Detector Plants for Agriculture, Food and Environmental Monitoring

June Medford
Colorado State University
Fort Collins, Colorado
june.medford@colostate.edu

(No manuscript.)

[*Editors' note:* Dr. Medford described how techniques in synthetic biology—with funding from the Defense Advanced Research Projects Agency and the Department of Defense—are being developed to modify plants to detect with high sensitivity the presence of chemicals (*e.g.* pollutants, explosives) or pathogens and reveal their presence by rapid de-greening of chlorophyll, for use in agriculture and at high-population terrorist targets such as airports.]

Some of the information presented by Dr. Medford at NABC 23 has been published:

Antunes MS *et al.* (2006) A synthetic de-greening gene circuit provides a reporting system that is remotely detectable and has a re-set capacity. Plant Biotechnology Journal 4 605–622. doi: 10.1111/j.1467-7652.2006.00205.x

Summary: Plants have evolved elegant mechanisms to continuously sense and respond to their environment, suggesting that these properties can be adapted to make inexpensive and widely used biological monitors, or sentinels, for human threats. For a plant to be a sentinel, a reporting system is needed for large areas and widespread monitoring. The reporter or readout mechanism must be easily detectable, allow remote monitoring and provide a re-set capacity; all current gene reporting technologies fall short of these requirements. Chlorophyll is one of the best-recognized plant pigments with an already well-developed remote imaging technology. However, chlorophyll is very abundant, with levels regulated by both genetic and environmental factors. We designed a synthetic de-greening circuit that produced rapid chlorophyll loss on perception of a specific input. With induction of the de-greening circuit, changes were remotely detected within 2 h.

Analyses of multiple de-greening circuits suggested that the de-greening circuit functioned, in part, via light-dependent damage to photosystem cores and the production of reactive oxygen species. Within 24–48 h of induction, an easily recognized white phenotype resulted. Microarray analysis showed that the synthetic de-greening initiated a process largely distinct from normal chlorophyll loss in senescence. Remarkably, synthetically de-greened white plants re-greened after removal of the inducer, providing the first easily re-settable reporter system for plants and the capacity to make re-settable biosensors. Our results showed that the de-greening circuit allowed chlorophyll to be employed as a simple but powerful reporter system useful for widespread areas.

Antunes MS *et al.* (2009) Engineering key components in a synthetic eukaryotic signal transduction pathway. Molecular Systems Biology 5; Article number 270; doi:10.1038/msb.2009.28.

Abstract: Signal transduction underlies how living organisms detect and respond to stimuli. A goal of synthetic biology is to rewire natural signal transduction systems. Bacteria, yeast, and plants sense environmental aspects through conserved histidine kinase (HK) signal transduction systems. HK protein components are typically comprised of multiple, relatively modular, and conserved domains. Phosphate transfer between these components may exhibit considerable cross talk between the otherwise apparently linear pathways, thereby establishing networks that integrate multiple signals. We show that sequence conservation and cross talk can extend across kingdoms and can be exploited to produce a synthetic plant signal transduction system. In response to HK cross talk, heterologously expressed bacterial response regulators, PhoB and OmpR, translocate to the nucleus on HK activation. Using this discovery, combined with modification of PhoB (PhoBVP64), we produced a key component of a eukaryotic synthetic signal transduction pathway. In response to exogenous cytokinin, PhoB-VP64 translocates to the nucleus, binds a synthetic PlantPho promoter, and activates gene expression. These results show that conserved-signaling components can be used across kingdoms and adapted to produce synthetic eukaryotic signal transduction pathways.

Antunes MS *et al.* (2011) Programmable ligand detection system in plants through a synthetic signal transduction pathway. PLoS ONE 6(1): e16292. doi:10.1371/journal.pone.0016292.

Background: There is an unmet need to monitor human and natural environments for substances that are intentionally or unintentionally introduced. A long-sought goal is to adapt plants to sense and respond to specific substances for use as environmental monitors. Computationally re-designed periplasmic binding proteins (PBPs) provide a means to design highly sensitive and specific ligand sensing capabilities in receptors. Input from these proteins can be linked to gene expression through histidine kinase (HK) mediated signaling. Components of HK signaling systems are evolutionarily conserved between bacteria and plants. We previously reported that in response to cytokinin-mediated HK

activation in plants, the bacterial response regulator PhoB translocates to the nucleus and activates transcription. Also, we previously described a plant visual response system, the de-greening circuit, a threshold sensitive reporter system that produces a visual response which is remotely detectable and quantifiable.

Methodology/Principal Findings: We describe assembly and function of a complete synthetic signal transduction pathway in plants that links input from computationally re-designed PBPs to a visual response. To sense extracellular ligands, we targeted the computational re-designed PBPs to the apoplast. PBPs bind the ligand and develop affinity for the extracellular domain of a chemotactic protein, Trg. We experimentally developed Trg fusions proteins, which bind the ligand-PBP complex, and activate intracellular PhoR, the HK cognate of PhoB. We then adapted Trg-PhoR fusions for function in plants showing that in the presence of an external ligand PhoB translocates to the nucleus and activates transcription. We linked this input to the de-greening circuit creating a detector plant.

Conclusions/Significance: Our system is modular and PBPs can theoretically be designed to bind most small molecules. Hence our system, with improvements, may allow plants to serve as a simple and inexpensive means to monitor human surroundings for substances such as pollutants, explosives, or chemical agents.

June Medford, a professor of biology at Colorado State University, is a leader in the field of plant synthetic biology. She received her BS in botany from the University of Maryland and a PhD in biology from Yale, followed by postdoctoral training with the Plant Molecular Biology group at Monsanto.

Dr. Medford's research focus is on plant synthetic biology, the forward engineering of plants for specific purposes, both basic and applied. She has developed a synthetic signal-transduction system based on conserved histidine kinase components and a field-level synthetic readout system. By linking these synthetic systems together with computationally re-designed receptors, the Medford lab has produced the first sentinels to allow plants to serve as inexpensive and highly specific detectors of substances such as explosives, environmental pollutants and chemical agents. Detection levels are approximately 10- to 100-fold better than the detection abilities of dogs. Work is in progress to add ultra-sensitivity and memory for specific application (*e.g.* transportation hubs) and expand the detection platform to biological agents. Furthermore, the synthetic system is a biological input-output system and, hence, is being used to control biofuel and agronomic traits.