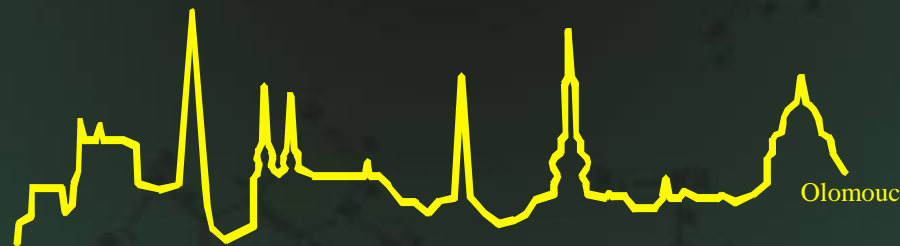


Laboratoř růstových regulátorů

Miroslav Strnad

Klíčení
[kap. 21



- Univerzita Palackého & Ústav experimentální botaniky AV CR



Semeno (caryopses)

- 3 části – diploidní embryo, triploidní endosperm a fúzaná testa-perikarp
- Embryo má skutelum – absorbční orgán, který asimiluje rozpuštěné zásobní látky z endospermu, an povrchu endospermu aleuronová vrstva, endosperm – škrobová zrna, kdežto aleuron - proteinová tělíska (obsahují phytin)
 - Po nabobtnání dochází k hydrolýze zásobních látek na cukry, AK, atd. Skrob rozkládám α - a β -amylasou
 - α -amylasa sekretována scutelem a aleuronem. Aleuron hlavní zdroj hydrolitických enzymů, nefunguje ale bez embrya (Haberlant 1890). Embryo produkuje GA, posléze zjištěno že GA1. Indukuje expresi řady dalších hydrolytických enzymů.
 - Indukce transkripce mRNA pro α -amylasu – inhibitory transkripce a translace blokuje, GA-responsive element (200-300 bp upstream)

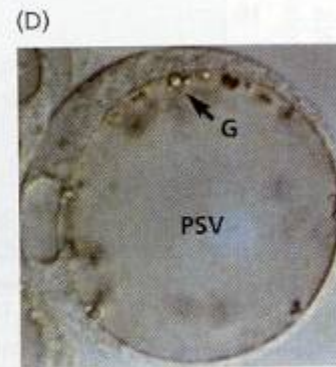
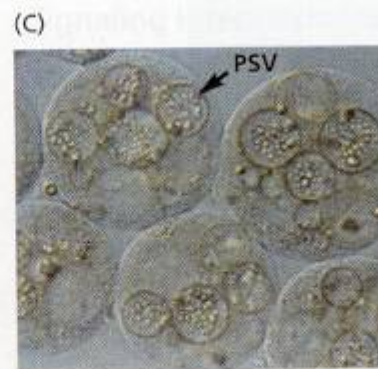
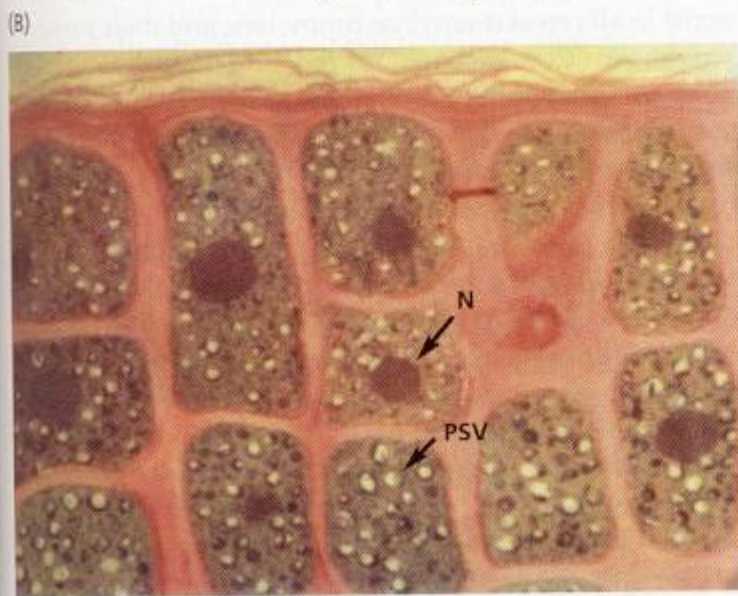
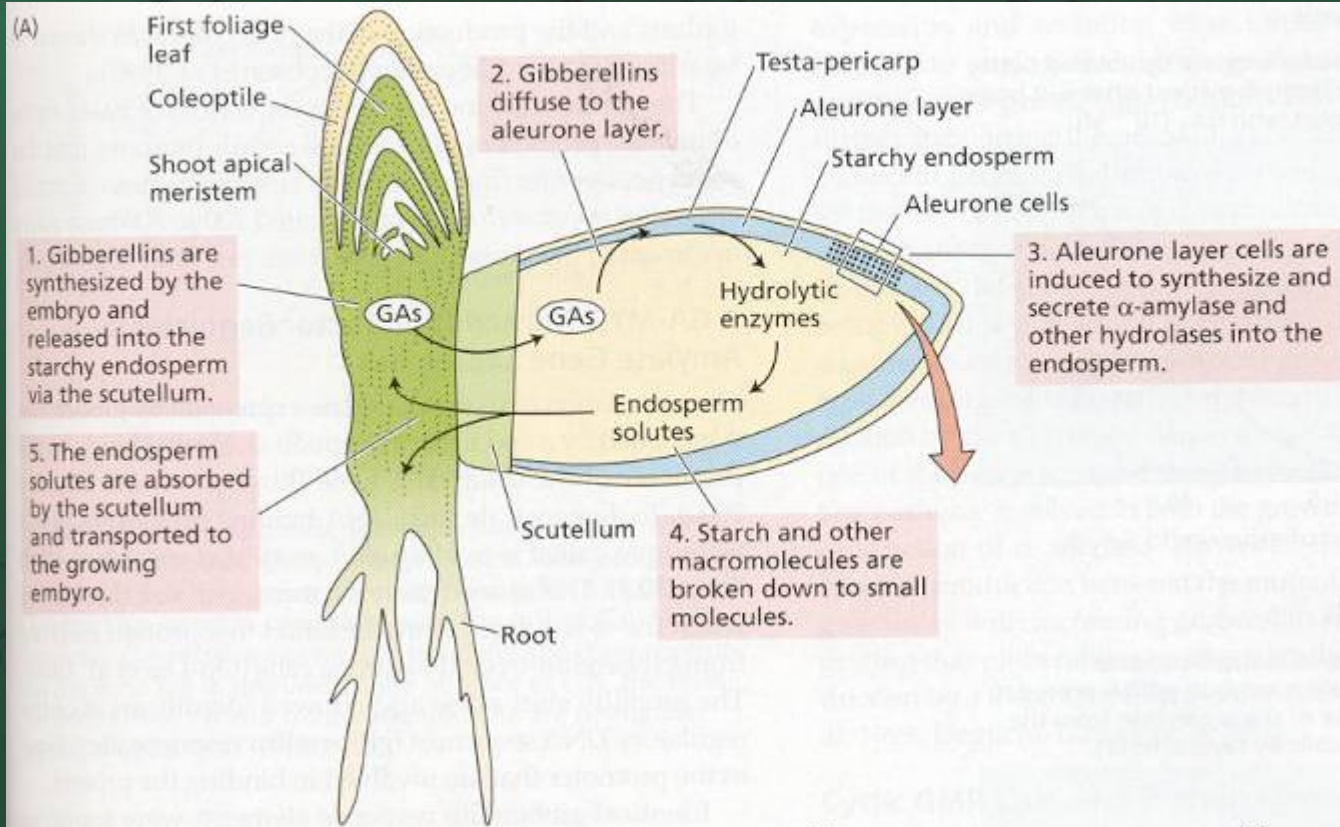
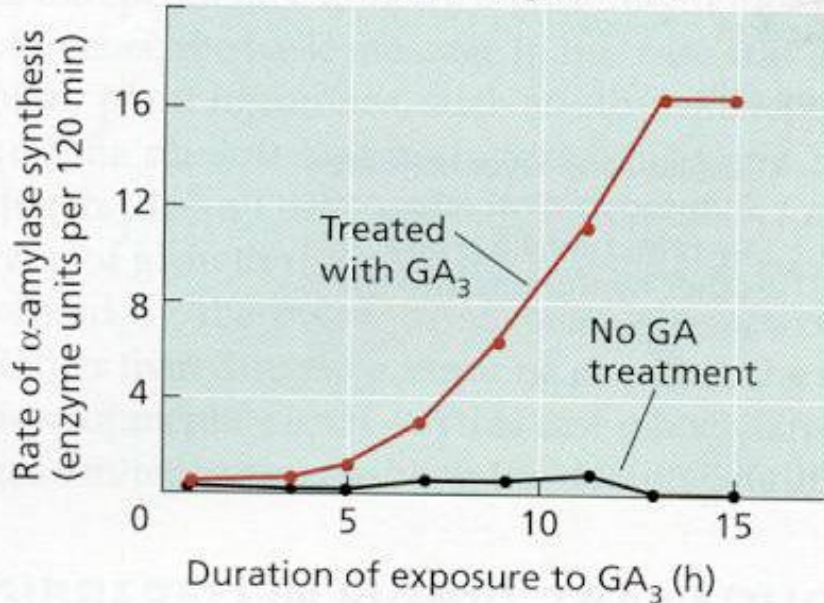


FIGURE 20.33 Structure of a barley grain and the functions of various tissues during germination (A). Microscope photos of the barley aleurone layer (B) and barley aleurone protoplasts at an early (C) and late stage (D) of amylase production. Protein storage vesicles (PSV) can be seen in each cell. G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

(A) Enzyme synthesis

Synthesis of α -amylase by isolated barley aleurone layers is evident after 6–8 hours of treatment with GA_3 (10^{-6} M).



(B) mRNA synthesis

A gibberellin-induced increase in translatable α -amylase mRNA precedes the release of the α -amylase from the aleurone cells by several hours.

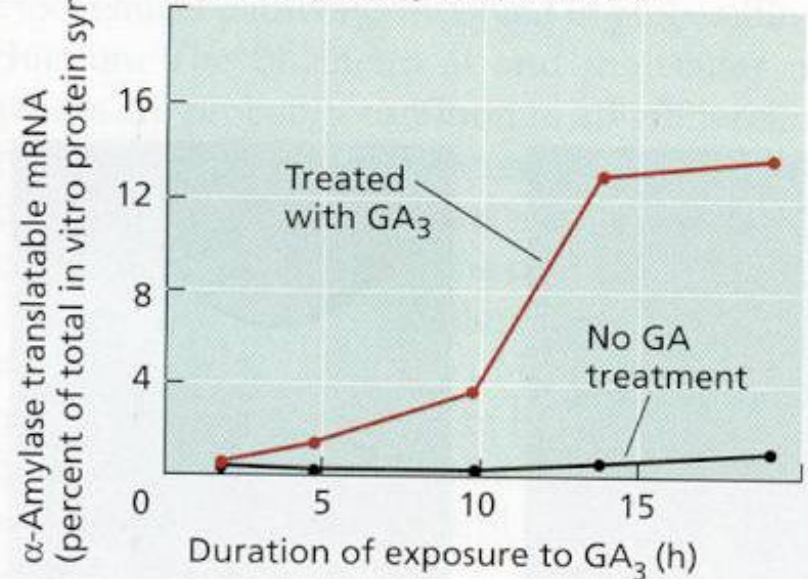


FIGURE 20.34 Gibberellin effects on enzyme synthesis and mRNA synthesis. The α -amylase mRNA in this case was measured by the in vitro production of α -amylase as a percentage of the protein produced by the translation of the bulk mRNA. (From Higgins et al. 1976.)

Signální dráhy v klíčícím semeni

- GA-MYB transkripční faktor reguluje expresi genu pro α -amylasu, váže se v promotorové, je to gen primární odpovědi (indukován do 3 h), kdežto gen pro α -amylasu je sekundární. Indukce je zprostředkována receptory v membránách aleuronových buněk, zatím nejsou detailně popsány, pouze izolovány GBP, které zřejmě interagují s GTP-vazebnými proteiny, kativují adenyl anebo guanylylcyklasu, která produkuje cGMP, který otevírá Ca^{2+} kanály a reguluje tak aktivitu protein kinas, Ca^{2+} se poté váže na kalmodulin (sekundární přenašeč)

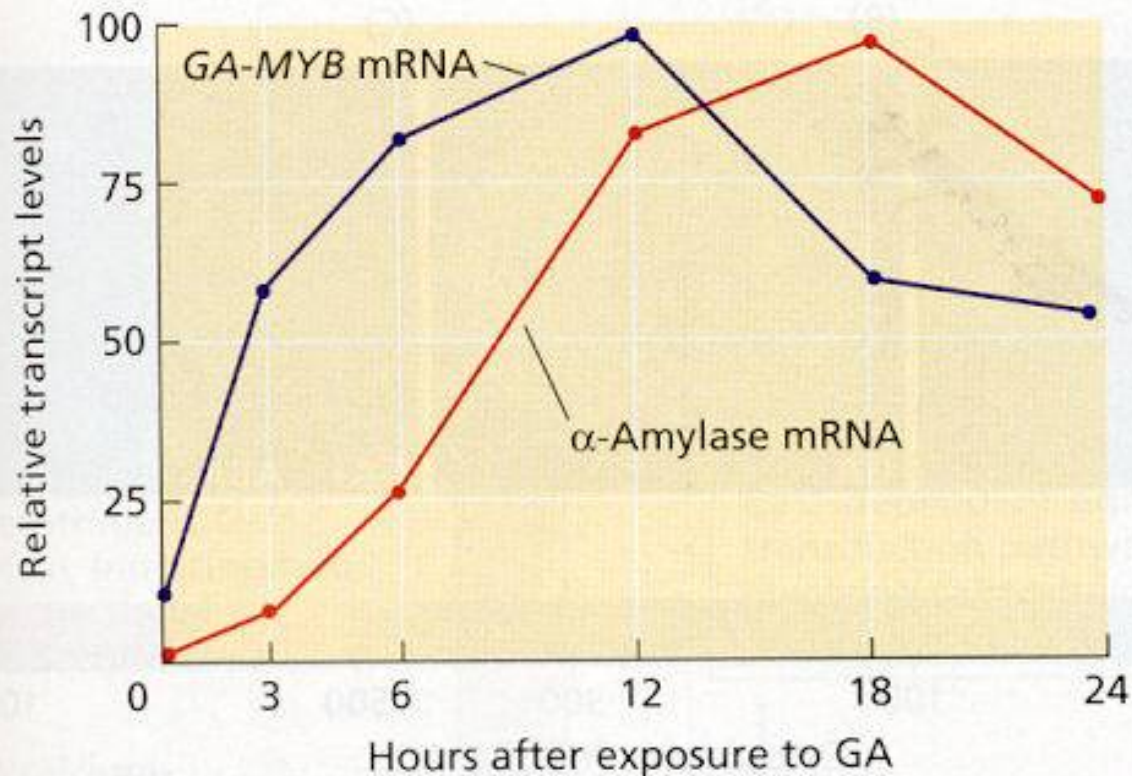


FIGURE 20.35 Time course for the induction of *GA-MYB* and α -amylase mRNA by gibberellic acid. The production of *GA-MYB* mRNA precedes α -amylase mRNA by about 5 hours. This result is consistent with the role of *GA-MYB* as an early *GA* response gene that regulates the transcription of the gene for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

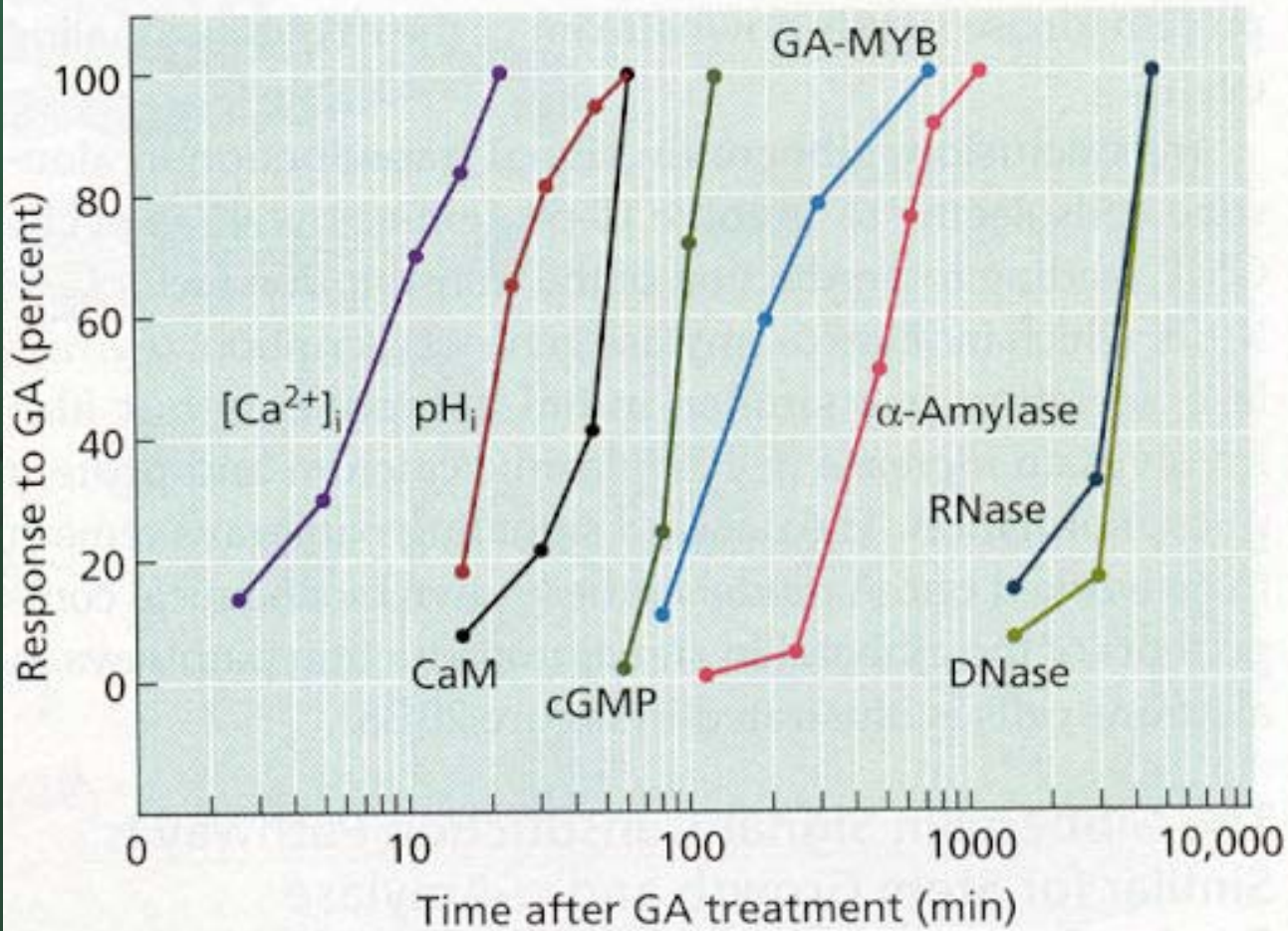


FIGURE 20.36 Following the addition of GA to barley aleurone protoplasts, a multiple signal transduction pathway is initiated. The timing of some of these events is shown. (From Bethke et al. 1997.)

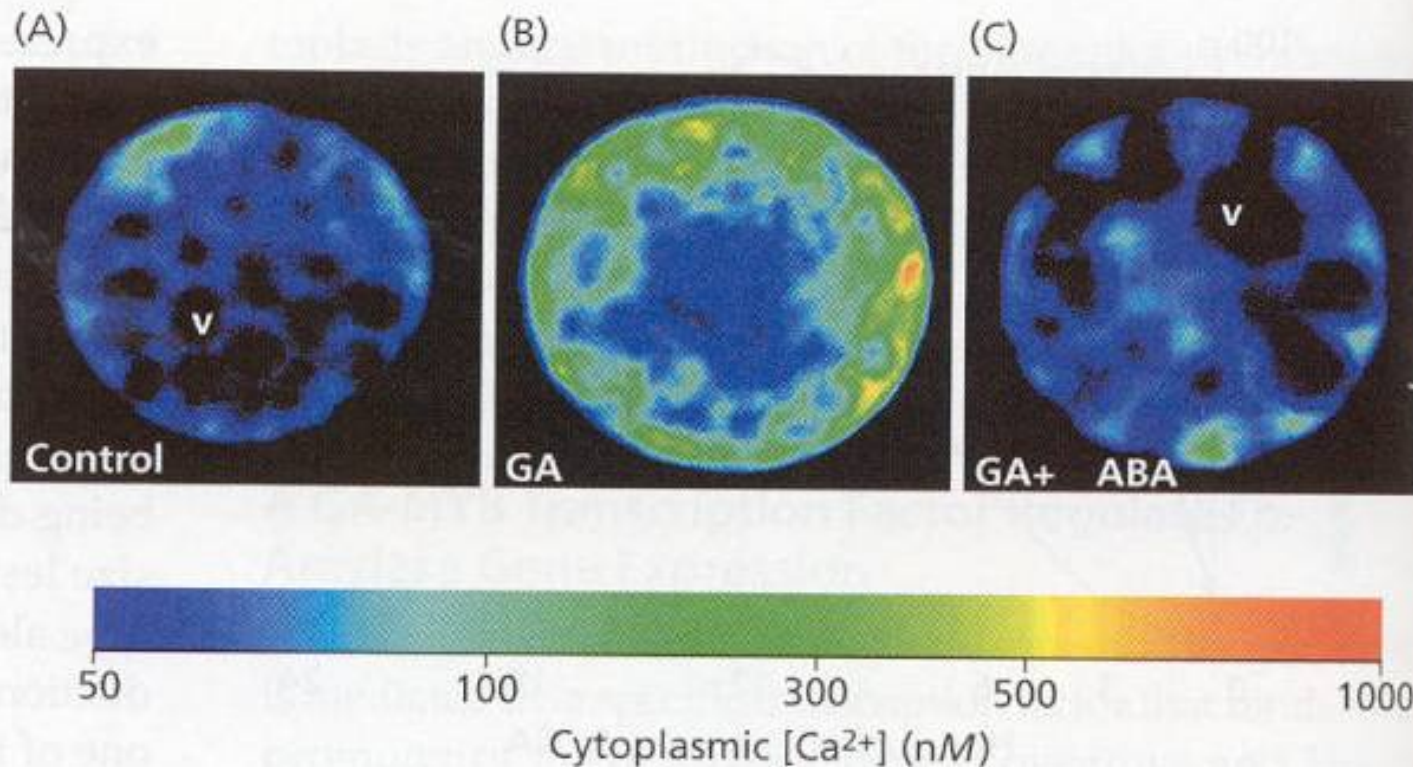


FIGURE 20.37 Increase in the calcium in barley aleurone protoplasts following GA addition can be seen from this false color image. The level of calcium corresponding to the colors is in the lower scale. (A) Untreated protoplast. (B) GA-treated protoplast. (C) Protoplast treated with both abscisic acid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

1. GA₁ 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.

