Arabidopsis histidine kinase

CONSTRUCT

What’s already known:

-PhoB works as a response regulator in plants and localize to the nucleus

Rationale for using Cph1: already a two-component signaling system. Phytochromes lack the 5 domains critical for histidine kinase activity. Need histidine kinase activity.

CONSTRUCT:

1. Chimeric transmembrane receptor:
   1. Clone histidine kinase domain of Arabidopsis Histidine Kinase (AHK) into a plant expression vector.
   2. Conduct CLUSTALW alignment of Cph1 against Arabidopsis Histidine Kinase (AHK) to figure out which section of Cph1 to use (i.e. take out the histidine kinase domain of Cph1). Conduct a small library screen by varying the number of Cph1 amino acids included (ex. +/- 9 amino acids as done for the bacterial photography system).
   3. Also put in accessory plasmid that makes haem 🡪 PCB.
2. Couple to PhoB induced expression of luciferase
   1. Promoter design: 4 PhoB boxes + relevant plant promoter
3. Transform plasmid with Luciferin Regenerating Enzyme
   1. Regenerates luciferin from oxyluciferin (So need luciferase in the first place)
4. Transform into plants with Agrobacterium

EXPERIMENTS:

1. Confocal microscopy study: Assay to make sure that in presence of light, PhoB doesn’t localize to the nucleus (and that it localizes to the nucleus without light).
   1. Stain with DAPI to make sure it is indeed the nuclei
2. Luciferase assay to make sure light-responsive enzymatic activity occurs.
3. Make sure promoter is not constitutively on
4. Mutate the aspartate on response regulator to alanine to see if phosphorelay is responsible for this signal
5. Measure luciferase expression as a function of decreasing input light stimulus.
6. Measure power output and metabolism effects.
   1. LU per leaf/stem/root area 🡪 Scale up for the entire plant
   2. Compare this to the LU required by human eyes to be considered a noticeable light.
   3. CCD counter: digitally capture optical counts
7. Test ideal concentration of luciferin (tobacco plants – after 400 uM was toxic)
8. Increasing kinase function (or decreasing intensity of light):
   1. Means more luciferase is made
   2. So 1) higher light intensity because higher enzymatic activity because more enzymes
   3. 2) Shorter time of light expression. Faster depletion of substrate.\* (Depends: will we make LRE dependent on light stimulus too?)
      1. This depends on relative strengths of LRE and luciferase.
9. Determine energy balance of plants (i.e. from using ATP in luciferase activity)

FUTURE STEPS

1. Try to find homology to put in larger plants (like trees!)
2. Find luciferin coding gene.
   1. Ex. Look for actively transcribed mRNA in luminous organs of fireflies as a starting point.
3. Try to tune the activity to make more intense light (by genetic screen?)
4. Elucidate why PhoB translocates to the nucleus (it does not have a Nuclear Localization Signal)
   1. The paper admits they don’t know. First time a bacterial component translocates to the nucleus.

BIG SIGNIFICANCE:

-Reliable genetic components/parts to modify in plants (eukaryotes).