Final Progress Report (98ER20312)

This DOE grant supported studies focused on understanding the role of the Arabidopsis protein AINTEGUMENTA (ANT) in organ initiation and growth. The experiments were initially aimed in four directions: 1) identification of downstream targets of ANT regulation, 2) initial characterization of *AINTEGUMENTA*-like (*AIL*) gene expression and function, 3) investigation of the role of ANT using a cell culture system, and 4) structural studies of ANT protein. Summarized below are our significant findings during the period of funding.

Aim 1: Identification of downstream targets of ANT regulation

A. ANT contributes to organ polarity and upregulates expression of the polarity gene *PHABULOSA* (*PHB*)

We have found that loss of *ANT* activity in combination with mutations in one or more *YABBY* genes results in organ polarity defects greater than those observed in *yabby* mutants alone. Our results suggest that *ANT* acts in combination with *FILAMENTOUS FLOWER* (*FIL)* to promote organ polarity by upregulating the expression of the adaxial specifier *PHB*. We do not know if this regulation is direct as we have not been able to detect binding of ANT to the *PHB* promoter in vitro. ANT may also act with other factors to upregulate *FIL* and *YAB3* expression as ANT binds in vitro to a conserved sequence within the *FIL* and *YAB3* promoters. This work was published in Plant Physiology.

B. Floral homeotic genes are targets of ANT and AIL6 regulation

fil ant double mutants completely lack normal petals and stamens and expression of the class B gene *AP3* is reduced in *fil ant* flowers. Petals and stamens are also absent in *ant ail6* flowers. Correspondingly, we find that the levels of *AP3* and *AG* mRNA are reduced in these flowers and their spatial expression domains altered (Figure 1). Thus, *ANT* and *AIL6* act redundantly to regulate floral homeotic gene expression in young flowers. This work was published in Plant Physiology.

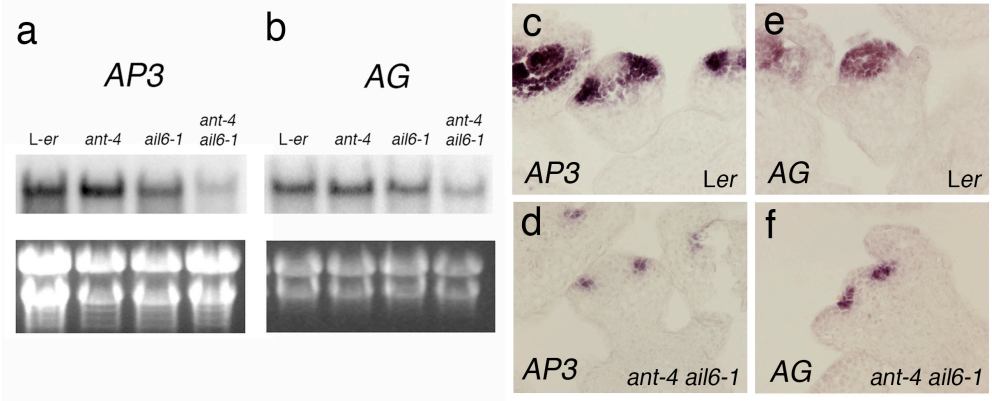

Figure 1. Regulation of floral homeotic gene expression by *ANT* and *AIL6*. a. and b. RNA blots showing *AP3* and *AG* mRNA in L*er*, *ant-4*, *ail6-1*, and *ant-4 ail6-1* inflorescences. c. In situ hybridization of *AP3* in a stage 3 L*er* flower. d. In situ hybridization of *AP3* in a stage 3 *ant-4 ail6-1* flower. e. In situ hybridization of *AG* in a stage 4 L*er* flower. f. In situ hybridization of *AG* in a stage 4 *ant-4 ail6-1* flower.

D. Global expression analysis upon induction of ANT activity

To identify direct targets of ANT regulation, we constructed *ANT:ANT-GR ant-4* plants in which activity of the ANT transcription factor is regulated by the steroid dexamethasone. These transgenic plants will be used in future RNA-Seq studies.

Aim 2: Characterization of *AIL* gene expression and function

A. Members of the *AINTEGUMENTA*-like (*AIL*) gene family are expressed in young developing plant tissues

We carried out an expression analysis of a small family of genes related to *ANT*, named *AINTEGUMENTA*-like (*AIL*) genes (Figure 2). Using real time PCR and in situ hybridization, we demonstrated that *AIL* genes are most highly expressed in young, still-dividing plant tissues. *ANT*, *AIL5*, *AIL6*, and *AIL7* exhibit partially overlapping spatial patterns of expression in inflorescences and flowers. We also showed that ectopic expression of *AIL5*, like *ANT*, is sufficient for organ growth. This work was published in Plant Molecular Biology.

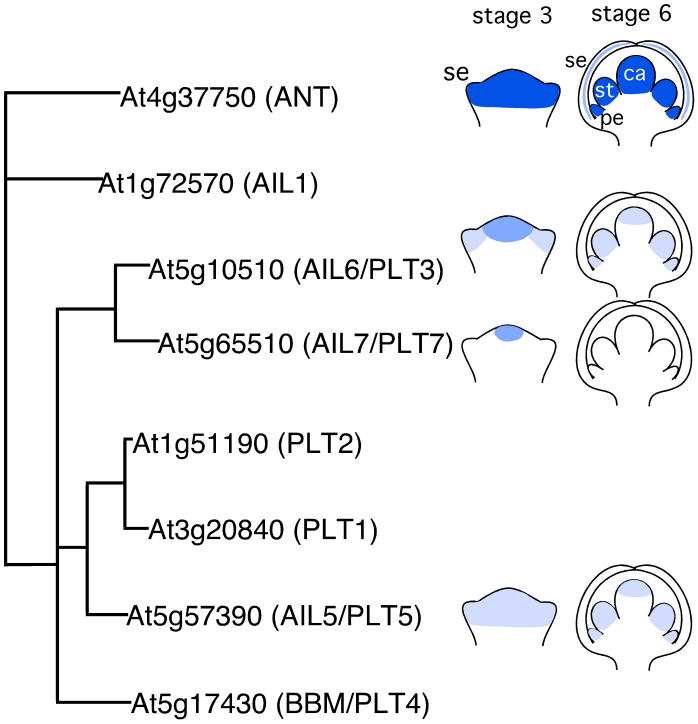

Figure 2. AIL/PLT family. Phylogenetic tree and mRNA expression patterns of *ANT*, *AIL5*, *AIL6* and *AIL7* in stage 3 and 6 flowers. Stage 3 flowers have initiated sepal (se) primordia while stage 6 flowers are composed of sepal, petal (pe), stamen (st) and carpel (ca) primordia. Intensity of the blue color reflects relative expression levels.

B. *ANT* and *AIL* genes display functional redundancy

We generated transgenic plants downregulated for *AIL5* expression (*ail5IR*) and have identified four T-DNA insertional alleles of *AIL6*. None of the *ail5* or *ail6* single mutants display obvious developmental defects. However, generation of *ant ail5* and *ant ail6* double mutants has revealed partially overlapping functions between *ANT* and *AIL5* and between *ANT* and *AIL6*.

Aim 3: Investigation of the role of ANT using a cell culture system

We obtained a cDNA of the tobacco *ANT* homolog (*NtANTL*) from Richard Feron at Radboud University. We planned to investigate the consequences of altering the levels of *NtANTL* in BY-2 cells using overexpression and RNAi constructs. However, these studies were discontinued in order to focus our efforts on the *AIL* genes, which act redundantly with ANT to regulate organ initiation and growth.

Aim 4: Crystallization of ANT protein

ANT was crystallized in the presence of the following oligos LL-1/LL-2 (LL-1: 5'-GCA TCG GGA TAT GTG CTT-3' and LL-2: 5'-GCA CAT ATC CCG ATG CAA-3'). Both native and heavy atom soaked crystals were tested at Argonne National Lab, but no diffractions were observed. Room temperature measurements in oil were also tried and no diffractions were observed. Unfortunately, we were not able to grow larger crystals suitable for x-ray diffraction.

Other significant findings

A. Amino acids 252 to 255 are required for the nuclear localization of ANT

Using a leek bombardment assay, we showed that ANT is nuclear localized and that amino acids 252 to 255 (KKKR) act as a nuclear localization signal. This work was published in Planta.

B. Amino acids 134 to 213 of ANT are required for transcriptional activation

We have shown that amino acids 134 to 213 of ANT are required for ANT to activate transcription in yeast and for its in vivo activity. Part of this sequence is conserved in ANT orthologs from other plants. This work was published in Planta.

C. *AVP1* is expressed in young developing plant organs

This work was carried out in collaboration with Roberto Gaxiola. Dr. Gaxiola's lab has found that ectopic expression of the vacuolar pyrophosphatase (H^+-PPase) *AVP1* produces plants with larger lateral organs, similar to the phenotype of *35S::ANT* plants. To better understand the normal role of *AVP1* during organ development, we examined *AVP1* mRNA expression in different tissues and at different developmental stages. *AVP1* expression is high in shoot meristems and young organ primordia and decreases during later stages of organ development. The *AVP1* expression pattern is similar to that of *ANT* and supports a role for *AVP1* in organ development and size regulation. Data from other labs that were part of this study suggests that AVP1 acts to regulate auxin fluxes during organogenesis. This work was published in Science.

Publications acknowledging DOE support

Research Papers

1. Krizek, B.A. and Sulli, C. (2006) Mapping sequences required for nuclear localization and the transcriptional activation function of the Arabidopsis protein AINTEGUMENTA. Planta 224, 612-621.

2. Nole-Wilson, S. and Krizek, B.A. (2006) *AINTEGUMENTA* contributes to organ polarity and regulates growth of lateral organs in combination with *YABBY* genes. Plant Phys. 141, 977-987.

3. Li, J., Yang, H., Peer, W.A., Richter, G., Blakeslee, J., Bandyopadhyay, A., Titapiwantakun, B., Undurraga, S., Khodakovskaya, M., Krizek, B., Murphy, A., Gilroy, S., and Gaxiola, R. (2005) Arabidopsis H^+-PPase AVP1 regulates auxin-mediated organ development. Science 310, 121-125.

4. Nole-Wilson, S., Tranby, T., and Krizek, B.A. (2005) *AINTEGUMENTA*-like (*AIL*) genes are expressed in young tissues and may specify meristematic or division-competent states. Plant Mol. Biol. 57, 613-628.

Review papers and book chapters

1. Krizek, B.A. (2006) Molecular biology of floral organogenesis. In *The molecular biology and biotechnology of flowering*, 100-123, Brian Jordan (ed), CABI Publishing, Oxfordshire.

2. Krizek, B.A. and Fletcher, J.C. (2005) Molecular mechanism of flower development: An armchair guide. Nat. Rev. Genet. 6, 688-698.