"The Role of ATHK1, a histidine kinase, as an osmosensor in *Arabidopsis thaliana*: studies using mutant alleles"

Keywords: *Arabidopsis thaliana, ATHK1, osmosensor, histidine kinase, water stress, agriculture, biofuels.*

Broader Impacts of Research:

In a recent letter in The New York Times entitled "Farmer in Chief," Michael Pollen addresses the future president elect: "Which brings me to the deeper reason you will need…to make the reform of the entire food system one of the highest priorities of your administration: unless you do, you will not be able to make significant progress on the health care crisis, energy independence or climate change." As Pollen goes on to expound, both food and biofuel crop research can be directly linked to national security and independence, environmental issues, and societal health. However, simply increasing crop yield is not the answer, as more and more land must be cleared, usually from previously undisturbed ecosystems in South America and Southeast Asia. This type of land use actually increases the "carbon debt" of the land, a debt which production of even the most efficient biofuel may take over 50 years to repay (Fargione, 2008). However, farming on degraded and abandoned land significantly reduces the carbon debt.

Degraded farmland has been abandoned for just that reason: it has been leached of nutrients, lost its water source, or significantly eroded, as often happens in tropical land cleared for agriculture. Much of this land could be reclaimed if crops were engineered to be more drought resistant and/or salinity tolerant. In addition, as climate change continues to occur, farming on already drought-prone land will become exceedingly difficult. Therefore, my proposed research is designed to help elucidate drought resistance in crop plants. More narrowly, I will focus on the ATHK1 gene in *Arabidopsis*.

Research Design:

ATHK1 is a histidine kinase in *Arabidopsis* thought to sense osmotic stress. Histidine kinase signal transduction pathways are common in many eukaryotes and involve three proteins: the sensor, the phototransfer protein, and the response regulator. The His kinase is a membrane-bound enzyme that, in response to stress, autophosphorlyates a conserved His residue, which is then transferred intramolecularly to a conserved Asp residue. First discovered in 1999, ATHK1 acts to complement to yeast deletion mutants deficient in the known osmosensor Sln1, and the ATHK1 transcript accumulates in *Arabidopsis* root tissues in response to high or low osmolarity (Urao, 1999). Recent ATHK1 research in the XXX lab at the University of Wisconsin-Madison has suggested that the abiotic stress activation of the ATHK1 signal transduction pathway induces abscisic acid biosynthesis, which in turn upregulates athk1 (Wolbach, 2008). In this way, the osmotic stress signal is propagated.

It is therefore likely that ATHK1 functions as an osmosensor in *Arabidopsis* and warrants more in depth research. First, I propose to investigate the function of the conserved Histidine and Aspartate residues in ATHK1. Using site directed mutagenesis, I will change each of these residues to several different residues (neutral, charged, bulky, etc.), transform knockout plants to athk1 using agrobacterium, and assay phenotypic performance to osmotic stress. I hypothesize that, due to their conserved nature, both of these residues are necessary for ATHK1 response, and mutants will therefore show phenotypes characteristic of osmotic stress, such as poor growth, compared to wild type plants.

Illumination into the role of ATHK1 as an osmosensor can also be gained by complementarity experiments. Just as Urao et al. (1999) complemented yeast deletion mutants to Sln1 with athk1 cDNA, so *Arabidopsis* deficient in ATHK1 should be complemented by sln1 cDNA. In addition, a sampling of 32 *Arabidopsis* accessions from different ecoclimatic regions showed 39 polymorphisms and 24 haplotypes, suggesting that the ATHK1 gene is not constrained against amino acid changes (Zhang, 2008), and that some alleles may confer better drought tolerance. I propose to transform laboratory strains of *Arabidopsis* knocked out for athk1, using agrobacterium gene transfer. Candidates for complementarity include different natural alleles found in *Arabidopsis* as well as those found in yeast. Plants will then be exposed to osmotic stress and phenotypic responses observed, as described in Wolbach et al. (2008). I would expect athk1 alleles from plants found in more arid regions to confer the most drought tolerance and, as athk1 cDNA complemented yeast Sln1 mutants, I would expect sln1 transformation to complement ATHK1 *Arabidopsis* mutants.

Arabidopsis is an ideal model for the experiments proposed above as its genome has been sequenced, it is easily transformed using agrobacterium, has short generation times, and has a large reservoir of mutants. However, findings in *Arabidopsis* will not necessarily translate to crops pertinent to food or biofuel agriculture. Therefore, I will also investigate into an ATHK1-like protein in a crop plant such as corn through sequence similarity database searches, identification of any putative histidine kinases similar in primary sequence to ATHK1, and knockout experiments as those described in Wolbach et al. (2008) to elucidate if the gene(s) identified is a regulator in response to osmotic stress.

Intellectual Merit and Broader Impacts of Biotechnology Education:

Several years ago I took a night class at Harvard University Extension titled "History and Ethics of Biotechnology." My fellow classmates were old and young, scientists and simply interested citizens. After listening to their opinions and interpretations of the various ethical questions discussed in class, I concluded that biotechnology, as it applies to society, may take many years to gain acceptance, but science has already been reaping the benefits for many years. Biotechnology has met with much global resistance: golden rice, designed to supply precious beta-carotene to nutrient poor societies and mitigate child blindess, was met with significant resistance due in part to its color; viral vectors as delivery mechanisms for genetic therapy may never see use in humans due to "disease" stigmas. Yet as scientists, I feel we have a duty to explore all possible solutions to a problem. Crop and biofuel production have the potential to affect every person in the world, directly or indirectly, and while many potential avenues of solutions are currently being explored, there are many left untraveled.

In addition to my own research I aspire to bring biotechnology to the classroom. I hope to bring hands-on experiments to local middle and high schools (see "Personal Statement"), and through these experiments I can educate future leaders, both in and outside of science, about biotechnology. Once the public, especially the youngest people, are educated and informed, public decisions regarding biotechnology can be made for the benefit of society.

Originality: I state that the work presented here is my own.

References:

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