Progress Report for DOE DE-FG03-98ER20317
"Regulation of the floral homeotic gene *AGAMOUS*"
Current and Final Funding Period: September 1, 2002, to December 31, 2002

Results obtained during this funding period:

(1) Phylogenetic footprinting of AG *regulatory sequences*

Sequences necessary and sufficient for *AGAMOUS* (*AG*) expression in the center of *Arabidopsis* flowers are located in the second intron, which is about 3 kb in size. This intron contains binding sites for two transcription factors, LEAFY (LFY) and WUSCHEL (WUS), which are direct activators of *AG*. We used the new method of phylogenetic shadowing to identify new regulatory elements. Among 29 Brassicaceae, several other motifs, but not the LFY and WUS binding sites previously identified, are largely invariant. Using reporter gene analyses, we tested six of these motifs and found that they are all functionally important for activity of *AG* regulatory sequences in *A. thaliana*.

(2) Repression of AG *by MADS box genes*

A candidate for repressing *AG* in the shoot apical meristem has been the MADS box gene *FUL*, since it is expressed in the shoot apical meristem and since an activated version (*FUL:VP16*) leads to ectopic *AG* expression in the shoot apical meristem. However, there is no ectopic *AG* expression in *ful* single mutants. We therefore started to generate VP16 fusions of several other MADS box genes expressed in the shoot apical meristem, to determine which of these might be candidates for *FUL* redundant genes. We found that *AGL6:VP16* has a similar phenotype as *FUL:VP16*, suggesting that *AGL6* and *FUL* interact. We are now testing this hypothesis.

(3) Two candidate AG *regulators,* WOW *and* ULA

Because the phylogenetic footprinting project has identified several new candidate regulatory motifs, of which at least one (the CCAATCA motif) has rather strong effects, we had decided to put the analysis of *WOW* and *ULA* on hold, and to focus on using the newly identified motifs as tools. We conduct ed yeast one-hybrid screen with two of the conserved motifs, and identified several classes of transcription factors that can interact with them. One of these is encoded by the *PAN* gene, previously known to be expressed in a domain that overlaps the *AG* domain, but not known before to regulate *AG*.

(4) New genetic modifiers of AG

This part of the project was concluded in the previous funding period.

Publications:

Parcy, F., Bomblies, K., and Weigel, D. (2002) Interaction of *LEAFY*, *AGAMOUS*, and *TERMINAL FLOWER1* in maintaining floral identity in *Arabidopsis*. **Development** *129*, 2519-2527.

Hong, R. L., Hamaguchi, L., Busch, M. A., and Weigel, D. (2003) Regulatory elements of the floral homeotic gene *AGAMOUS* identified by phylogenetic footprinting and shadowing. **Plant Cell** *15*, 1296-1309.