Bio 328. Spring 2005 Name: KEY

Test #2

Provide concise answers in the space provided after each question, or, if more space is needed, continue on the back side of the page. The potential value of each answer is 4 points unless otherwise noted in the margin.

1. (a) After describing the upward gravitropic curvature of horizontally positioned coleoptiles, a Plant Biology text book just published noted: "A reasonable explanation is that gravity caused a movement of the growth-promoting substance to one side of the shaft. The faster growth at that side causes the bending." Would this explanation be true for root gravitropism? Explain.

Ans.: No, because in roots the growth affecting substance (auxin) accumulates on the lower side where it inhibits growth.

(b) What experimental treatment could you use to make a vertically growing root bend to the left without moving the root to a different position?

Ans.: Apply auxin (or calcium) to the left side of the root, making it grow slower than the right side.

(c) Plants genetically engineered to be defective in starch synthesis are defective in gravitropism. Some have argued that this result means that statoliths are the gravity sensors in plants, and their downward movement is what triggers gravitropism. Describe experimental evidence that argues against this hypothesis, and provide an alternative explanation for why statoliths are important for gravitropism.

Ans.: Rice roots growing in a medium of equal density to the density of the protoplasm show severely reduced gravitropism. In this situation the protoplasm floats, but the amyloplasts in the root cells sink, indicating that the more important cellular structure that has to fall to cue roots as to which way is up is the protoplasm not the amyloplasts. In these cells the primary function of the amyloplasts would be to add mass or weight to the protoplasm thus helping it to create more tension and compression forces to trigger the gravitropic response.

2. (a) What are integrins, to what are they connected, and what do they "integrate"?

Ans.:Integrins are transmembrane proteins that connect to cytoskeletal proteins on the cytoplasmic side of the membane and to large ECM proteins on the outside of the membrane. Their connection to the cytoskeleton integrates the entire protoplasm as a single mass unit.

(b) A group at Cornell used a peptide to inhibit the gravity response in *Chara* cells, but the peptide worked only when it was applied to the ends of vertically-oriented cells. What was this peptide, what specific biochemical activity did it inhibit in order to block the gravity response, and why did it block the gravity response when applied on the ends and not when applied on the sides of the cells?

Ans.: When Chara cells are in the vertical orientation all the tension and compression generated by the protoplasm is on the horizontal (top and bottom) walls, and none is on the side. Thus using RGD peptides to disconnect integrins from the protoplasm along the sides of the cells would not affect the tension and compression forces, but using it to disconnect integrins along the top of the cell would.

3. (a) Name two photoreceptors whose activation inhibits hypocotyl elongation, and describe one main difference between them.

Ans.: Phytochrome and cryptochrome. Phy is photoreversible and in the Pr form absorbs mainly red light; Cry is not photoreversible and absorbs mainly blue light.

(b) Knocking out the genes encoding two closely related proteins in a plant would make that plant defective in its ability to grow toward the light and its ability to begin breathing in CO_2 at dawn. What are these two proteins, and why do the genes encoding both of them have to be mutated for the defects to show up?

Ans.: Phototropin 1 and 2, and both have to be mutated because they can complement each other's function.

Question 3, continued:

(c) If you irradiated a purified plasma membrane preparation with blue light in the presence of ATP that had its terminal (γ) phosphate radioactively labeled, what key biochemical change would take place in the membrane and why?

Ans.: A protein in the membrane would be phosphorylated, because the blue light photorceptor, phototropin is a plasma membrane protein, and it autophosphorylates itself when it is activated by blue light.

4. (a) In what kind of plant would you observe the highest concentrations of phytochrome (phy), which phy would be dominant in this plant, and what is the survival benefit to the plant to have this much phy?

Ans.: Etiolated (dark-grown) seedlings have very high concentrations of phy, and most of it is PhyA. The high concentration of phy makes the seedling more sensitive to light, so that the seedling can be induced to start making its photosynthetic machinery when it encounters even the dim light that penetrates through an inch of soil. This gives the seedling a head start on becoming autotrophic, which could be important for survival, because its seed food reserve is depleted the longer it grows in darkness.

(b) Phy activation dramatically changes the metabolism of a cell. Do those changes typically spread to surrounding cells? Give evidence for your answer.

Ans.: No. Activating phy in the skin of an unripe (green) apple that is partially covered by black tape will allow the exposed skin to turn red, but not the adjoining covered skin.

(c) What survival benefit does the photoreversibility characteristic of phy confer to plants? Your answer should include information on where red and far-red light occur in nature.

Ans.: The red light that activates phy is a major component of unfiltered sunlight and the far-red light that reverses this effect is highly enriched in light filtered through green leaves. Seeds receiving primarily FR in nature are most likely surrounded by mature plants and are at a competitive disadvantage, so there is an adaptive advantage for these seeds not to germinate. Because in nature wind can temporarily move leaves allowing unfiltered sunlight to penetrate, seeds surrounded by other plants would occasionally receive unfiltered sunlight. The stimulatory effects of this light would have to be reversible by FR when the wind dies down and the leaves of the surrounding plants once again shade the seeds, otherwise occasional sunflecks could induce the seeds to germinate even though they are still at a competitive disadavantage.

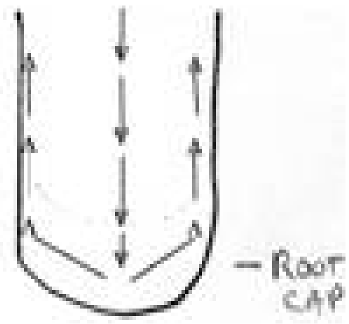
5. (a) What are the PIFs ,where do they occur in cells, and what do they regulate after they become active?

Ans.: PIFS are phytochrome-interacting factors, proteins that bind to phytochrome in the nucleus. Their interaction with phy can promote the transcription of some genes and inhibit the transcription of others.

(b) Among the genes regulated by phy, give an example of one that is up-regulated and one that is down-regulated, and for each describe the benefit of this response to plants.

Ans.: The β-hydroxylase gene whose product catalyzes the production of GA_1, a growth promoting hormone, is up regulated by phy; the phyA gene is down-regulated by phy in etiolated seedlings that don't need more phy once it has been activated. The down regulation helps assure that the plant will not be allocating valuable energy reserves to produce more phy when it doesn't need it.

6. (a) Auxin moves in a polar fashion both basipetally and acropetally. In the stick diagram on the right, use arrows to mark the tissue regions in which auxin flows, and in which direction it moves in those regions.



Question 6, continued

6(b) The movement of auxin from cell to cell requires that it enters cells and leaves cells. Briefly describe the main information scientists have about the transporters that mediate this movement.

Ans.: The transport of auxin into cells is through the AUX1 transporter protein, and the movement of it out is through a complex of proteins that is likely to include members of the PIN family of proteins and an MDR protein.

(c) An indirect method was used to monitor where auxin moved when roots are placed in a horizontal position. What is this method, and what did it reveal?

Ans.: A promoter-green fluorescent protein (GFP) construct was used in which the promoter, DR5, was very sensitive to auxin levels and increased GFP expression the more auxin was present. The results showed increased green fluorescence on the lower side of root tips, corresponding to the gravity-directed movement of auxin to that side of the root

7. (a) What roles do AUX/IAA and ARF1 proteins play in auxin-induced growth responses?

Ans.: AUX/IAA proteins repress the activity of ARF1, which is a positive-acting transcription factor. Auxin must promote the removal of AUX/IAA to relieve ARF1 of this repression and induce genes needed for growth responses.

(b) Aux/IAA proteins have 4 conserved domains. What are the main functions of Domain II and of Domains III and IV?

Ans.; Domain II is the instability domain that allows it to bind to the F-box protein Tir1 and become ubiquitinated and thus targeted for destruction. Domains III and IV are the dimerization domains that allow Aux/IAA proteins to dimerize either with other Aux/IAA proteins or with ARF1

(c) What is the main function of TIR1, and what are its various binding partners when it carries out its function?

Ans.: TIR1 is an F-box protein that is part of the SCF E3 ligase complex. Auxin binding to TIR1 increases the affinity of TIR1 for Aux/IAA proteins and thus increases the efficiency of ubiquitination of these proteins.

(d) How did the use of insect cells clarify how the function of TIR1 was enhanced by auxin?

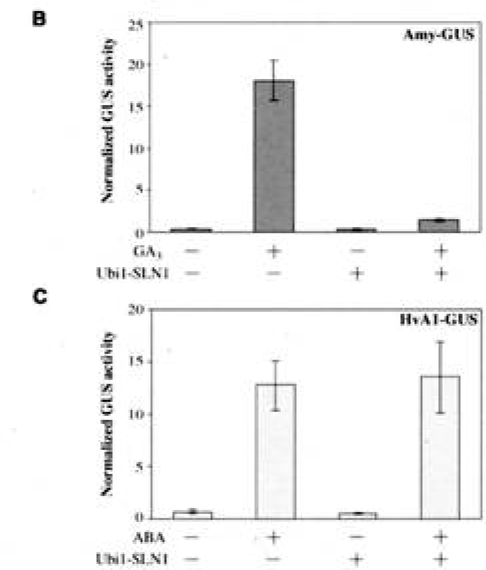
Ans.: Scientist knew that auxin bound to a TIR 1 preparation that was purified from plants, but they could not be sure it was binding to the TIR1 in the preparation or to some other plant protein. To address this question they expressed TIR1 in insect cells, which do not make plant proteins, and found that the affinity of auxin for this preparation of TIR1 was still very high, making it almost sure that auxin was binding to TIR1 and not to some contaminant protein.

8 (a) Three different seed tissues are involved in GA action in barley seeds. What are these tissues and how do they participate in the induction of seed germination by GA?

Ans.: The embryo of the seed makes GA, which then is transported to the aleurone layer where it turns on the transcription of alpha amylase (and other hydrolytic enzymes), and where it also promotes the secretion of alpha amylase into the endosperm of the seed, where it digests starch to glucose. The glucose is used as nutrition by the mature embryo to begin its growth during germination.

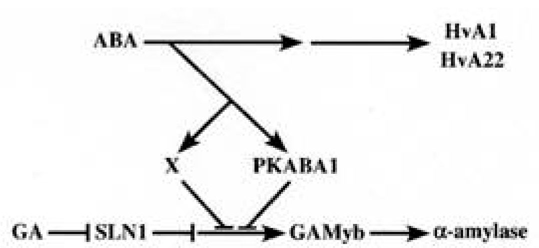
Question 8, continued

8 (b) In the Figure below, what are the main questions, what are the main conclusions, and how do the data shown support the conclusions?



Ans.: The main questions are whether SLN1 over-expression can reverse the promotive effects of GA on Amy-GUS expression and whether SLN1 has any effect on the expression of ABA-regulated genes. Fig. B shows in the bar on the far right that when SLN1 is overexpress in a plant GA cannot induce Amy-GUS activity. Fig. C shows in the bar on the far right that the overexpression of SLN1 has no effect on the ability of ABA to induce th HvA1-GUS gene.

(c) In the Figure below, what is the role of GAMyb, and what is the evidence that it plays this role?



Ans.: GAMyb is a positive regulator of GA-induced α-amylase gene expression. In mutant plants that have their GAMyb expression suppressed by RNAi GA cannot induce the expression of α-amylase.

9 (a) What are DELLA proteins, why are they given that name, and what are two DELLA proteins that play a role in GA responses?

Ans.: DELLA proteins, so-called because they all have the amino acid sequence DELLA in their primary structure, are repressors of transcription. SLN1 in barley and SLR1 in rice are two DELLA proteins.

(b) What alteration does GA induce in DELLA proteins, and by what mechanism does this alteration influence DELLA protein stability?

Ans.: GA induces the phosphorylation of the SLR1 DELLA proteins. This enhances the affinity of these proteins for F-box protein GID2, which binds to SLR1 and promotes its ubitquitination and destruction.

(c) What is GID2, what is its target of action, and how does its activity promote rice growth?

Ans.: GID2 is an F-box protein. Its target of action is the suppressor SLR1. The GID2-mediated ubiquitination and subsequent destuction of SLR1 relieves the suppression of GAMyb, a positive promoter of genes needed to promote rice growth.