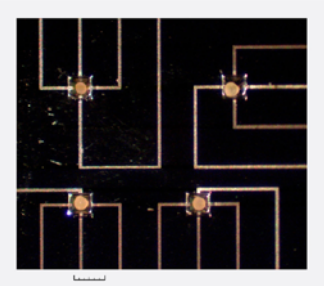
Bio 328, Fall 2009, Test #2 Name: KEY

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

1 (a) In the gravity response of Ceratopteris, the equipment illustrated below was used to measure different responses to gravity. What were two different effects of gravity demonstrated by this equipment?



Ans.: Using this microchip scientists were able to show that: 1) when the chip was turned upside down a calcium current across the spores on the chip also inverted and did so within less than 30 seconds (began to invert in less than 5 seconds); and 2) when spores on the chip were flown on a DC9, during parabolic flight the calcium current across the spores went up when the g-force went up and decreased to near zero when the g-force decreased to near zero, and these changes also occurred in just a few seconds.

(b) State evidence that asymmetric ion transport is important for the gravity response in fern spores.
Ans. Blocking calcium uptake along the bottom of the spore with nifedipine blocks the gravity response.

(c) State evidence that an asymmetric ion distribution could be important for a gravity response in roots.
Ans.: Asymmetric application of calcium along one side of a vertically growing root can make it bend toward the side calcium was applied.

2. (a) What biochemical activity does blue light induce in phototropin?
Ans.: Blue light induces protein kinase activity in phototropin, resulting in autophosphorylation.

(b) Describe an experiment that indicates the activity noted in your answer to 2(a) is important for downstream blue light responses. Your answer should include the term *phot1phot2*.
Ans.: *Scientists identified several serine (S) and threonine (T) residues that are autophosphorylated in the PHOT1 protein, then created mutated versions of the PHOT1 protein by substituting Alanine (A) for S or T in the phosphorylated residues of PHOT1, or by substituting Aspartic acid (D) for these S and T amino acids. They then tested whether the mutated version of PHOT1, when inserted into the phot1phot2 mutant, could complement the mutant and thus allow it to promote stomata opening by blue light (BL). Their results showed S851A could not complement, but S851D could*.

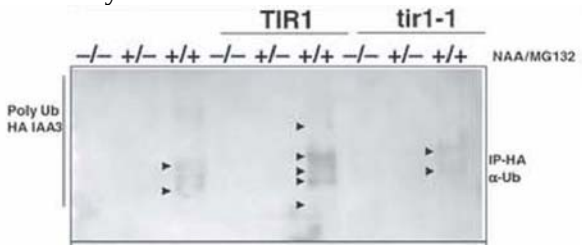
(c) When blue light activates phototropin, this hyperpolarizes guard cells by altering the activity of a transport protein. What is this transport protein, and why does a change in its activity hyperpolarize the guard cells?
Ans.: The transport protein is a proton-pumping ATPase, and its activation pumps protons (positive charges) out of the cell, thus making the membrane potential inside the cell more negative.

3. (a) When phytochrome induces seed germination it alters the level of GA4. How does it do this? Your answer should include the term transcription factor.
Ans.: Photoactivated phytochrome (Pfr) promotes the destruction of the transcription factor PIF1 thereby promoting the transcription of biosynthetic genes for enzymes that catalyze the production of GA4, and inhibiting the transcription of genes encoding enzymes that convert GA4 to an inactive GA.

b) Typically when white light activates phytochrome, other photoreceptors "cooperate" with phytochrome in inducing physiological responses. Give two examples of white light-induced phytochrome responses that typically involve the activity of other photoreceptors.
Ans.: White light both activates phytochrome and the light harvesting complexes that drive photosynthesis, and both are needed to promote the synthesis of anthocyanin for the red color of apples. White light also activates both phytochrome and cryptochrome and both pigments promote the inhibition of hypocotyl growth.

(c) What are the relative levels of HY5 and PIF1 in dark-grown plants, and how do these levels change when phytochrome is converted to the Pfr form?
Ans.: In the dark, HY5 levels are low and PIF1 levels are high; in the light, when phytochrome is converted to Pfr, HY5 levels are high and PIF1 levels are low.
4. (a) How do AUX/IAA proteins alter the function of ARFs, and how does auxin affect the AUX/IAA-ARF interaction?
Ans.: Some AUX/IAA proteins bind to ARFs and block their ability to turn on auxin-regulated genes. Auxin promotes the destruction of AUX/IAA proteins, and thus releases ARFs from AUX/IAA suppression.

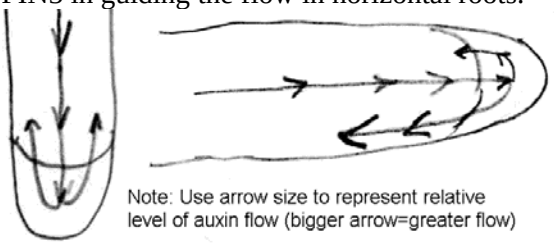
(b) In the Figure below, what is being visualized in the dark bands and why does the presence of MG132 increase the intensity of these bands?



Ans.: The dark bands represent ubiquitinated IAA3, and the presence of MG132 prevents the proteolysis of this protein, thus allowing its visualization on the gel.

The evidence

(c) A "fountain" model describes auxin transport into and out of root tips. In the root tips below, use arrows to describe auxin flow when the root is in a vertical and horizontal position, and describe evidence for the role of PIN3 in guiding the flow in horizontal roots.



Ans.: The evidence that PIN3 helps guide the flow is that it facilitates the export of auxin from cells, and upon gravi-stimulation it becomes asymmetrically distributed primarily along the bottom side of root cap cells, which would promote the movement of auxin preferentially toward the bottom flank of the horizontally positioned root.

5. (a) Stephen Spielberg, who works as a plant physiology lecturer in evenings to help pay the bills, once speculated to his class that DELLA was so named because this was the name of the discoverer's mother. What is the function of DELLA proteins, and why is DELLA an appropriate name for these proteins?
Ans.: DELLA proteins are negative regulators of the transcription of genes that would be turned on by GA. DELLA proteins all have a DELLA sequence of amino acids in their primary structure.

(b) What is the sequence of events in seeds that leads from GA treatment to the destruction of SLR1? Include in your answer the term "GID2".
Ans.: GA binds to GID1 and this complex associates with SLR1, targeting it for binding to GID2, an F-box protein in an SCF E-3 ligase comples. This interaction results in the ubiquitination of SLR1 and its destruction by proteolysis.

(c) How does ABA antagonize GA effects on seed germination?
Ans.: ABA promotes the production of two nuclear proteins (OsWRK51 and OsWRK71) which form a complex that binds to a promoter region for the Amy32b gene, blocking its transcription, whether or not GA is present and SLR1 is absent. The Amy32b gene encodes alpha amylase, and without production of this enzyme, seed germination is suppressed.

6. (a) AIP2 and KEG are two RING-type E3 ligases. How does ABA influence their level or activity, and how do they regulate ABA responses?
Ans.: ABA increases the level of AIP2 which increases the ubiquitination and turnover of AIB3. AIB3 helps promote the trascription of AIB5, but its turnover helps turn off ABA responses that have been activated. ABA decreases the ability of KEG to ubiquitinate AIB5, which increases its level. AIB5 is a positive regulator of ABAS responses

(b) During drought stress ABA levels go up. Describe two adaptive tissue-level changes induced in plants by ABA in response to this stress, and indicate the survival benefit of each change.
Ans.: 1) Root growth is inhibited. This allows the plant to divert more of its resources to supportive stress responses. 2) Stomates are induced to close. This reduces water loss by transpiration.

7. (a) What is a key rate-limiting enzyme in ethylene biosynthesis, what are the substrate and product of this enzyme, and what inhibitor blocks its activity?
Ans.: Rate-limiting enzyme is ACC synthase,whose substrate is SAM and whose product is ACC. AVG blocks ACC synthase activity.

(b) In describing the structure and activity of ethylene receptors the terms copper, histidine, aspartic acid, and CTR1 are used. Use all these terms in describing the structure/activity of ethylene receptors.
Ans.: The receptor has an essential copper ion bound to it, and it is a histidine kinase that phosphorylates itself at a histidine residue. This phosphate is then transferred to an aspartic acid on the receptor to fully activate it. The activated receptor inactivates the negative regulator kinase, CTR1, which allows activation of the positive regulators of the signaling pathway.

(c) There has to be a mechanism for turning off what hormones turn on. Describe the feed-back mechanism that helps turn off responses turned on by ethylene.
Ans.: Ethylene binding allows the positive transcription factor EIN3 to turn on genes that promote ethylene responses, but EIN 3 also turns on the production of EBF1 and EBF2, F-box proteins that target EIN3 for ubiquitination and destruction, thus turning off the ethylene response.

8. (a) In plants hormones typically act in concert with other hormones. What experimental observation illustrates this principle in the case of cytokinin-auxin interaction?
Ans.: The ratio of cytokinin to auxin determines whether roots or shoots will develop from cells in tissue culture.

(b) Describe the feed-back mechanism that helps turn off responses turned on by cytokinin.
Ans.: The signaling pathway that is activated by cytokinins ultimately leads through phospho relays to the phosphorylation of an A-type ARR in the nucleus, which can serve as a feed-back negative regulator of cytokinin-activated genes.

(c) There are multiple receptors for cytokinin, but all are not equally important for inducing different cytokinin responses. Point out one cytokinin receptor that appears to be more important for one cytokinin response, and give evidence for your answer.
Ans.: As judged by a cytokinin assay based on chlorophyll retention, the knockout of the AHK3 receptor for cytokinin lowers chlorophyll content more than the KO of two other cytokinin receptors, indicating that AHK3 plays a more important role than the other two for this response.

9. (a) Describe two lines of evidence that BRI1 is a brassinosteroid receptor.
Ans.: Plasma membranes isolated from plants overexpressing BRI1 bind more labeled brassinosteroid than wild-type plants;.BRI1 overexpressing plants are longer/taller than wild-type; bri-1 mutant plants are smaller than wt plants and are insensitive to added brassinosteroids.

(b) What is *det2,* what is its defect, and what is its main phenotypic difference from wild-type in darkness?

Ans.: *det2 is a mutant that cannot make brassinosteroid, and consequently has a phenotype in darkness that shows a short hypocotyl, as if it has already been de-etiolated by light.*