Bio 328, Spring 2006, Test #3 Name: KEY

Provide concise answers in the space provided after each question, or, 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 ethylene signaling pathway, name three proteins that function downstream of CTR1, and for each one indicate its function.
Ans.: EiN2, positive regulator of ethylene responses; EBF1, 2: F-box proteins that negatively regulate the stability of EiN3 and EiLs, which are transcription factors that turn on ethylene regulated genes; ERF1, transcription factor that regulates the transcription of ethylene responsive genes.

(b) What would be three features of dark-grown seedlings that have a knock-out mutation in CTR1?
Ans.: Shorter, thicker hypocotyls; disoriented growth of hypocotyls.

2. (a) What are two responses that whole plants show to cytokinin treatment?
Ans: Inhibits root growth and branching, alters light sensitivity for phytochrome responses, retards senescence and increases retention of chlorophyll.

(b) In tissue culture what hormone interacts with cytokinins in controlling root and shoot development, and how would you change the ratio of these two hormones to favor (i) root development or (ii) shoot development?
Ans.: Auxin interacts with cytokinins in controlling root and shoot development. Higher ratio of auxin to cytokinin promotes root development; higher ratio of cytokinin to auxin promotes shoot growth.

3. (a) What is the main phenotype of knock-out mutants that cannot express a functional version of AHK3? AHK3 mutants have other less-dramatic phenotypes, but a second mutation can enhance or exaggerate these phenotypes. What is this second mutation and why does it enhance the effects of mutating AHK3?
Ans.: Main phenotype of AHK3 knock-out mutants is accelerated senescence. Second mutation would be to knock out one of the other two his kinases that serve as cytokinin receptors, AHK2 or AHK4, because these receptors can partially complement or replace the function of AHK3.

(b) What is ARR2, and what does it have to do with cytokinin signaling?
Ans.: ARR2 is a response regulator that serves as the receiver domain for AHK3, and so it is necessary to transduce cytokinin effects on downstream cytokinin responses such as delayed senescence of leaves.

(c) In ethylene signaling, what peptide functions in the equivalent role of ARR2, and how is its position relative to the kinase active site different than that of ARR2?
Ans.: The peptide that functions in the equivalent role is the receiver domain of the ETR1 receptor, which, unlike ARR2, which is a separate protein, is positioned as part of the same receptor that houses the kinase active site.

4. (a) The systemin defense system in plants slows the growth rather than kills plant herbivores. Explain.
Ans.: The systemin defense system primarily promotes the production of protease inhibitors, which suppress the protein digestion of the feeding herbivore. This reduces the nutrition and growth of the herbivore, but does not kill it.

(b) Ibuprofen is a lipoxygenase inhibitor. Why would this interfere with plant wound responses?
Ans.: Lipoxygenase activity is crucial for JA production, which is crucial for mediating wound responses.

(c) The treatment of plant A with linolenic acid induces plant B to begin making defense compounds. Why?
Ans.: Increased linolenic acid in plant A induces increased in JA production. JA is readily converted to methyl jasmonates, a volatile hormone that can induce defense compounds (protease inhibitors) in plant B.

(d) Describe experimental evidence that Jasmonic acid is actually an effective defense compound in plants.
Ans.: Mutant plants that cannot make linolenic acid in response to a feeding insect also cannot make JA, and this defect results in the mutant plants showing higher mortality when exposed to herbivores. This mortality is reduced if the mutants are treated with JA.

5 (a) Animal cells have two broad classes of receptors for eATP. What are these two classes and how do they differ in the way they mediate downstream signaling steps?
Ans.: The two classes are P2X and P2Y which both induce the downstream signaling stem of increased cytosolic calcium. P2X receptors function as cation channels that open and lets calcium into cells when it is activated by eATP. P2Y is a G-protein linked receptor. When it is activated it increases the cytosolic calcium level through the mediation of IP3 which releases calcium from internal stores.

(b) An ATP receptor has not yet been found in plants, but what are two lines of evidence that, if plants have this type of receptor, it will resemble the animal receptor somewhat in its properties?
Ans.: If plant responses to eATP are mediated by a receptor, they are likely to resemble animal receptors in that. like animal receptors, their activation leads to an increase in cytosolic calcium, and they are blocked by the same antagonists as the animal receptors.

(c) Normally levels of extracellular ATP (eATP) are extremely low. What stimulus often experienced by plants would be expected to increase the [eATP], and what is the evidence that eATP can help mediate the typical downstream responses induced by this stimulus?
Ans.: A wound stimulus increases [eATP], and eATP can induce the same responses as a wound stimulus when applied to an unwounded leaf.

6. (a) What is WRKY70, and why is it said to occupy a "node" or "crossroad" position in plant responses to pathogen attack? Your answer should include what happens upstream and downstream of the WRKY70 "node".
Ans.: WRKY70 is a protein kinase. Those pathogens that upregulate WRKY70 induce salicylic acid (SA) production and the defense responses induced by SA; those pathogens that downregulate WRKY70 upregulate JA production and the defense responses induced by JA.

(b) Salicylic acid (SA) both suppresses a certain kind of plant defense and activates other kinds. Describe one activity SA suppresses and two that it activates.
Ans.: SA suppresses lipoxygenase activity and, consequently, JA production, and it activates the production of PR proteins, the hypersensitive response (localized programmed cell death), and the systemic acquired resistance response.

7. (a) If you wanted to lock a G-protein mediated response in an activated mode, what bacterial toxin would you use and why does this toxin "freeze" the G-protein in an active state?
Ans.: Cholera toxin locks the $G\alpha$ subunit of G-proteins in an activated state because it blocks the GTPase activity of $G\alpha$.

(b) In animal cells an eATP stimulus can turn on a calmodulin-regulated enzyme even if plasma membrane calcium channels are blocked. Describe four intermediate transduction steps that would help convert the receptor activation to enzyme activation.
Ans.: In animal cells that have blocked plasma membrane calcium channels, eATP can active a P2Y type receptor. This would lead, sequentially, to activation of a heterotrimeric G protein, activation of a phospholipase C, production of IP3, and release of calcium from internal stores, which would activate calcium-dependent enzymes (like CDPK).

8. (a) Define what is CDPK, and name one transport activity it regulates.
Ans.: CDPK is a calcium-dependent (or calmodulin-domain) protein kinase. In stomatal cells, calcium-activated CDPK can phosphorylate and depress the K+-import activity of an inward K+ channel.

(b) Why does the transport activity identified in your answer to 8(a) alter transpiration rates in plants?
Ans.: The closing of inward K+ channels in guard cells together with the activation of outward directed K+ transporters promotes the loss of water from the guard cells and closing of stomates, which reduces transpiration.

(c) In the cells expressing CDPK, the same signaling step that activated CDPK could also activate other molecules. Name two other molecules this signaling step could activate, and for each describe a possible downstream effect of that activation.
Ans.: Two other molecules are: (1) calmodulin, which can bind to and activate enzymes like NADPH oxidase or transporters like Ca ATPase, and (2) annexins, which can promote the fusion of secretory vesicles or serve as calcium channels.

9. (a) Various stimuli (gravity, light, wounding, etc.) induce an increase in cytosolic calcium concentration ($[Ca^{2+}]_{cyt}$) as a necessary step in the signal transduction chain leading to the specific responses they generate. Describe three ways such a common, generic signaling step can be interpreted in such a way as to lead to the specific responses generated by each stimulus.
Ans.: Specificity of the calcium signal can be achieved by (1) differences in the molecular signaling components of the microdomain in which the calcium changes take place; (2) differences in the magnitude of the calcium increase and (3) differences in the rhythm or pulse or frequency of successive calcium spikes.

(b) The earliest report of phytochrome-induced change in calcium transport in 1980 revealed that red light induced an efflux of calcium from cells. Later in 1992 another paper reported that phytochrome induced a rapid increase in $[Ca^{2+}]_{cyt}$. Explain why the results of these two papers are not contradictory.
Ans.: Both responses could be induced by phytochrome, because the increase in $[Ca^{2+}]_{cyt}$ reported in the 1992 paper could be expected to be followed by an increase in the efflux of calcium reported in the 1980 paper, because increased $[Ca^{2+}]_{cyt}$ can activate calmodulin which can activate calcium pumps that pump calcium out of cells.

(c) Det 3 mutants are defective in a vacuolar-type proton pumping ATPase, and when they are stimulated by ABA their stomates fail to close. Scientists also noted that in the guard cells of these mutants ABA induces an increase in $[Ca^{2+}]_{cyt}$, but, unlike in wild-type guard cells, the calcium concentration stays high and does not rapidly return to base-line levels. Why is this result considered important in considering how calcium induces both stomatal closure and stomatal opening?
Ans.: Data comparing certain mutants to wild-type cells suggest that the rhythm or frequency of calcium spikes appears to be a critical aspect of whether or not the increased $[Ca^{2+}]_{cyt}$ results in stomates staying open or closing. In the Det3 mutant there are no successive spikes of calcium changes, and this lack is considered to be a plausible explanation for why the stomates do not close.

(d) (2-point BONUS) In the lecture yesterday, Prof. Raoul Ranjeva described changes in $[Ca^{2+}]$ in two different cellular compartments. What were these two compartments, and what evidence was shown that these compartments change their $[Ca^{2+}]$ independent of each other?
Ans.: The two compartments are the cytosol and the nucleus, and the evidence that these two compartments change their $[Ca^{2+}]_{cyt}$ independent of each other is that isolated nuclei (with no surrounding cytoplasm) can be induced by various stimulit to change their $[Ca^{2+}]_{cyt}$.