Molecular Mechanism of Shoot Determinacy and Flowering in Arabidopsis

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Flowering is controlled by a number of endogenous and environmental factors (Martinez-Zapater et al., 1994). The environmental factors include day-length, temperature, and stress conditions, while the endogenous factors include age, circadian rhythm, hormone, sugar content, etc.. These factors or signals are perceived and transmitted to the nucleus to cause changes in gene expression that would lead to flower development.

Flower development is usually preceded by changes in shoot development. In Arabidopsis, the vegetative shoot does not undergo internode el*ongation and is called a rosette shoot. Upon floral induction, the shoot bolts and produce an inflorescence on which flowers arise. The Arabidopsis shoot undergoes two major phase changes (Poethig, 1990). The first phase change is the vegetative to reproductive switch. It involves the switch from the rosette to the inflorescence development which results in the lengthening of the stem internode (bolting), shortening of the leaf petiole, and the development of the axillary branches. This phase transition is regulated by a large number of flowering time genes. In Arabidopsis, a facultative long day plant, more than 50 genes have been identified that are involved in the signaling pathway of flowering and flower development (Blasquez 2000). Many of these genes are transcription factors, such as the *FLOWERING LOCUS C* (*FLC*, Sheldon et al., 2000), *CONSTANS* (*CO*, Putterill et al., 1995); others may be involved in signaling processes, such as *FLOWERING LOCUS T* (*FT*, Kardialsky et al., 1999), *TERMINAL FLOWER 1* (*TFL1*, Shannon and Meeks-Wagner, 1991; Bradley et al., 1997), etc. If these genes are rendered nonfunctional, the plant will flower later or earlier than wild-type (WT) by producing more or fewer rosette leaves than WT. The second phase change involves the development of the flower rather than the branches on the main inflorescence shoot. Genes such as *LEAFY* (*LFY*, Weigel et al, 1992), *APETALA 1* (*AP1*, Schultz and Haughn, 1991), are important in specifying flower initiation. A large number of genes are involved in specifying flower organ identity. Many of these encode the "MADS" box genes, such as *AGAMOUS (AG), AP1, AP3* and *PISTILLATA (PI)* (Ng and Yanofsky, 2000; Ng and Yanofsky, 2001a). The flower meristem identity genes were shown to activate *AP3* and *PI*. In spite of their role in the second phase transition, *AP1* and *LFY* are implicated in flowering, the first phase transition, as evidenced by early flowering in transgenic plants that express *AP1* or *LFY* ectopically (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995). Thus there appears to be interaction between the regulation of the two phases. In addition, flower meristem is formed at the time of bolting or stem elongation. Thus, while the three distinct morphological features indicate two phase transitions, it was also proposed that there is only one major transition, the vegetative to flower transition in Arabidopsis (Fig. 1, Hempel and Feldman, 1994).

There are several models of flowering. In general, the models propose the presence of floral repressors or floral activators that, in response to the flowering signals, regulate the flowering pathway (Sheldon et al., 2000, Simpson et al., 2001). A more comprehensive model proposes flowering as a part of shoot maturation process (Schultz and Haughn, 1991). Current research aims at clarifying the relationship among the various genes and group them into different, yet overlapping, signaling pathways, and deciphering the molecular mechanism of how the repressors work, e.g., what genes they repress, and how the repression is overcome to allow flowering.

The *EMBRYONIC FLOWER (EMF)* genes are required for vegetative development . Molecular characterization of these two genes (Aubert et al., 2001; Yoshida et al., 2001) have provided information on the mechanism of floral repression during vegetative development.

The *EMF* genes

The *EMF* genes were identified by the isolation of the seedling lethal mutants with very distinct features. The weak mutants produce a small inflorescence upon germination and a few flowers that were sterile (Fig. 2). All leaves are small and petiole-less, thought to be cauline leaves. The strong mutants cannot even produce inflorescence or flower, all lateral organs eventually develop into carpels (Sung et al., 1992; Yang et al., 1995). The mutants have embryonic phenotypes (Bai et al., 1995), indicating that these genes are expressed in the embryo and and implying that the mutants are committed to flowering or reproductive state during embryogenesis. There are 13 mutants isolated to date that belong to two complementation groups, *EMF1* and *EMF2* (Table 1).

Cloning of the *EMF1* gene revealed that it encodes a novel protein with little homology to any genes of known function (Aubert et al., 2001). The predicated amino acid sequence contains certain motifs that include nuclear localization signals, phosphorylation sites, an ATP/GTP binding motif, and a LXXLL motif. The LXXLL motif has been demonstrated to mediate the binding of steroid receptor coactivator complexes to a nuclear receptor (Heery et al., 1997; Torchia et al., 1997). In plants, it has been identified in the RGA and GAI

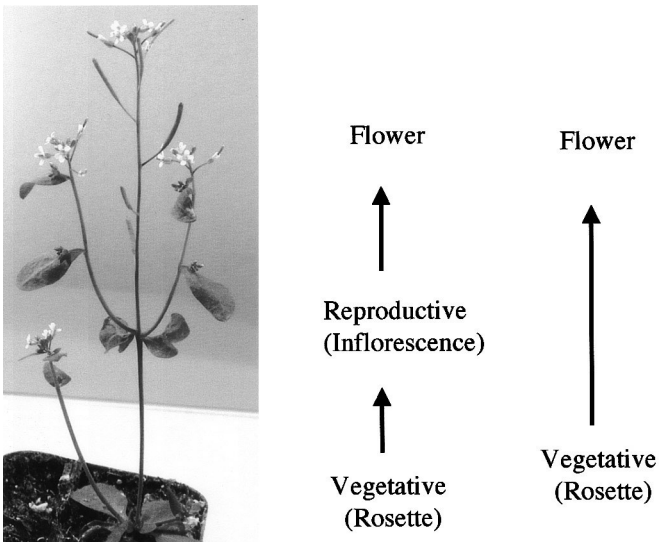

Fig. 1. Possible major phase transitions during Arabidopsis main shoot development.

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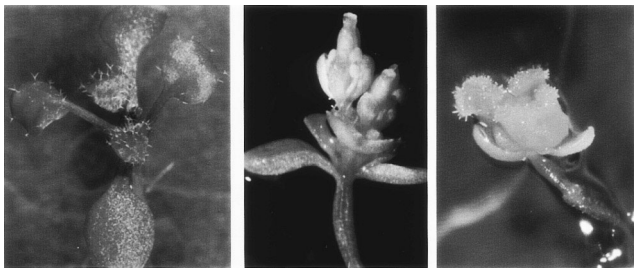

Fig. 2. Phenotypes of *EMF* mutants. (**left**) wild-type; (**middle**) weak phenotype, e.g., *emf1-1*, *emf2-1*; (**right**) strong phenotype, e.g., *emf1-2*, *emf1-3*.

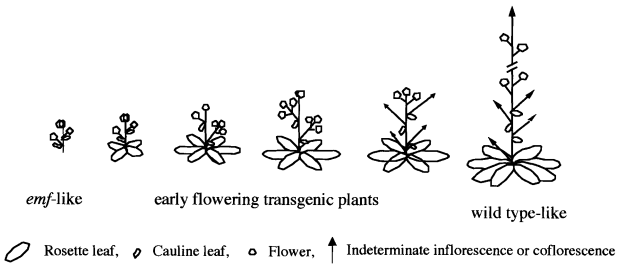

Fig. 3. *EMF* transgenic plants. (**left**) *emf*-like transgenic plants, (**middle**) early-flowering terminal flower transgenic plants, (**right**) wild-type-like plant.

Table 1. *emf* mutant alleles (Goodrich, personal communication).

Gene	Allele	Phenotype	Original designation	Mutagen	Source	Genetic background
EMF1	*emf1-1*	weak	501	EMS	Sung lab, UC Berkeley	Col
	emf1-2	strong	G3-152	γ - ray	same as above	Col
	emf1-3	strong	4902	Ac/Ds[z]	C. Dean, John Innes Institute	Ler
	emf1-4	strong	---	T-DNA[z]	P. Springer, UC Riverside	Ler
EMF2	*emf2-1*	weak	298	EMS	Sung lab, UC Berkeley	Col
	emf2-2	weak	G29-49	γ - ray	same as above	Col
	emf2-3	weak	G14-138	γ - ray	same	Col
	emf2-4	weak	D513	DEB	same	Col
	emf2-5	weak	SP1	EMS	S. Poethig, U. of Penn.	Ler
	emf2-6	weak	SP2	DEB	S. Poethig, U. of Penn.	Ler
	emf2-7	weak	DEB		K. Barton, U. of Wisconsin	Ler
	emf2-8	weak	cyr	EMS	J. Deikman, Penn State U.	Col, gl1
	emf2-9	weak	mpc1	EMS	N. Yoshida, Mitsui Chem. Inc.	Col

[z]Not tagged by transposon or T-DNA (Goodrich, personal communication).

proteins, both putative transcriptional regulators in the gibberellic acid signal transduction pathway (Peng et al., 1997; Silverstone et al., 1998). These two proteins and *EMF1* also display homopolymeric stretches of serine residues. In addition, a region of the *EMF1* protein between amino acids 901 and 1034 displays similarity (identities: 23%, positives: 37%) with two members of a nuclear receptor gene family. This gene family comprises one of the most abundant groups of transcriptional regulators in mammals with members involved in various developmental processes (Sluder et al., 1999). The identification of these motifs indicates that *EMF1* could function as a transcriptional regulator during shoot development.

EMF2 encodes a 71.7 kDa protein with sequence homology to two known Arabidopsis proteins, *FERTILIZATION INDEPENDENT SEED (FIS)* and *VERNALIZATION (VRN)2*, in the region containing C_2H_2 and an acidic W/M domain (Yoshida et al., 2001). These proteins also share homology with a newly identified Polycomb Group (Pc-G) protein Su(z)12 of Drosophila. Mutations of the *Su(z)12* gene cause typical phenotypes of Pc-G mutants such as homeotic transformations and misexpression of homeobox genes in the developmental process of Drosophila. Currently, three classes of Pc-G gene homologs are reported in Arabidopsis. These are *CURLY LEAF* (*CLF*) (Goodrich et al., 1997), *FIS1*/*MEDEA* (*MEA*) (Luo et al., 1999), and *FIS3*/*FERTILIZATION-INDEPENDENT ENDOSPERM* (*FIE*) (Ohad et al., 1999). Thus, *EMF2*, *VRN2*, and *FIS2* are the fourth class of Pc-G gene homologs in Arabidopsis. In animals, Pc-G proteins form large protein complexes (Tie et al., 1998, Tie et al., 2001; Shao et al., 1999) and act to remodel chromatin structures altering the accessibility of DNA to factors required for transcription. In plants, protein-protein interactions were examined among the *FIS* genes and only *MEA*-*FIE* interaction was detected by the yeast two-hybrid system (Luo et al., 2000; Yadegari et al., 2000). The role of the Arabidopsis Pc-G homologs in chromatin-remodeling remains to be investigated, protein interaction between *CLF* and *EMF2* is an intriguing proposition.

The expression pattern of the *EMF1* and *EMF2* RNA is highly similar; both RNAs are found in most plant organs and at all ages of the plant. Since the mutant phenotypes are very similar, these genes display functional similarity. The predicted protein sequences suggest that they are both likely to be nuclear proteins. Together with the genetic interaction studies (Chen et al., 1997), these findings indicate that the two proteins may interact to regulate gene expression. We compared global gene expression pattern of *emf* mutants with that of WT. While there are many genes that show differential expression patterns between the mutants and WT, the most notable finding is that many flower-specific homeotic genes are highly expressed in the mutants shortly after gemination (data not shown). This is consistent with our earlier results showing ectopic *AP1* and *AG* expression in *emf* mutants (Chen et al., 1997). Since mutant seedlings are morphologically similar to WT, our results suggest that the loss-of-function *emf* mutations enabled flower-specific homeotic gene expression in the seedlings. The *CLF* gene is also known to suppress *AG* expression, and when mutated, causes ectopic *AG* expression, early flowering and curly leaves.

EMF transgenic plants

Transgenic plants harboring CaMV 35S promoter::*EMF1* or *EMF2* in sense and antisense directions have been analyzed. Again, *EMF1* and *EMF2* transgenic plants have similar phenotypes. The antisense plants displayed a spectrum of *emf1*-like, early-flowering and WT-like phenotypes (Fig. 3). The *emf1*-like plants were sterile, but the early-flowering plants were fertile and could grow in soil. The *emf1*-like transgenic plants, like *emf1* mutants, lacked rosette leaves and flowered at 14–16 d after sowing. Early-flowering transgenic plants produced 2–8 rosette leaves and flowered at 16–20 d after sowing. In the same growth conditions WT-like plans produced 10–13 rosette leaves and flowered at ≈25 d after sowing. The endogenous *EMF1* transcript levels of the early-flowering and *EMF1*-like antisense plants were greatly decreased relative to WT-like antisense plants and WT plants. The fact that fertile early-flowering plants with normal leaves and flowers were found in plants with reduced EMF activities indicate that *EMF* genes are indeed involved in regulating flowering time.

Possible mechanism of floral repression

All the *emf1*-like and early-flowering transgenic plants made the shift from an indeterminate to determinate growth habit by producing terminal flowers (Fig. 2). Additionally, some early-flowering plants showed a sympodial branching phenotype during shoot development, a phenotype seen in nature (Foster and Gifford, Jr., 1974) but never observed in WT Arabidopsis. The activation of MADS box genes in the *emf* mutant seedlings suggests that EMF1 and EMF2 are involved in the

repression of MADS box gene expression. Ectopic expression of some MADS box genes causes early flowering and produces terminal flowers as in *35S::AP1*, *35S::AG* transgenic plants (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995), consistent with the notion that the flower homeotic genes act downstream from *EMF*. Because *EMF2* encodes a PcG-like protein, thus it may function like the Drosophila PcG protein by maintaining the repression of homeotic genes via chromatin remodeling. To explain flowering in mature Arabidopsis plants or plants grown under long day conditions, we propose that the floral activators such as *LFY, AP1*, etc. may gain access to the flower homeotic genes, and overcome the floral repression. The diverse phenotypes seen among the *EMF* transgenic plants suggest that *EMF* proteins may interact with different proteins and form different complexes during development, thus explaining the pleiotropic effects of *EMF* on multiple phases of shoot development.

In summary, we found that 1) suppression of *EMF* activities shortens the vegetative phase in transgenic plants, 2) alteration of *EMF* activities causes shoot determinacy and affects inflorescence architecture, 3) EMF1 encodes a putative transcription regulator, *EMF2* a potential Pc-G protein, and 4) The two proteins may modulate chromatin structure and repress flower homeotic gene expression. The diverse phenotypes seen among the *EMF* transgenic plants suggest that *EMF* proteins may interact with different proteins and form different complexes during development.

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