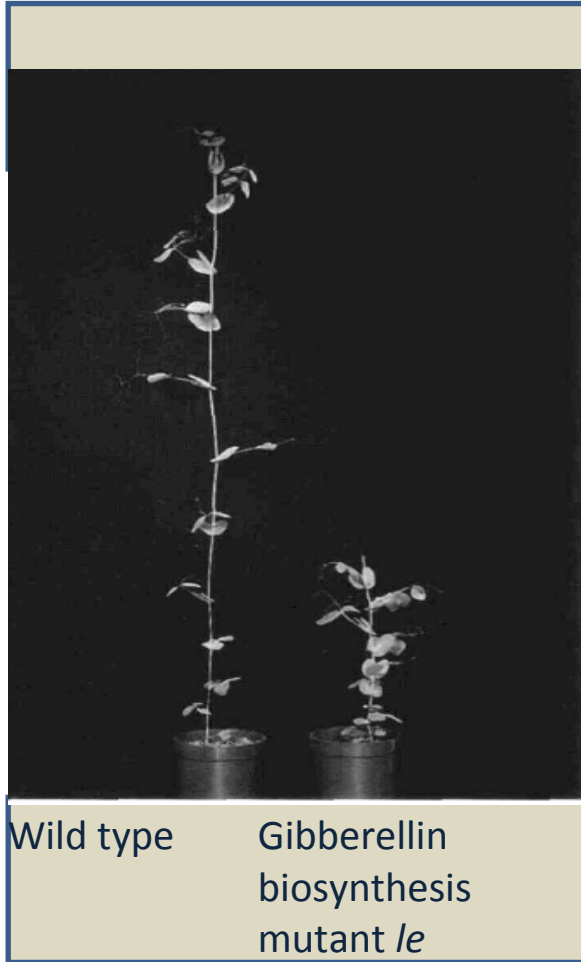
Gibberellins also have been identified in root exudates and root extracts, suggesting that roots can also synthesize gibberellins and transport them to the shoot via the xylem.



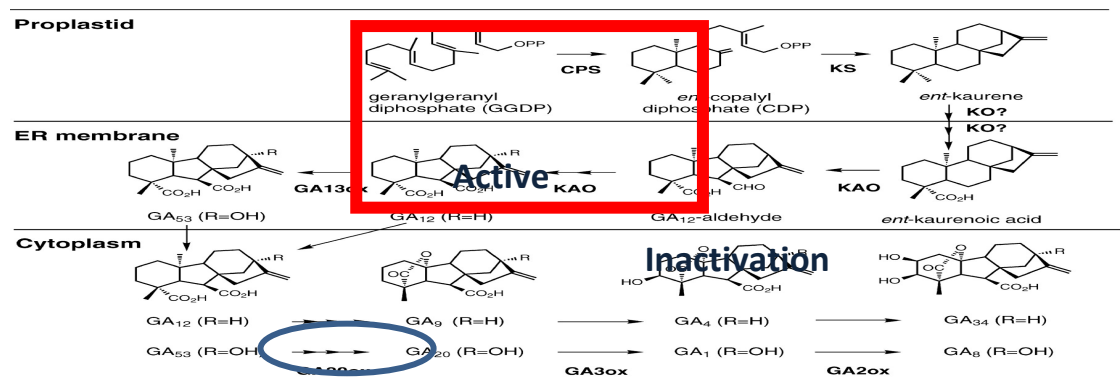
*Gibberellin is synthesized mainly in the shoot apex and in young developing leaves. This false color image shows light emitted by transgenic Arabidopsis plants expressing the firefly luciferase coding sequence coupled to the GA20ox gene promoter. The emitted light was recorded by a CCD camera after 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.*

## GA1 Is the Biologically Active Gibberellin Controlling Stem Growth

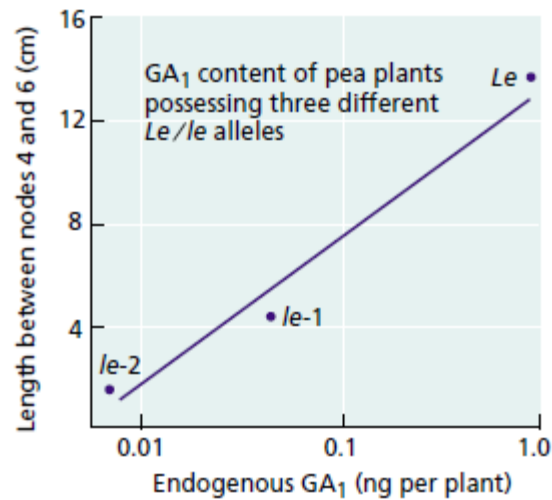
The pea mutant *le*, studied by Mendel, encodes enzyme which produces active GA. Loss of function of *le* reduces active GA levels and makes plants dwarfed



*Le* gene enables the plants to convert GA<sub>20</sub> to GA<sub>1</sub>. Metabolic studies using both stable and radioactive isotopes demonstrated conclusively that the *Le gene encodes an enzyme that* 3β-hydroxylates GA<sub>20</sub> to produce GA<sub>1</sub> .



# Endogenous GA1 Levels Are Correlated with Tallness



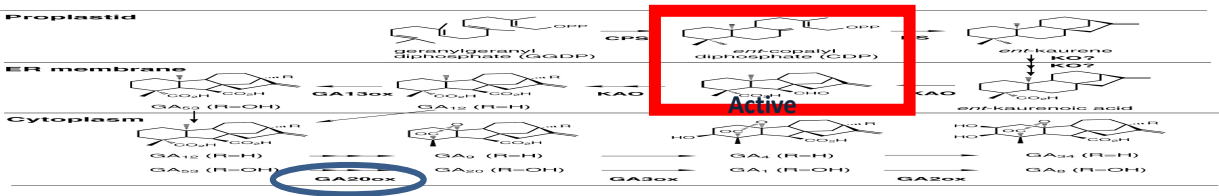
There is also an extreme dwarf mutant of pea that has even fewer gibberellins. This dwarf has the allele *na* (the wild-type allele is *Na*), which completely blocks gibberellin biosynthesis between *ent-kaurene* and *GA12-aldehyde*. As a result, homozygous (*nana*) mutants, which are almost completely free of gibberellins, achieve a stature of only about 1 cm at maturity.





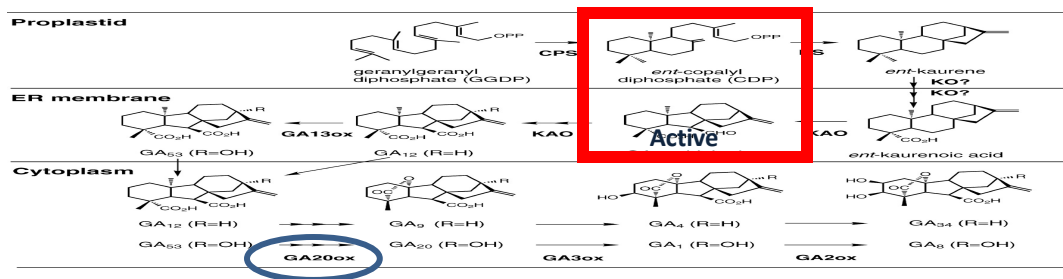
Tremendous increases in crop yields (the Green Revolution) during the 20<sup>th</sup> century occurred because of increased use of fertilizer and the introduction of semidwarf varieties of grains.

The semidwarf varieties put more energy into seed production than stem growth, and are sturdier and less likely to fall over.



Semidwarf rice varieties under produce GA because of a mutation in the GA20 oxidase biosynthetic gene.





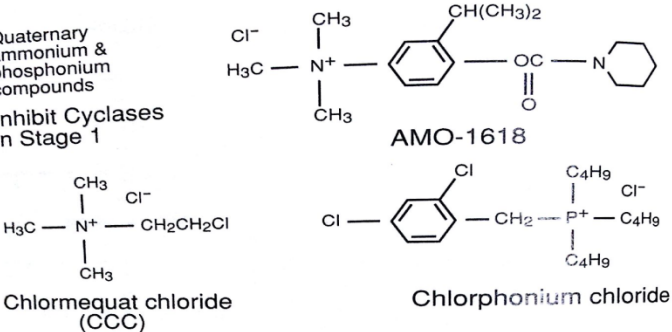
Semidwarf rice varieties under produce GA because of a mutation in a the GA20 oxidase biosynthetic gene.

Wild-type      Semidwarf

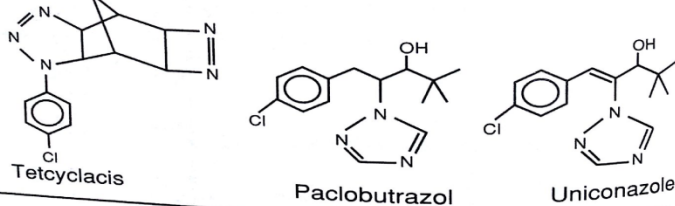


# Growth retardants inhibiting GA synthesis.

Quaternary ammonium & phosphonium compounds  
Inhibit Cyclases in Stage 1



N-heterocyclicals  
Inhibit P450 Monooxygenases in Stage 2



Acylcyclohexanediones  
Inhibit Dioxygenases in Stage 3

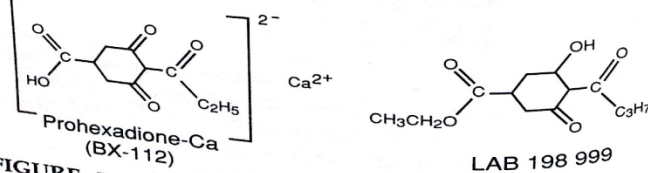
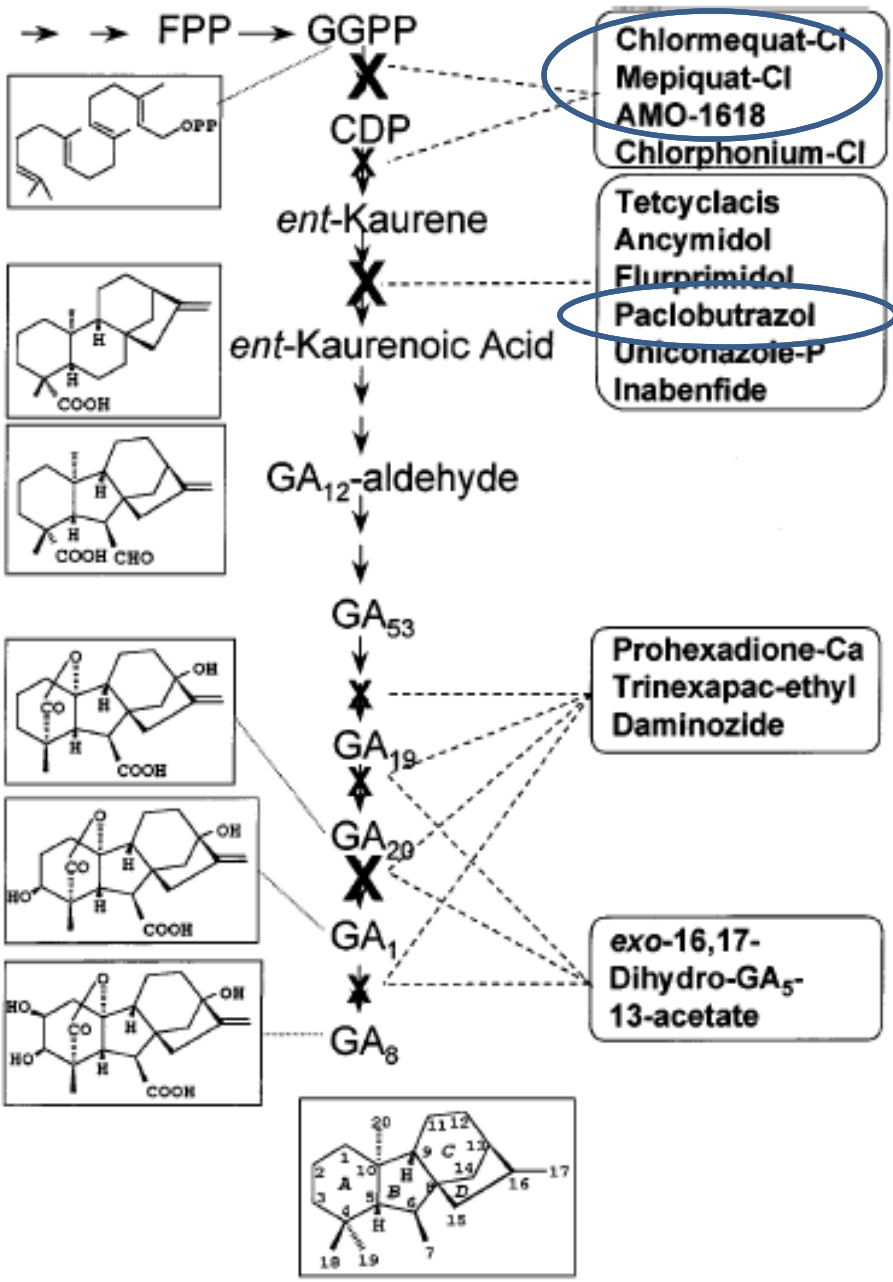


FIGURE 7-11 Based



# REGULATION OF GA LEVEL IN THE PLANT:

Regulation of synthesis:

Multiple steps biosynthesis enzymes may be turned on by developmental or environmental cues.

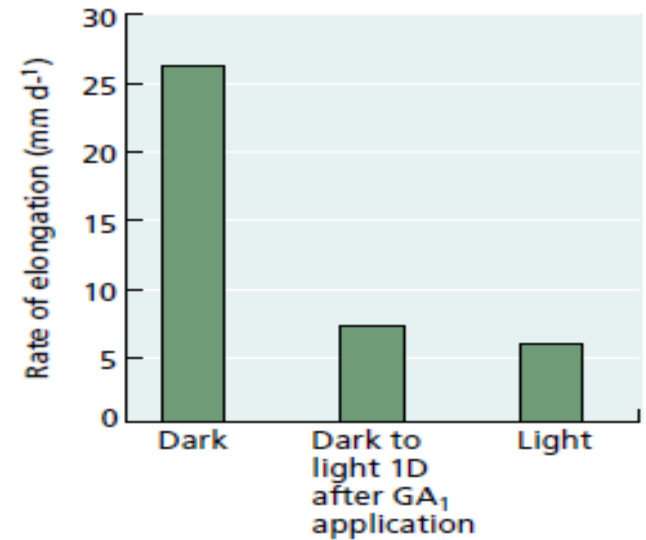
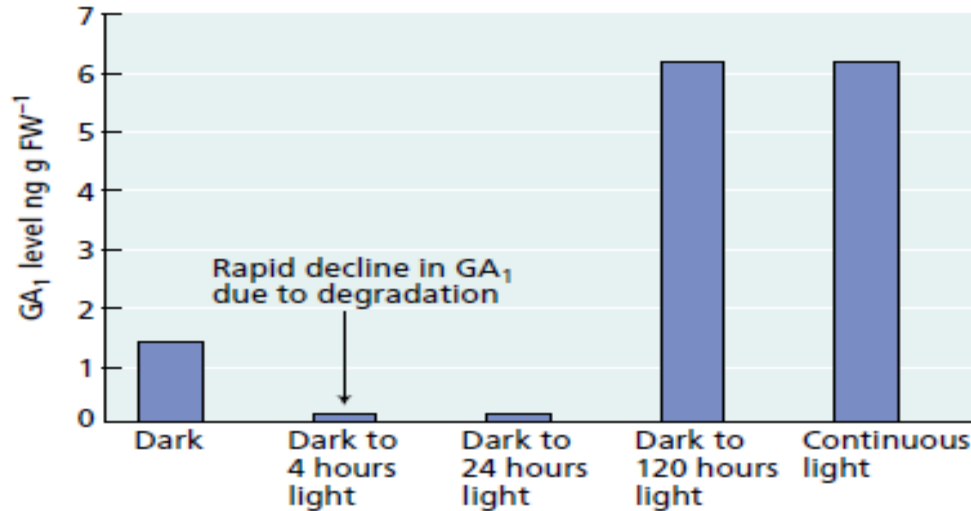
GGPP is abundant in plant tissues but CPS (copal diphosphate synthase) occurs in low abundance as its transcripts are expressed only in young growing tissues.

Light and temperature are known to affect GA levels and/or sensitivity of plant response.

## Light regulation of GA<sub>1</sub> biosynthesis.

Seed germination in some species

De-etiolation



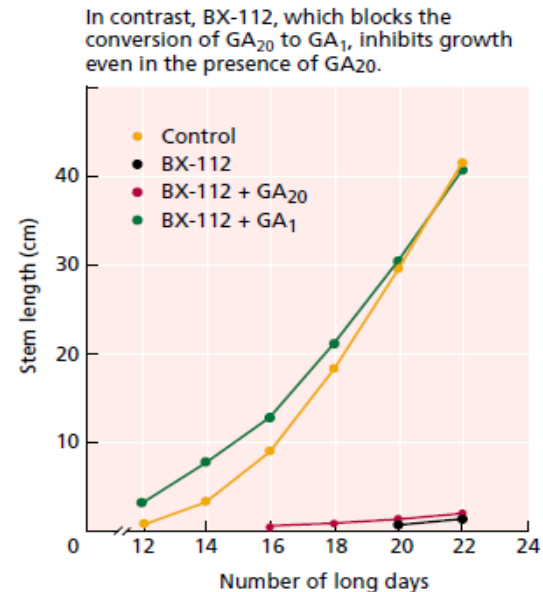
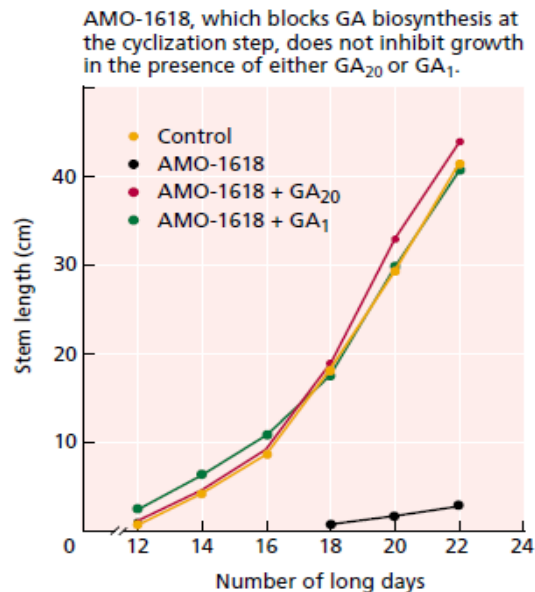
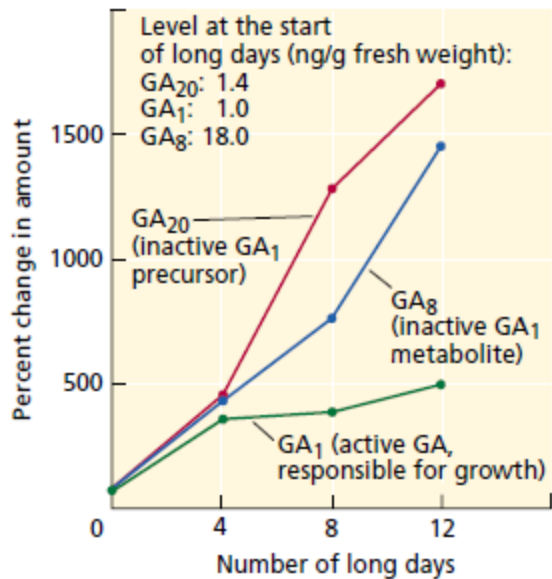
When a plant grows in the light, the rate of extension slows down through regulation by changes in hormone levels and sensitivity.

Exogenous GA was applied to 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<sub>1</sub> content is higher.

# Photoperiod regulation of GA1 biosynthesis.



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.



## ***Photoperiod control of tuber formation.***



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 GA1 levels.

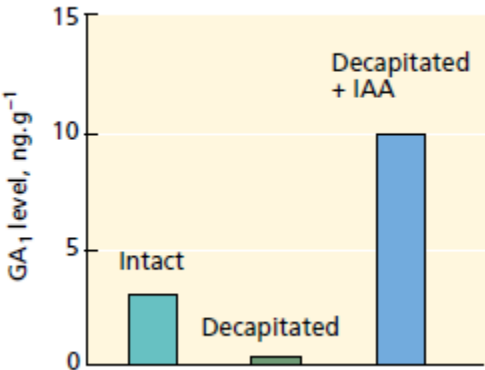
***In general, de-etiolation, light-dependent seed germination, and the photoperiodic control of stem growth in rosette plants and tuberization in potato are all mediated by phytochromes . There is mounting evidence that many phytochrome effects are in part due to modulation of the levels of gibberellins through changes in the transcription of the genes for gibberellin biosynthesis and degradation.***

## ***Temperature effects.***

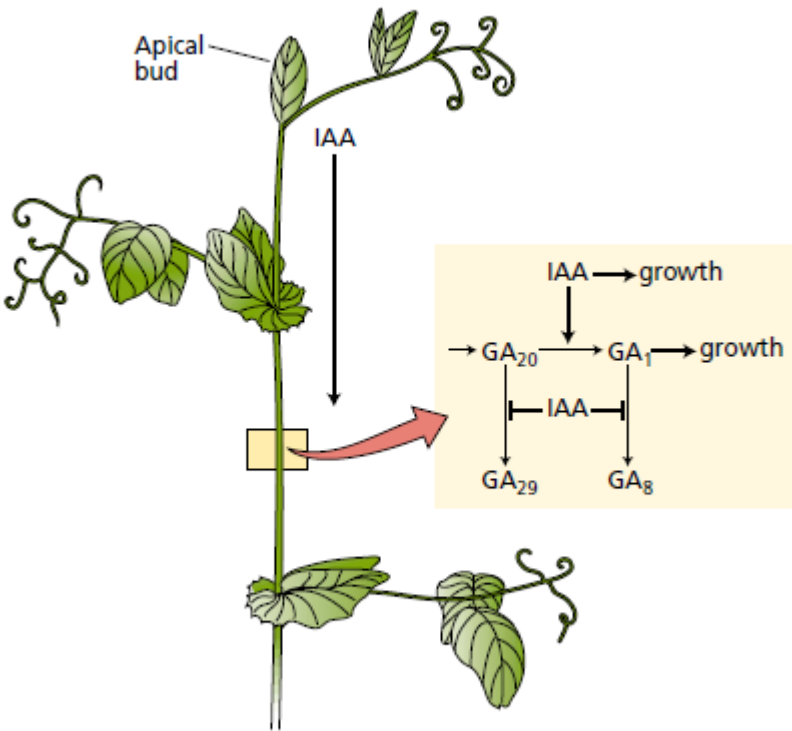
- Stratification and vernalization

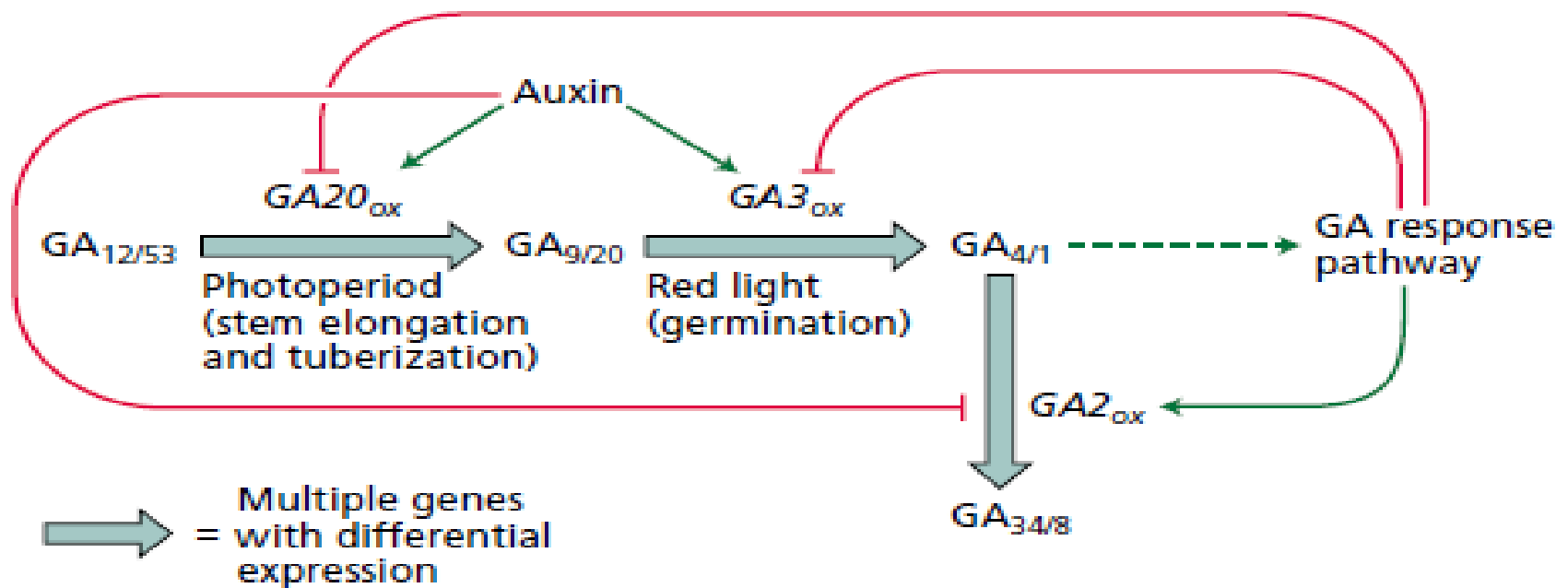
*In the absence of the cold treatment, ent-kaurenoic acid accumulates to high levels in the shoot tip, which is also the site of perception of the cold stimulus. After cold treatment and a return to high temperatures, the ent-kaurenoic acid is converted to GA9, the most active gibberellin for stimulating the flowering response. These results are consistent with a cold-induced increase in the activity of ent-kaurenoic acid hydroxylase in the shoot tip.*

# Auxin Promotes Gibberellin Biosynthesis



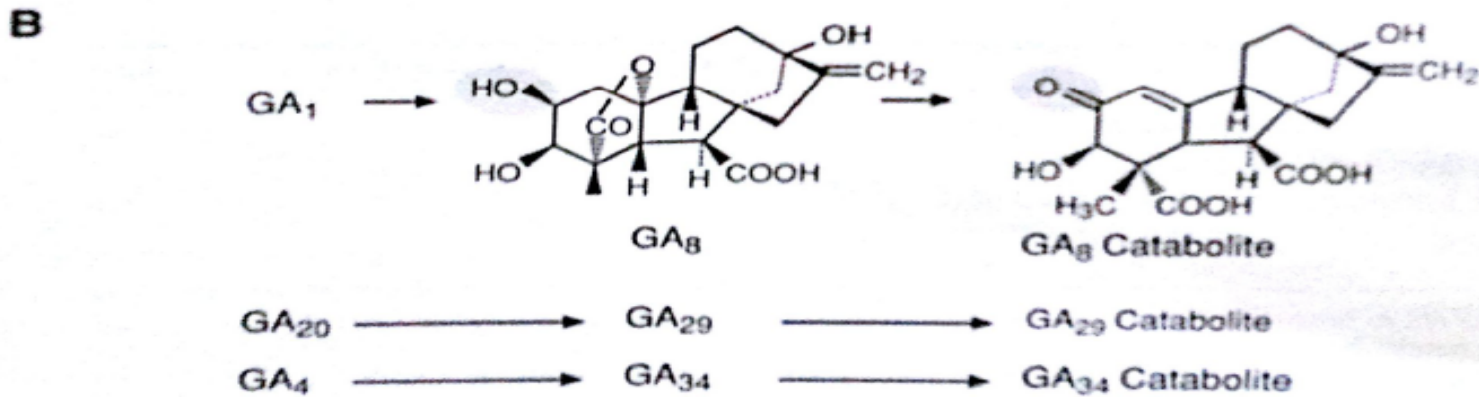
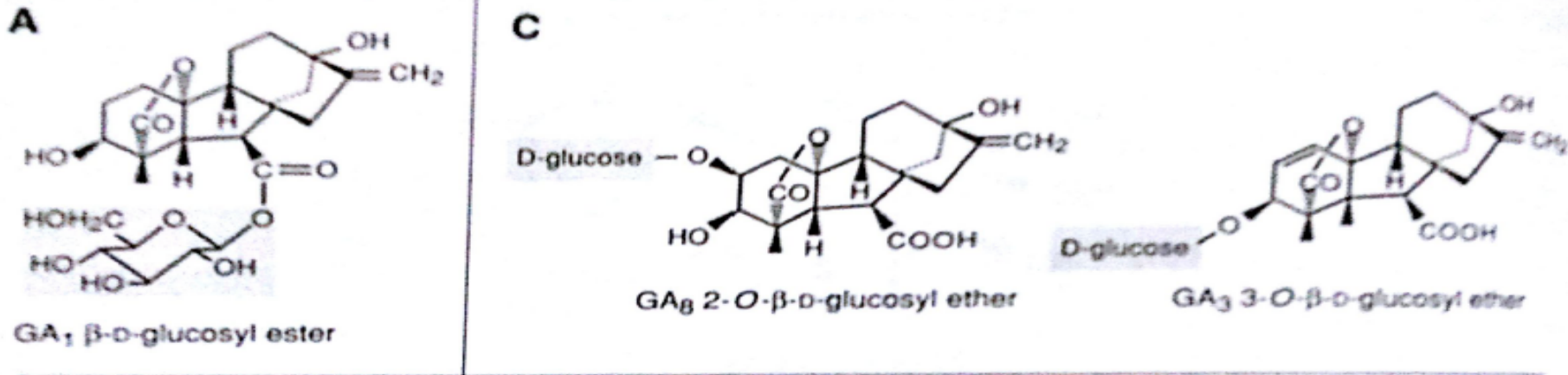
Pea plants are decapitated, leading to a cessation in stem elongation, not only is the level of auxin lowered because its source has been removed, but the level of GA<sub>1</sub> in the upper stem drops sharply.





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.

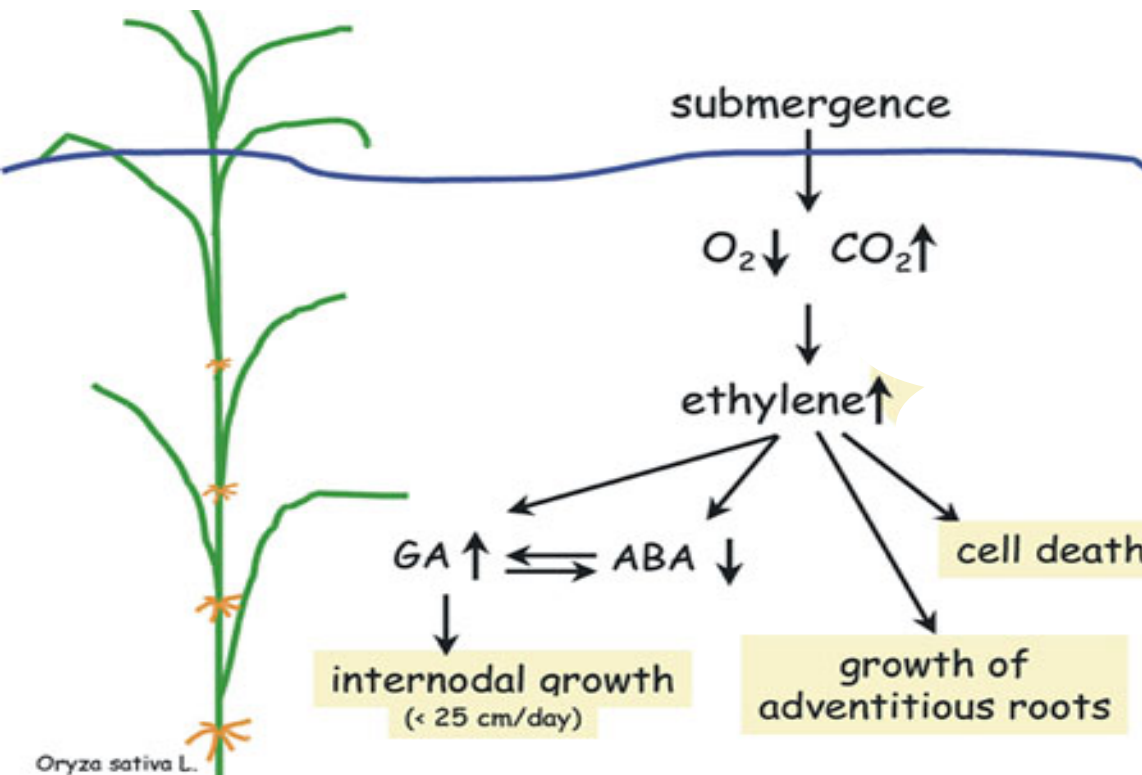
# GA conjugates and irreversible deactivation



# PHYSIOLOGICAL MECHANISMS OF GIBBERELLIN-INDUCED GROWTH

*Deep-water rice has the greatest potential for rapid internode elongation. Under field conditions, growth rates of up to 25 cm per day have been measured.*

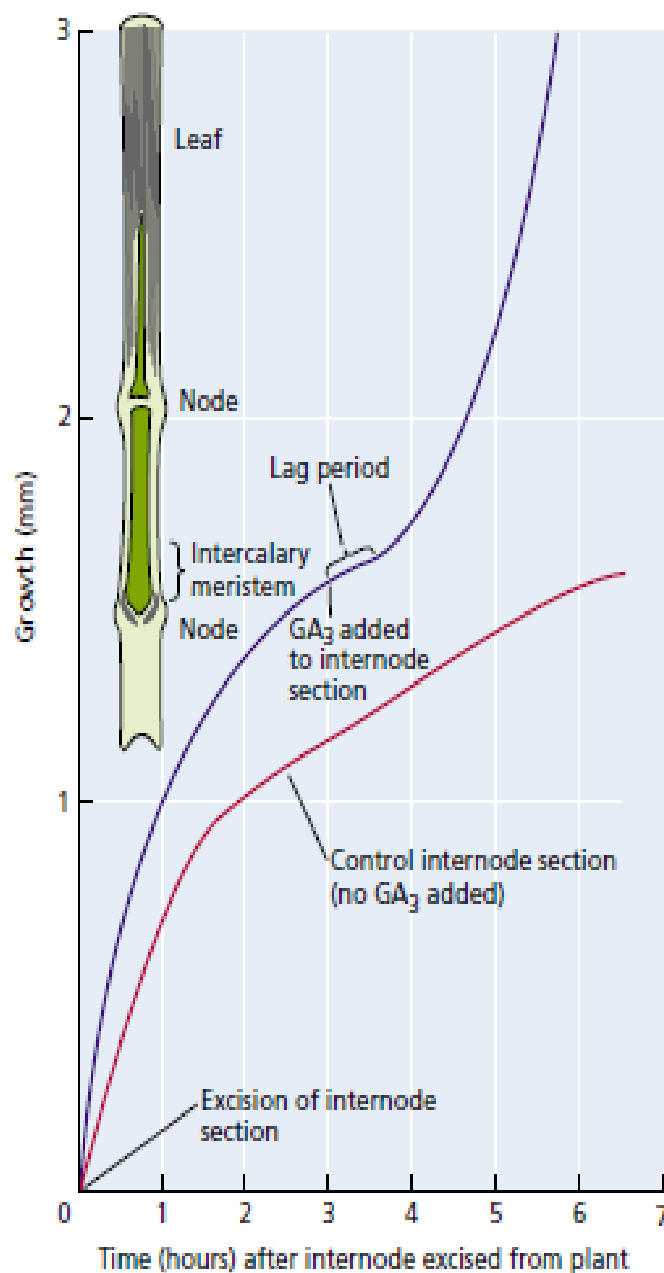
Reduced partial  $O_2$  pressure → Increase ethylene biosynthesis → ABA decreases  
→ Tissues become more responsive to GA.



**GA is primarily responsible for elongation.**

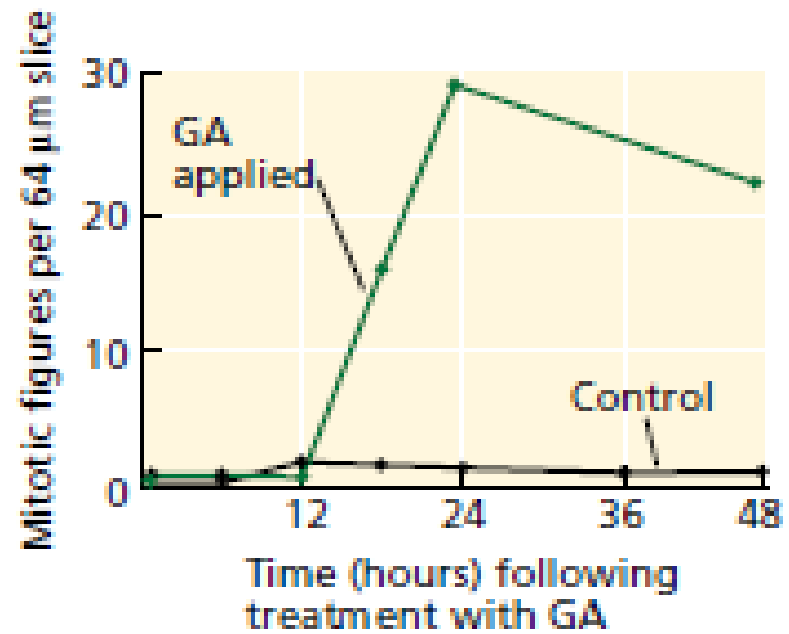
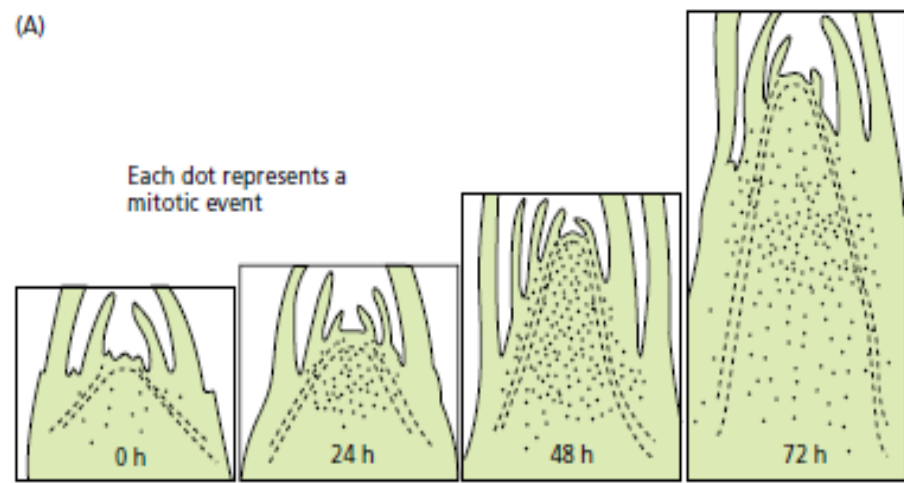
- GA inhibitors block the stimulatory effect of submergence.

- Exogenous GA stimulate the growth in absence of submergence.



The addition of gibberellin causes a marked increase in the growth rate after a lag period of about 40 minutes. Cell elongation accounts for about 90% of the length increase during the first 2 hours of gibberellin treatment.

# Gibberellins Stimulate Cell Elongation and Cell Division



# Gibberellins Enhance Cell Wall Extensibility without Acidification

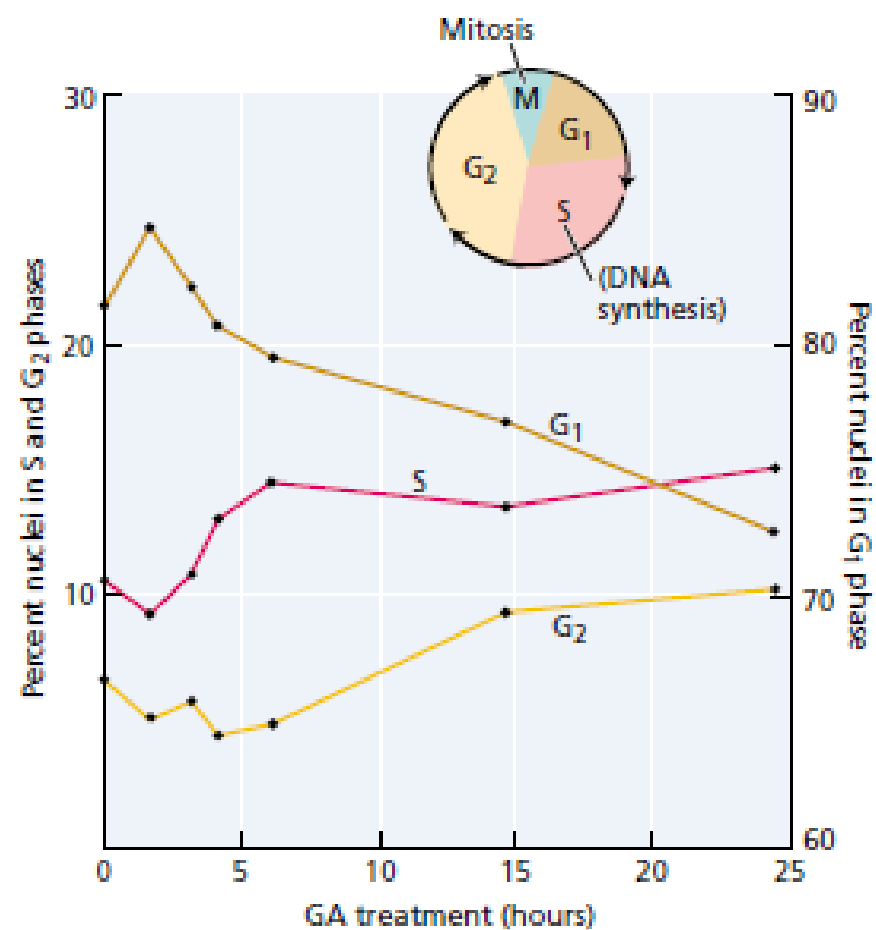
An analysis of pea genotypes differing in gibberellin content or sensitivity showed that gibberellin decreases the minimum force that will cause wall extension (the wall yield threshold).

In no case has a gibberellin-stimulated increase in proton extrusion been demonstrated. On the other hand, gibberellin is never present in tissues in the complete absence of auxin, and the effects of gibberellin on growth may depend on auxin-induced wall acidification.

Longer lag period than auxin.

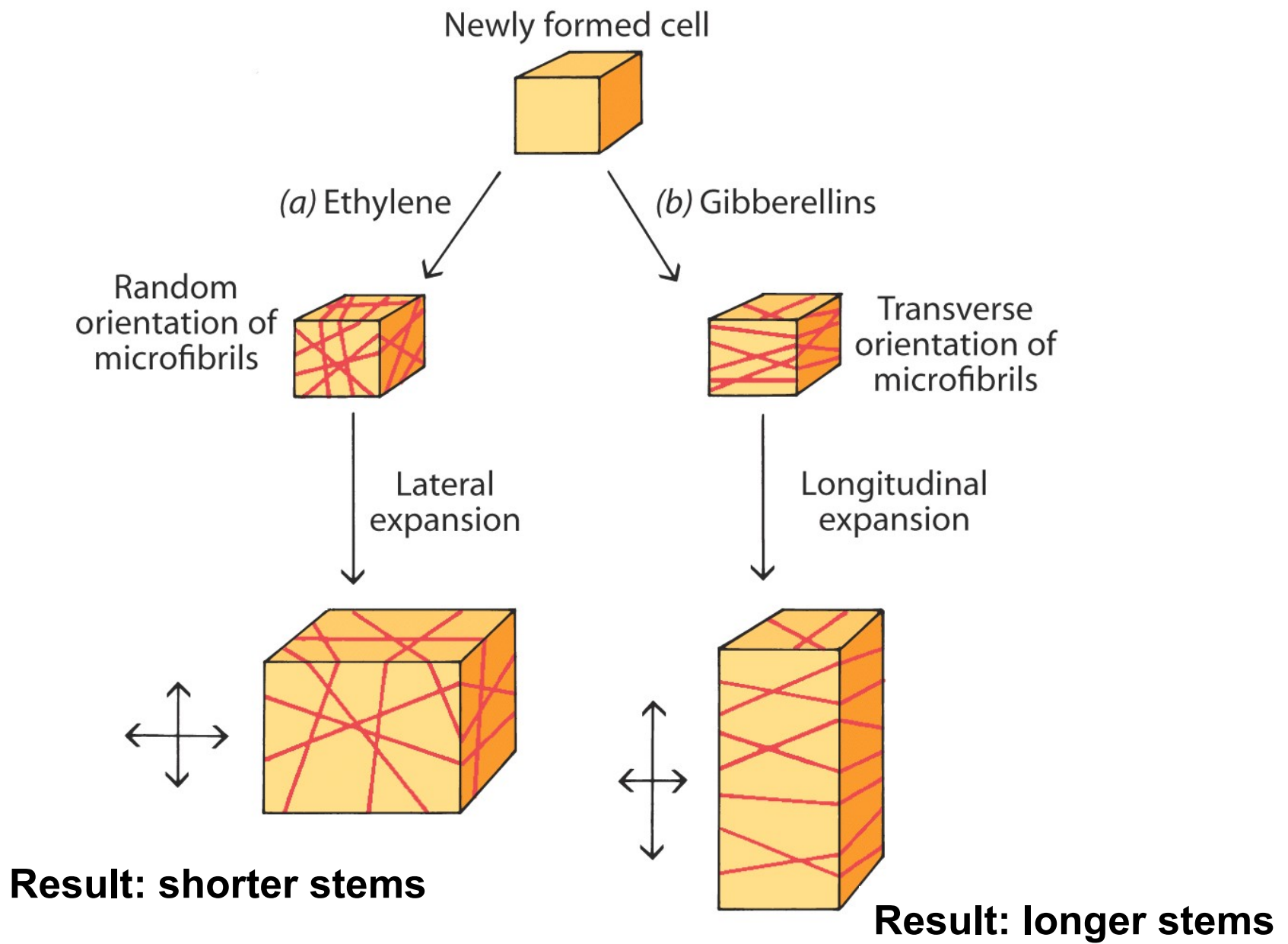
There is evidence that the enzyme xyloglucan endotransglycosylase (XET) is involved in gibberellin-promoted wall extension. The function of XET may be to facilitate the penetration of expansins into the cell wall.

# Gibberellins Regulate the Transcription of Cell Cycle Kinases in Intercalary Meristems



The transcription of these genes—first those regulating the transition from G1 to S phase, followed by those regulating the transition from G2 to M phase—is induced in the intercalary meristem by gibberellin. The result is a gibberellin induced increase in the progression from the G1 to the S phase through to mitosis and cell division.

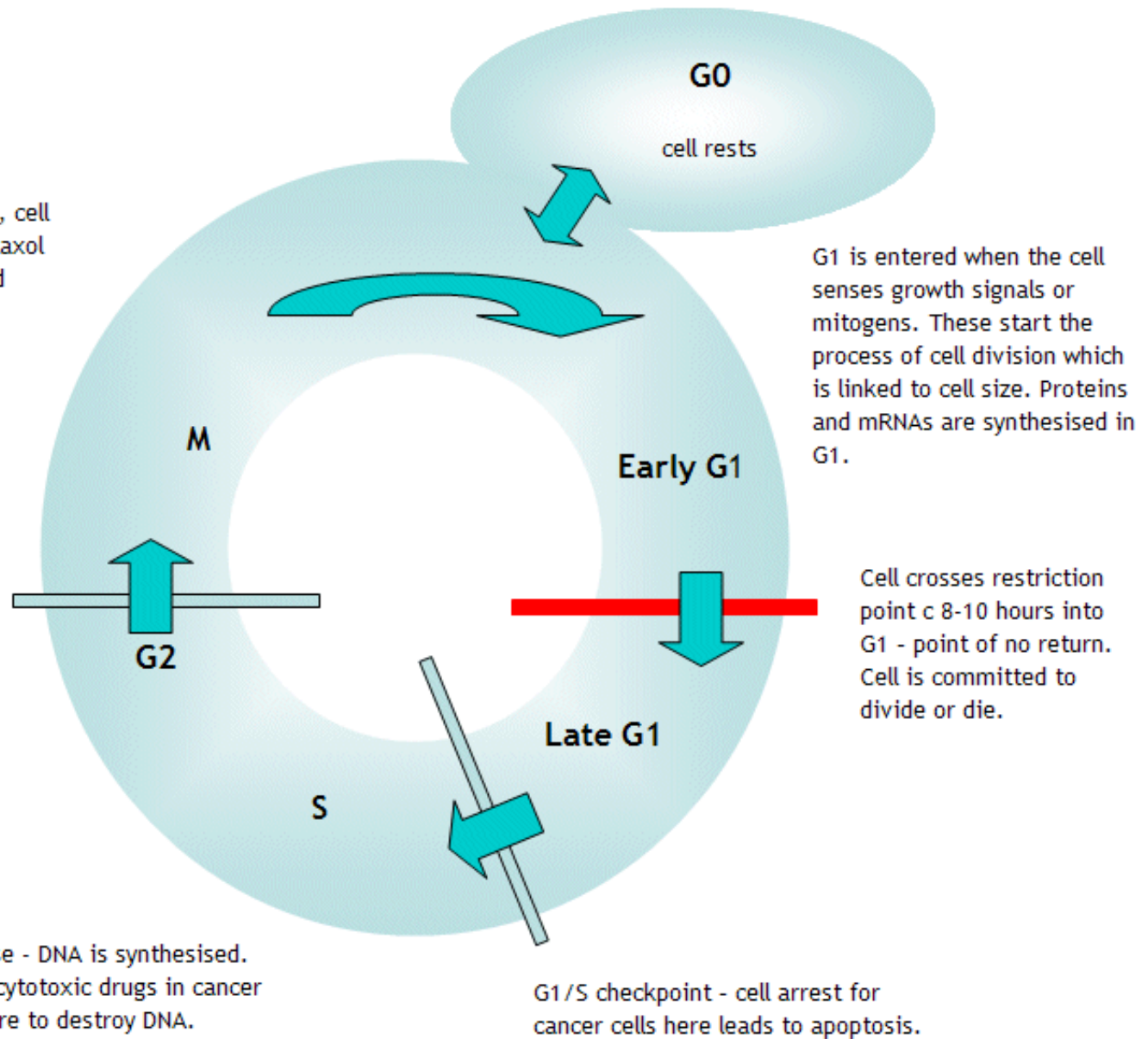
*Changes in the cell cycle status of nuclei from the intercalary meristems of deep-water rice internodes treated with GA3.*



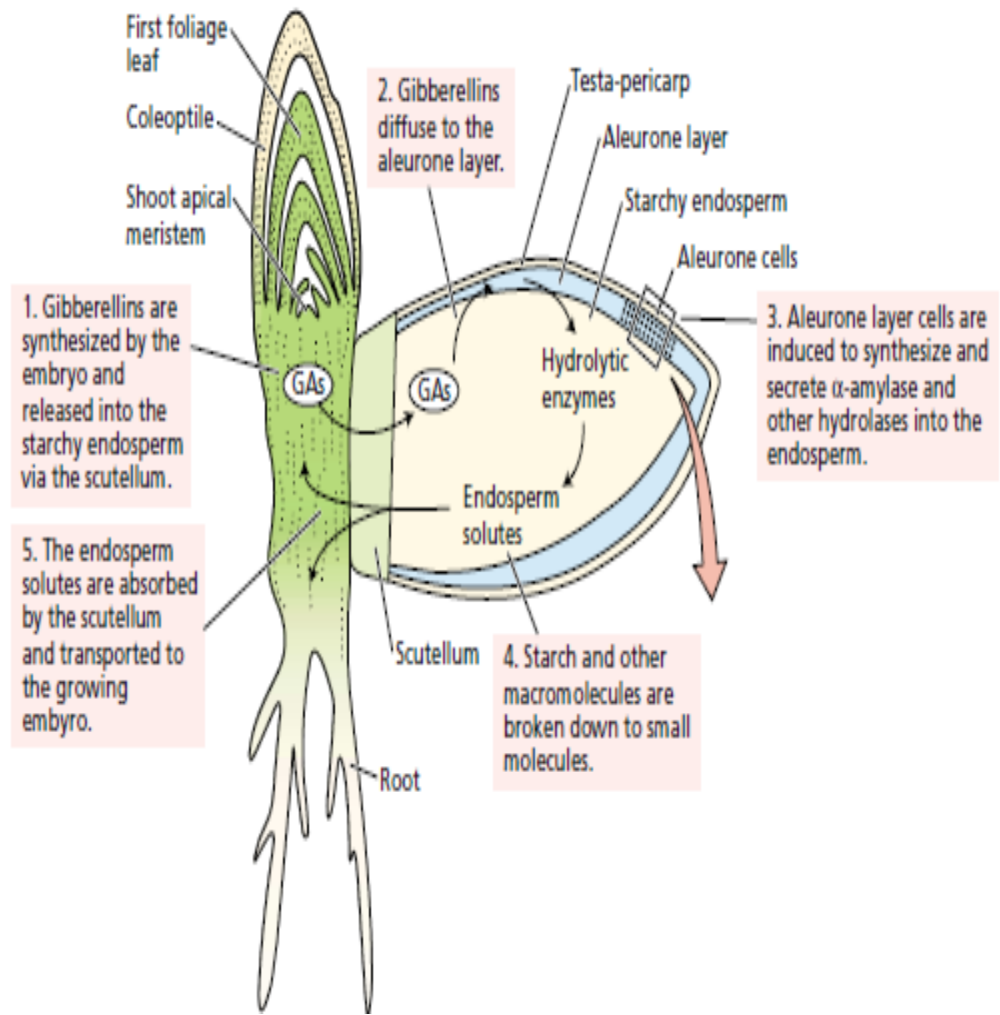
M phase - mitosis, chromosomes drawn apart by molecular motors, cell divides. Many cancer drugs like taxol act here freezing the process and causing apoptosis.

G2/M - cell arranges and checks chromosomes. There is a major checkpoint here to ascertain that DNA replication and chromosome segregation has successfully occurred. If not, a normal cell enters apoptosis.

S phase - DNA is synthesised. Many cytotoxic drugs in cancer act here to destroy DNA.

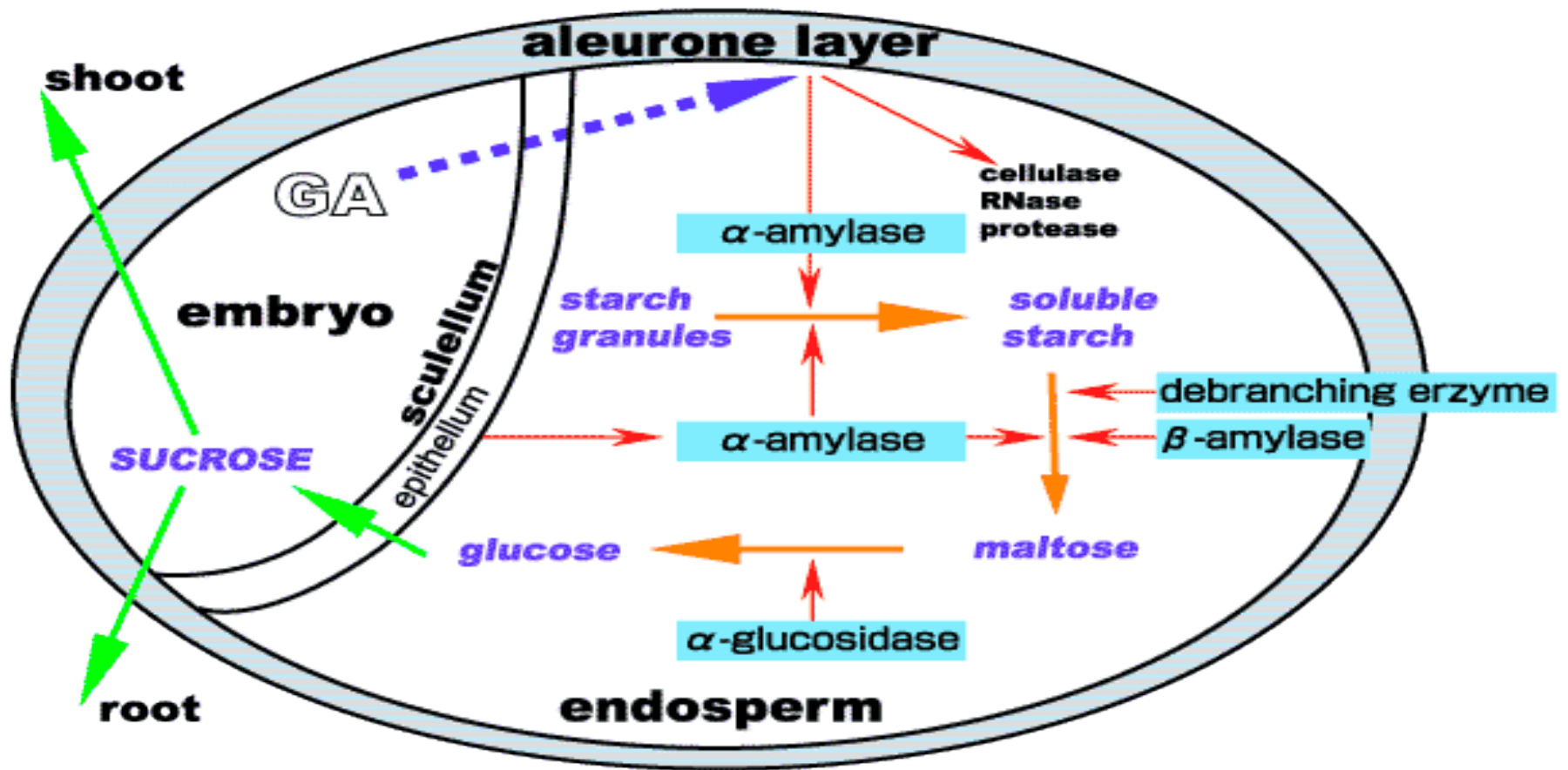


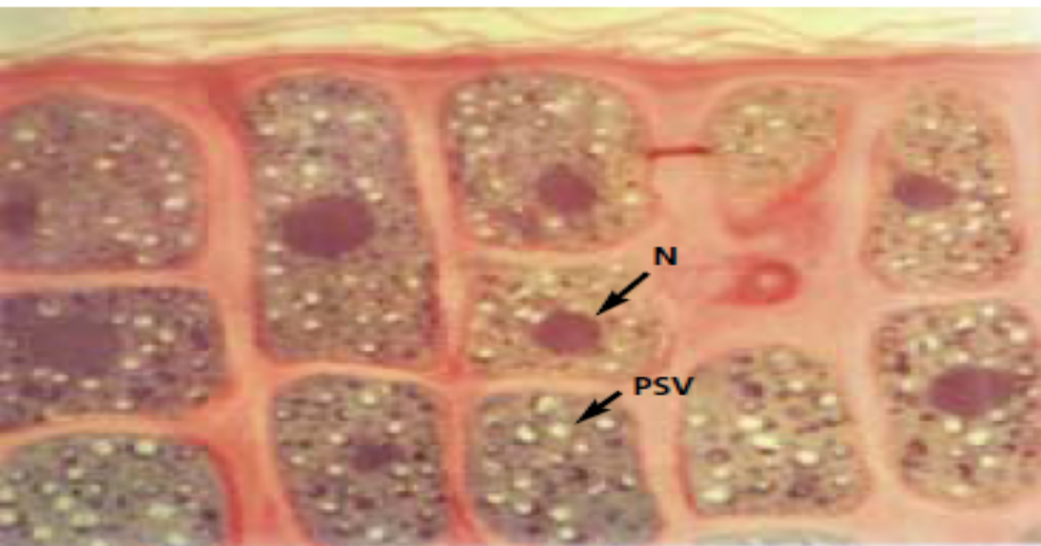
# Gibberellin from the Embryo Induces $\alpha$ -Amylase Production by Aleurone Layers



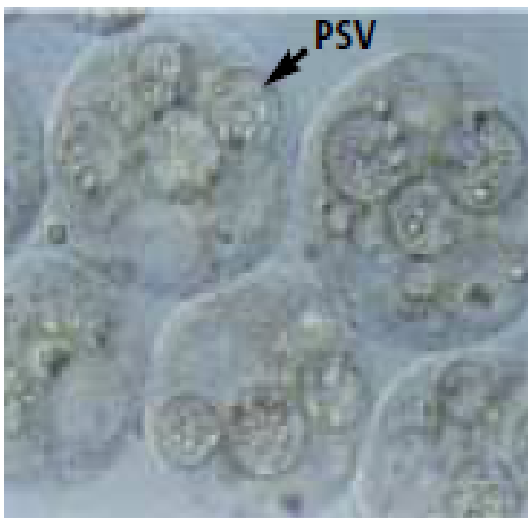
1890, “Half seeded” experiment of Haberlandt’s

The embryo part consists of the plant embryo proper, along with its specialized absorptive organ, the *scutellum* (plural *scutella*), which functions in absorbing the solubilized food reserves from the endosperm and transmitting them to the growing embryo. The endosperm is composed of two tissues: the centrally located starchy endosperm and the aleurone layer.





Microscope photos of the barley aleurone layer.



Barley aleurone protoplasts at an early and late stage

**Protein storage vesicles (PSV). G = phytin globoid; N = nucleus.**

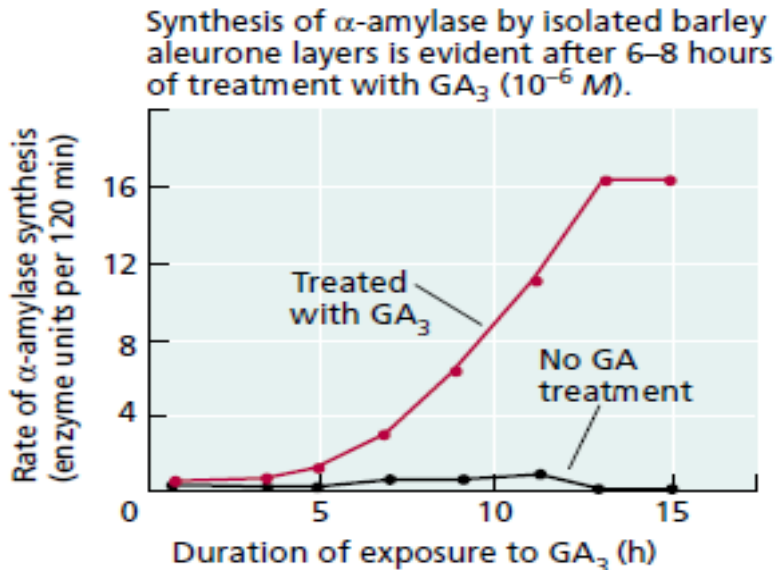
*The protein bodies also contain phytin, a mixed cation salt (mainly  $Mg^{2+}$  and  $K^{+}$ ) of myo-inositolhexaphosphoric acid (phytic acid).*

# GIBBERELLIN SIGNAL TRANSDUCTION: CEREAL ALEURONE LAYERS

1. How does gibberellin regulate the increase in  $\alpha$ -amylase?
2. Where is the gibberellin receptor located in the cell?
3. What signal transduction pathways operate between the gibberellin receptor and  $\alpha$ -amylase production?

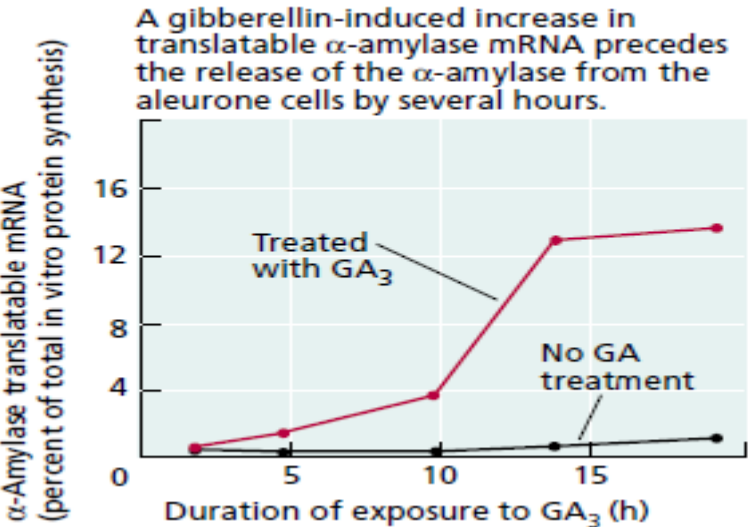
# Gibberellic Acid Enhances the Transcription of $\alpha$ - Amylase mRNA

## (A) Enzyme synthesis



1. GA<sub>3</sub>-stimulated  $\alpha$ -amylase production was shown to be blocked by inhibitors of transcription and translation.
2. Heavy-isotope- and radioactive-isotope labeling studies demonstrated that the stimulation of  $\alpha$ -amylase activity by gibberellin involved de novo synthesis of the enzyme from amino acids, rather than activation of preexisting enzyme.

## (B) mRNA synthesis



Transcription or decrease mRNA turnover?

# A GA-MYB Transcription Factor Regulates $\alpha$ - Amylase Gene Expression

The stimulation of  $\alpha$ -amylase gene expression by gibberellin is mediated by a specific transcription factor that binds to the promoter of the  $\alpha$ -amylase gene. (Mobility shift assay -regulatory DNA sequences **gibberellin response elements**)

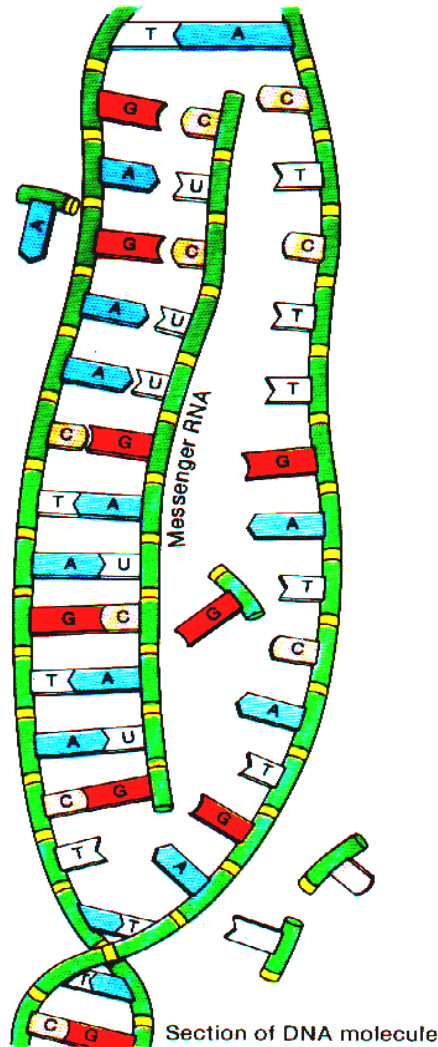
Studies demonstrated that gibberellin increases either the level or the activity of a transcription factor protein that switches on the production of  $\alpha$ -amylase mRNA by binding to an upstream regulatory element in the  $\alpha$ -amylase gene promoter.

The sequence of the gibberellin response element in the  $\alpha$ -amylase gene promoter turned out to be similar to that of the binding sites for MYB transcription factors .named GA-MYB, associated with the gibberellin induction of  $\alpha$ -amylase gene expression

# What is a Transcription Factor?

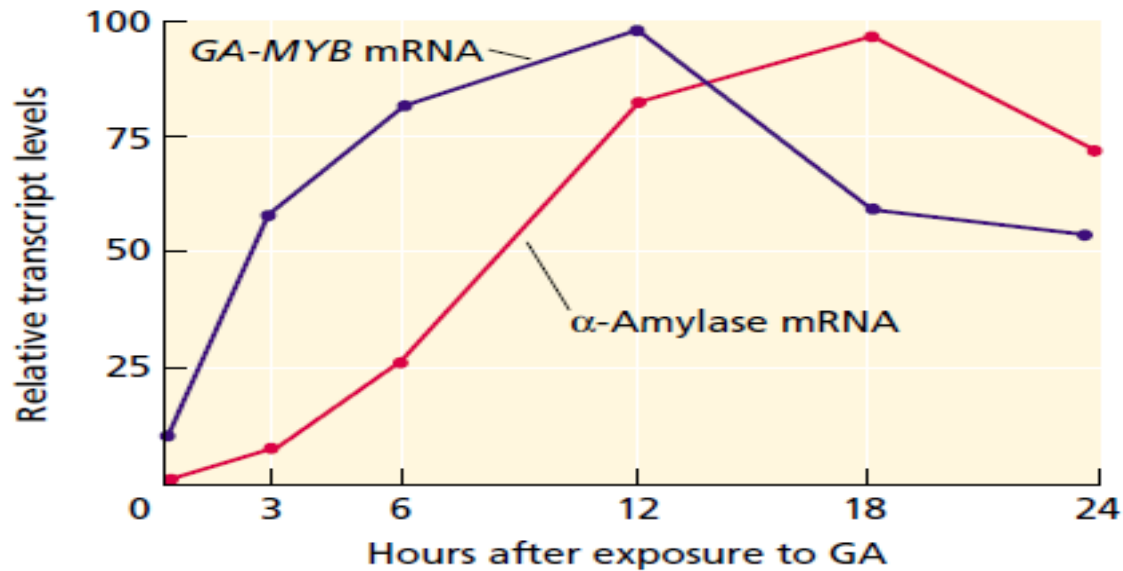
## KEY

T = thymine  
C = cytosine  
A = adenine  
G = guanine



- Transcription factor – any protein other than RNA Polymerase that is required for transcription

Myeloblast ---> MYB (came from first identified MYB which was in an avian oncogene)



The synthesis of *GA-MYB mRNA* in aleurone cells increases within 3 hours of gibberellin application, several hours before the increase in  $\alpha$ -amylase mRNA. The inhibitor of translation, cycloheximide, has no effect on the production of *MYB mRNA*, indicating that *GA-MYB* is a primary response gene, or early gene. In contrast, the  $\alpha$ -amylase gene is a secondary response gene, or late gene, as indicated by the fact that its transcription is blocked by cycloheximide.

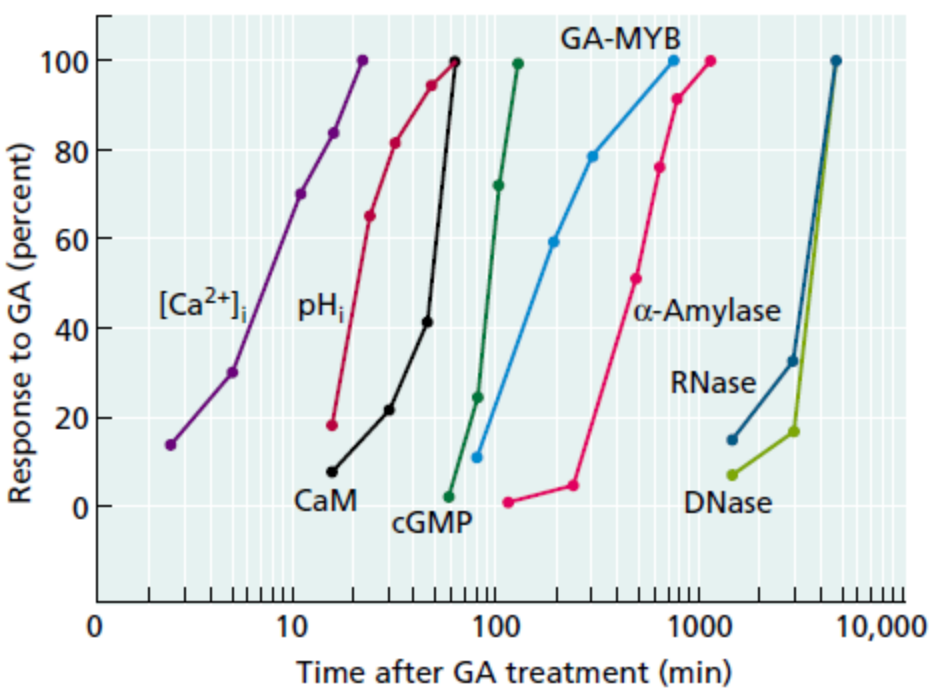
# Gibberellin Receptors May Interact with G proteins on the Plasma Membrane

GA that has been bound to microbeads that are unable to cross the plasma membrane is still active in inducing  $\alpha$ -amylase production in aleurone protoplasts.

In addition, microinjection of GA3 into aleurone protoplasts had no effect, but when the protoplasts were immersed in GA3 solution, they produced  $\alpha$ -amylase.

# Cyclic GMP, Ca<sup>2+</sup>, and Protein Kinases Are Possible Signaling Intermediates

In animal cells, G-proteins can activate the enzyme guanylyl cyclase, the enzyme that synthesizes cGMP from GTP, leading to an increase in cGMP concentration. Cyclic GMP, in turn, can regulate ion channels, Ca<sup>2+</sup> levels, protein kinase activity, and gene transcription. Gibberellin has been reported to cause a transient rise in cGMP levels in barley aleurone layers, suggesting a possible role for cGMP in  $\alpha$ -amylase production.



1. GA<sub>1</sub> 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.

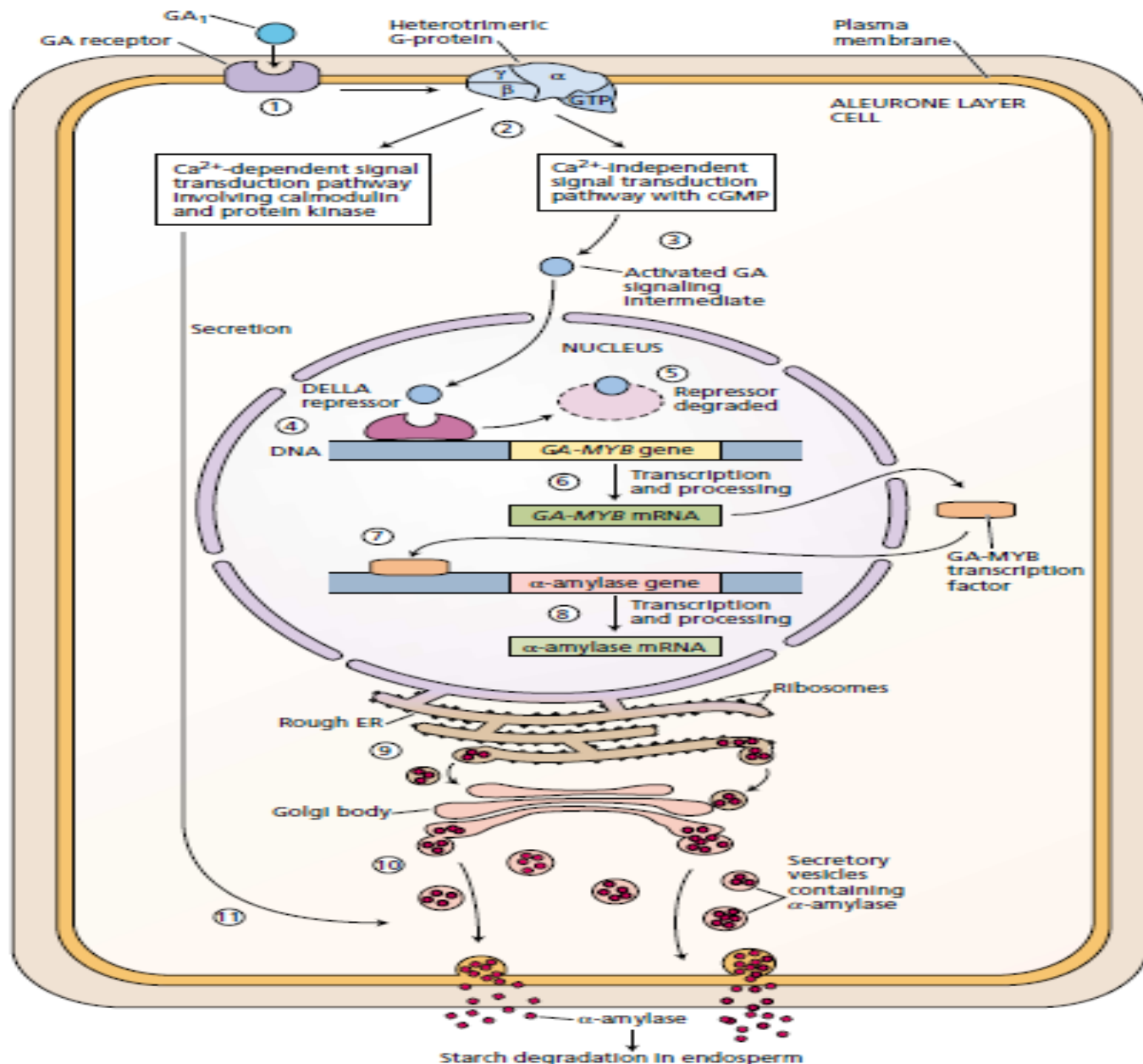
7. The newly synthesized MYB protein then enters the nucleus and binds to the promoter genes for  $\alpha$ -amylase and other hydrolytic enzymes.

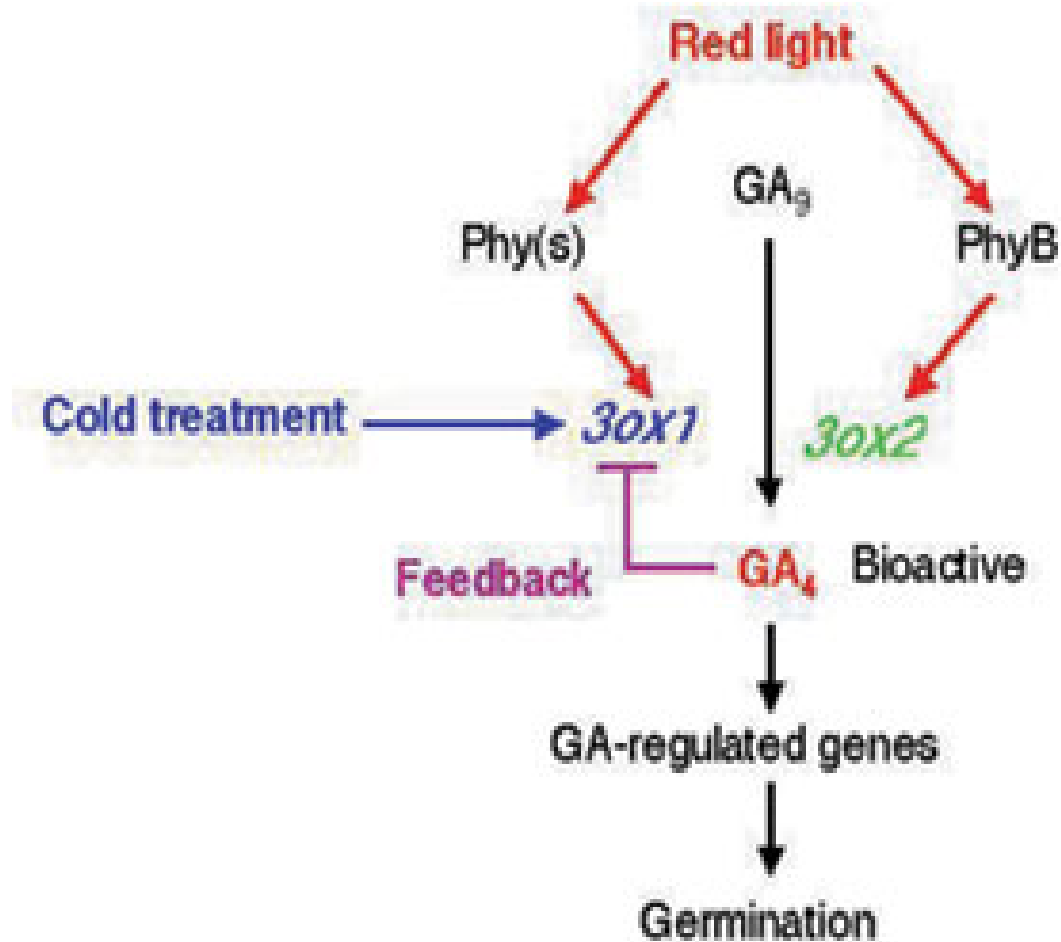
8. Transcription of  $\alpha$ -amylase and other hydrolytic genes is activated.

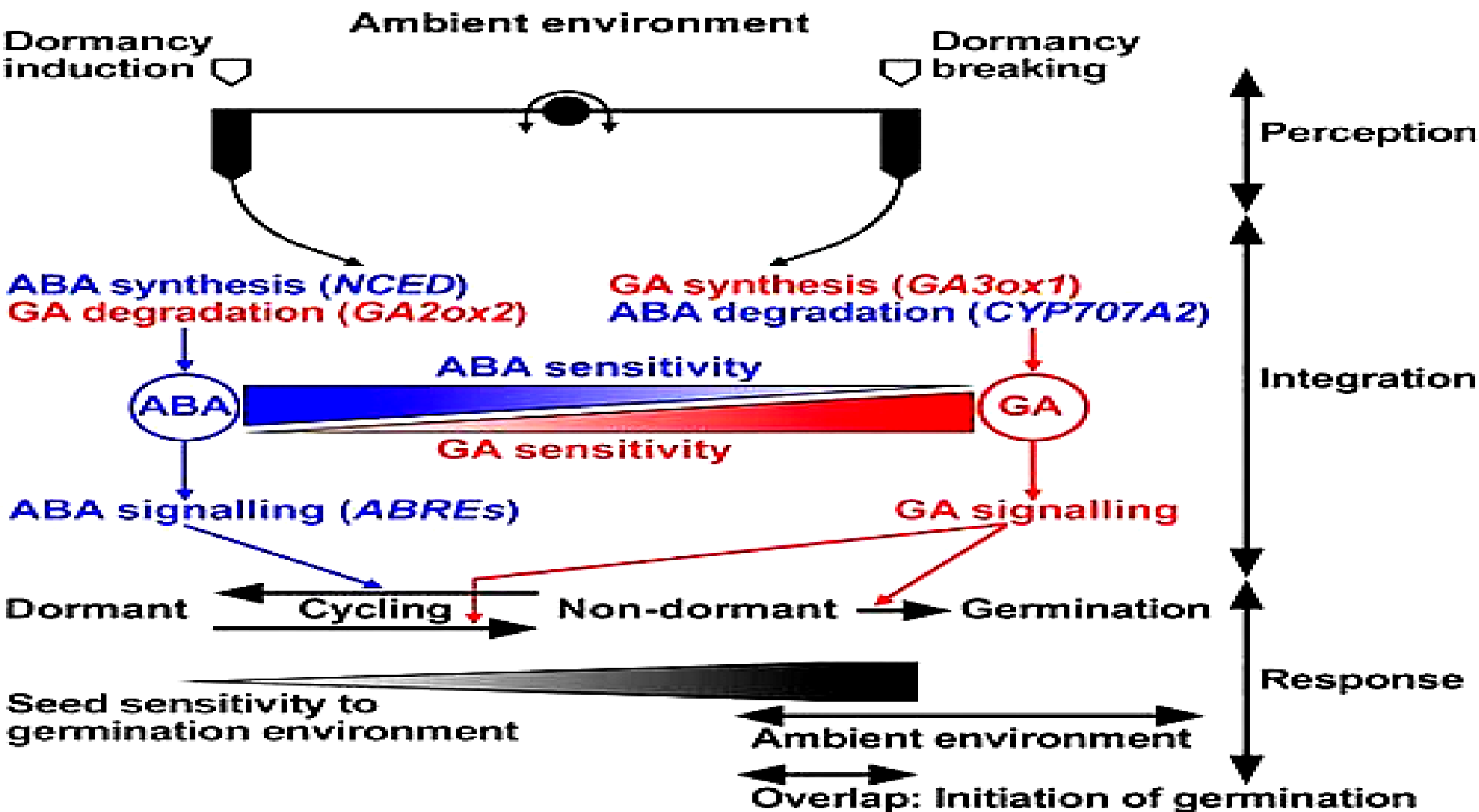
9.  $\alpha$ -Amylase and other hydrolases are synthesized on the rough ER.

10. Proteins are secreted via the Golgi.

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







Thanks