Final Project Report Grant #: DE-FG02-03ER15426, Role of the Arabidopsis *PINHEAD* gene in meristem function.

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1. Executive summary:

The shoot apical meristems of land plants are small mounds of hundreds of cells located at the tips of branches. It is from these small clusters of cells that essentially all above ground plant biomass and therefore much of our energy supply originates. Several key genes have been discovered that are necessary for cells in the shoot apical meristem to take on stem cell properties. The goal of this project is to understand how the synthesis and accumulation of the mRNAs and proteins encoded by these genes is controlled. A thorough understanding of the molecules that control the growth of shoot apical meristems in plants will help us to manipulate food, fiber and biofuel crops to better feed, clothe and provide energy for humans.

2. Project activities

When the aims of this grant were written, we knew that the Arabidopsis *PINHEAD* and *ARGONAUTE* gene products had similarity to factors known or suspected to play roles in the control of translation initiation and post transcriptional gene silencing. We also knew that these gene products were required for the accumulation of SHOOTMERISTEMLES protein, but not RNA. We thus hypothesized that these proteins were responsible for promoting translation of the SHOOTMERISTEMLESS mRNA.

1. Determining association of transcripts with polyribosomes One of the major aims of the project was to determine if the PHABULOSA, SHOOTMERISTEMLESS, CYCLIND and FILAMENTOUS FLOWER mRNAs showed different patterns of accumulation in profiles of polyribosomes from wild-type and a variety of *pinhead* and *argonaute* mutants. Aim 1 was to determine the connection between mRNAs and polysome distribution on three targets – PHB, STM and REVOLUTA and FILAMENTOUS FLOWER. In several carefully carried out experiments, Dr. Rachael Huntley in the lab was unable to identify reproducible differences in polysome profiles between wild type and mutants. Thus, using this technique we were unable to acquire evidence for a role in translational control for the PNH and AGO proteins. It is important to recognize that the polysome assay measures the steady state behavior of ribosomes and their associated mRNAs. For instance, it is possible that

although mRNAs targeted by PNH or AGO acquire their associated ribosomes more slowly than wild-type, they nonetheless achieve full loading over time. One of the ways we proposed to determine additional targets for PNH and AGO action was by isolating mRNA from wild-type and mutant polysomes. However, since we could not detect a difference in the behavior of our strongest candidate genes, we did not pursue this line of experimentation.

2. AGO and PNH activity are required for sensitivity to the translational inhibitor cycloheximide.

In the course of our studies on *argonaute* and *pinhead* mutants, we found that mutations in both genes allowed seeds to germinate in the presence of cycloheximide. When grown on concentrations of cycloheximide that delayed germination of wild-type seedlings, *argonaute* and *pinhead* mutants germinated more rapidly. Seedlings homozygous for *pinhead* and heterozygous for *argonaute* germinated even more rapidly. Conversely, seedlings overexpressing *PINHEAD* exhibited a stronger delay in germination than wild-type. Cycloheximide inhibits translation of mRNAs by inhibiting the translocation of the ribosome along the mRNA.

Thus, while we were unable to show an effect of mutations in *PINHEAD* and *ARGONAUTE* on the loading of ribosomes onto mRNA, we have shown that the known translational inhibitor cycloheximide requires PINHEAD and ARGONAUTE activity to block germination.

3. Discovery of alternative forms of the SHOOTMERISTEMLESS transcripts

One of the genes under study in this project is the *SHOOTMERISTEMLESS* locus. SHOOTMERISTEMLESS protein but not mRNA requires *PINHEAD* and *ARGONAUTE* activity. Dr. Rachael Huntley has discovered two forms of mRNA made at the *SHOOTMERISTEMLESS* locus. We have shown that these correspond to two forms of the protein. The shorter form includes the MEINOX dimerization domain and the homeodomain. The longer form includes these but also includes an N-terminal domain that is poorly conserved among related proteins. We have constructed inducible forms of these to compare their behavior in planta and have used them to identify downstream targets of the SHOOTMERISTEMLESS locus.

4. Mapping of enhancers of pinhead mutations

In order to understand what pathways in the cell the PINHEAD protein acts, we isolated dominant enhancers of pinhead mutations in a screen for narrow petals. 8 enhancers were chosen for further study. Genetic analysis was performed on

these mutants by Dr Debbie Alexander, a postdoc in the lab, to map and describe the behavior of these enhancers in wild-type and *pinhead* backgrounds. This genetic analysis was key to the ultimate cloning of these genes which was done by Ken-Ichiro Hibara. In total, these eight enhancers have been shown to correspond to two novel gain of function alleles of HDZIPIII genes, two novel loss of function alleles of ARGONAUTE, an allele each of genes involved in epigenetic control, transcript elongation/ tRNA modification, mRNA 3' end formation and a gene of unknown function.

Publications resulting from this work.

Huntley, R., Sharp, M., Fernandez, A.G. and M.K. Barton, SHOOTMERISTEMLESS function in the Arabidopsis Shoot Apical Meristem, in preparation.

Hibara, K.-I., Alexander, D., Lynn, K. and M.K.Barton, Enhancers of the Arabidopsis pinhead mutant identify genes involved in epigenetic and microRNA mediated regulation, in preparation.

Lye, K.-W. and M.K. Barton, The *Arabidopsis ARGONAUTE* and *PINHEAD* genes are required for germination inhibition and regulation of mRNA transcript levels in response to the translational inhibitor cycloheximide, in preparation.