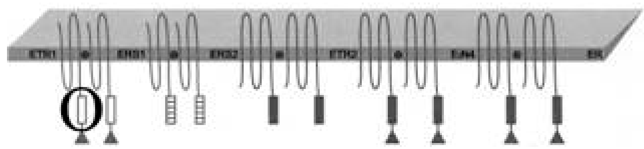
Bio 328. Spring 2005 KEY

Test #3

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

1. (a) In the Figure below, circle the histidine kinase domain of ETR1.



(b) Describe an experiment and results that revealed whether histidine kinase activity is required for ETR1 function.

Ans.: Scientists found that a modified gene that coded for an ETR1 protein that had an only slightly altered histidine kinase domain, but no histidine kinase activity, could substitute for a wild-type gene and maintain full ethylene sensitivity.

(c) What is the functional relationship of ETR1 to CTR1 and how does ethylene binding change this relationship, and thus change the function of CTR1?

Ans. : In the absence of bound ethylene ETR1 binds to CTR1 allowing CTR1 to inhibit the activity of a positive regulator EIN2. When ethylene binds to ETR1, it releases CTR1 and this de-represses EIN2.

2. (a) Define what is EIN3, compare its relative level in wild-type plants in the presence and absence of ethylene, and indicate by what mechanism ethylene changes the level of EIN3.

Ans.: EIN3 is a transcription factor that induces the transcription of ethylene responsive genes. Its level is higher in the presence than in the absence of ethylene, because ethylene presence on its receptors results in the inactivation of two F-box proteins, EBF1 and EBF2. In the absence of ethylene these proteins would promote the ubiquitination and subsequent destruction of EIN3.

(b) What is the functional relationship of EIN3 to ERF1?

Ans.: One of the genes turned on by EIN3 is the gene encoding ERF1.

(c) ERF1 is both a positive regulator and a negative regulator of ethylene action. Explain.

Ans.: ERF1 induces the increased transcription of a number of genes needed for ethylene effects on plant growth and development, so it is a positive regulator of ethylene action. It also increases the transcription of the genes for EBF1 and EBF2, which negatively regulate EIN3, so in this respect it is also a negative regulator of ethylene action.

3. (a) A wound in one part of a plant induces other parts of the same plant that are unwounded to make protease inhibitors. What are the 2 hormones that can carry the wound message from the wound site to other parts of the plant and what are the 2 routes by which these hormones travel?

Ans.: The two hormones are systemin, which travels through the vascular system of plants, and methyl jasmonate, which is a volatile hormone that travels through the air.

(b) Knocking out the lipoxygenase gene would block one of the two communication routes noted in your answer to 3(a). Explain why.

Ans.: Lipoxygenase is needed to convert linolenic acid to Jasmonic acid, so knocking out the gene that encodes this enzyme would block the formation of methyl jasmonate and the air route of communication.

(c) Plants transformed with the antisense to a gene that encodes an 18 amino acid peptide hormone look like wild-type plants. What treatment would reveal how they are different from wild-type plants? Explain your answer.

Ans.: Allowing Manduca larvae to feed on wild-type and antisense plants would reveal that these larvae do not grow as much on wild-type plants as they do on mutant plants.

4. (a)) Scientists have wondered what signals are released at a wound site that induce the transduction events leading to the production of protease inhibitors. Describe evidence that suggests ATP could be one of those signals.

Ans.: Concentrations of ATP that are released from wounded cells and accumulate at wound sites are sufficient to induce superoxide production, and superoxide production is an important prerequisite for systemin induction at the wound site. Systemin can induce protease inhibitors in wounded plants.

(b) Animals have known P2 receptors that respond to ATP. What is the evidence that plants may have receptors that are structurally and functionally similar to the animal receptors?

Ans.: Inhibitors that block the animal P2 receptors block ATP effects on plants and ATP induces an increase in $[Ca^{2+}]_{cyt}$ *in plant cells like it does in animal cells.*

(c) Does ATP have to be hydrolyzed to serve its signaling role? Give evidence for your answer.

Ans.: No, because non-hydrolyzable forms of ATP can induce the same responses as normal ATP.

5. (a) What is the hypersensitive response of plants to pathogens, and what is the survival benefit of this response for the plant?

Ans.: The hypersensitive response is the induction of programmed cell death in cells in and near the site of pathogen infection. This isolates the pathogen in dead cells, inhibiting its movement to surrounding cells and thus limiting the infection damage.

(b) What are three lines of evidence that salicylic acid may help mediate the hypersensitive response in plants?

Ans.: 1) salicylic acid levels increase at the site of infection; 2) plants expressing a bacterial enzyme that reduces salicylic acid content in cells are more susceptible to infection than wild-type plants; 3) treating susceptible plants with salicylic acid increases their ability to develop the hypersensitive response.

(c) What is systemic acquired resistance, and what changes occur in those parts of the plant that have acquired this resistance that help to explain why they have become more resistant?

Ans.: Systemic acquired resistance is a state of pathogen resistance in plants in which the original attack of a pathogen confers increased resistance to subsequent attack by pathogens in leaves and other parts of the plant that were not infected by the original attack. A key change in parts of the plants that have acquired resistance is that they are expressing pathogen resistance (PR) proteins.

5 pts. 6. (a) The binding of GTP activates G-proteins. What enzyme activity inactivates G-proteins, and what subunit has this activity?

Ans.: The GTPase activity of the α *subunit of G-proteins is the activity that inactivates G-proteins.*

5 pts. (b) In the Figure below, what protein serves the role of "relay" molecule, what is an example of an Effector target of the relay molecule, and how does the activation of that effector help further amplify the signal?

Ans.: The relay molecule is the α *subunit of G-proteins; an effector target is phosphlipase C, which generates from a PIP2 substrate IP3 and DAG, two agents that can amplify signals further.*

5 pts. 7. (a) If you knocked out a gene encoding a Gα protein in Arabidopsis, what is one response system you would disable in the mutated plant?

Ans.: ABA-induced stomal closure.

5 pts. (b) In the response system you noted in your answer to 7 (a), describe the signal transduction pathway starting with the inducer of the pathway through to the final response induced.

Ans.: In guard cells, ABA activates G protein, which induces increased $[Ca^{2+}]_{cyt,}$ which activates CDPK, which phosphorylates an inward K+ channel, which causes it to close. In the absence of K+ uptake the cell cannot take up water and swell, and the stomates cannot open.

8. (a) What function of calmodulin helps account for its name?

Ans.: Calmodulin modulates the $[Ca^{2+}]_{cyt}$ by activating calcium pumps that remove calcium from the cytosol by pumping it out of the cell or into vacuoles or ER.

(b) What enzyme in a proposed wound signaling pathway is activated by calmodulin, and what is the key signaling product of that enzyme.

Ans.: Calcium-activated calmodulin activates NADPH oxidase, and the main product is superoxide, which can induce downstream wound signaling events.

(c) Calmodulin is small enough to diffuse into the nucleus. What critical function is it known to play there?

Ans.: Nuclear calmodulin can bind to and activate transcription factors.

9. (a) There are hundreds of G-protein receptors in animals that initiate hundreds of different signal transduction chains leading to different responses. What is one way animals, but not Arabidopsis plants, can achieve specificity in the signaling response using the same kind of G-protein intermediate step?

Ans.: Some animals have 23 different Gα, six Gβ, and 12 Gγ subunits, and so have the potential to assemble more than a thousand different G proteins.

(b) Why does it appear that Arabidopsis plants cannot use the same strategy as animals in achieving specificity of response through G-protein intermediates?

Ans.: *In contrast to animals, plants have only one clearly identifiable Gα, one Gβ, and possibly two Gγ proteins, and so they cannot achieve much diversity of their G-proteins by different combinations of the alpha, beta, and gamma subunits.*

(c) Increases in $[Ca^{2+}]_{cyt}$ can promote both the opening and closing of stomata. How do guard cells figure out whether to swell or shrink when they experience an increase in $[Ca^{2+}]_{cyt}$?

Ans.: Guard cells can distinguish different calcium oscillation frequencies. Environmental signals that induce stomate closure have an oscillation period separating them of more than 5 min.; signals that induce stomate opening have a period of less than 5 min. Thus a calcium oscillation frequency of greater than 12/hr induces stomate opening, but a calcium-pulse frequency of less than 12/hr induces stomate closure.