An Overview of Flowering in *Arabidopsis thaliana*

By Christina M. Baker

For Developmental Genetics

Dr. Mackay

 The cell biology, life history, and evolutionary origins of plants argues that they will not utilize the same sets of pathways in the regulation of development as animals do. Plants do use hormones for a variety of reasons. Hormones can regulate gene activity, signal between cells utilizing as yet unknown signals, and by using transcription factors they can create differences in the fate of cells. Plants have very different organ systems from the systems seen in animals. They are dependent upon rigid cell walls for structural rigidity, they have a separate germ line from soma very late in development, and plants are very dependent upon the length and intensity of light received by the plant to trigger various developmental events.

 Plants play a vital role in our survival because they provide the oxygen we breathe, the food we eat, the fibers for our clothes, the materials to build our homes, and the raw goods for our industries. Plants also make up about one quarter of our medicinal drugs and give us paper to type reports and such on. Although they are of obvious importance, we know far less about them than about mice and fruit flies and other things. We can look at the genome of a plant and learn about how the plant interacts with its environment, and how it grows and develops. Plants can provide very useful chemicals to humans, they can protect themselves from pests, and they can sense, respond, and even alter our environment.

 Scientists realized that in order to learn the basics about plant growth and development and all they should pick a model species to study, *Arabidopsis thaliana*. All flowering plants are closely related and the complete sequencing of one representative would yield a lot of knowledge of higher plants also. *Arabidopsis* is often called a flowering weed although it is a member of the mustard family, Family Brassicaceae, and it is of no agronomic importance. This plants develops and responds to disease much the same way as crop plants but it takes little space to grow, prolific seed production, and has a short and rapid life cycle. A small genome size (five chromosomes with a haploid content of around 120 Mbp of DNA), and a large number of mutant lines that affect nearly every aspect of the plants growth are available making it a good organism for genetic and molecular study. The genome lacks a lot of repeated less-informative DNA sequences that complicate genome analysis. There are a number of tools including synthetic DNA markers for mapping the genome, a collection of new mutants, specialized transformation techniques, and a large collection of partially sequenced complementary DNAs, which represent genes that are expressed. These tools allow scientists to do further research and encourage them to work more with Arabidopsis. Another important resource is the combined collection of genetic maps, which are of critical importance for gene cloning and genetic analysis. Also significant is the establishment of databases, stock centers, and other research infrastructures which allow rapid dissemination of information and easy exchange of ideas and materials.

 Discoveries with *Arabidopsis* can be used to contribute to the development of improved lines of crops. Essentially the idea is that once the gene has been found in *Arabidopsis* it can more easily be found within other plants. Therefore scientists study the homologues within *Arabidopsis* of other crop genes so they can better understand the function of those genes. Knowledge has been gained on the defense mechanisms of *Arabidopsis* against pathogens and the knowledge is being used directly to develop disease-resistant strains of crops. Genetic comparisons between *Arabidopsis* and crops have been made with things like wheat, barley, soybean, rice, maize, pepper, and many other commercial species. Also, there is new work being done on producing less highly polyunsaturated vegetable and soybean oils since these, although not necessarily nutritionally healthy, make up about 1/3 of our diet. Genes for many of the fatty acid desaturases have been cloned in *Arabidopsis*. First these were used to identify the corresponding genes from soybean, canola, and several other crop species. Secondly, these were used to genetically engineer crop plants with reduced levels of polyunsaturation.

 Another interesting thing being done with *Arabidopsis* is with regards to the effort to produce biodegradable plastics in crop plants. Several genes from the bacterium *Alcaligenes eutrophus* were introduced into *Arabidopsis* so that the gene products built up in the cytoplasm and the result was the accumulation of small amounts of polyhydroxybutarate (PHB), which is a biodegradable plastic. Fairly recently experiments have raised the accumulating levels of PHB one hundred fold by transferring 3 genes from *A. eutrophus* to transgenic *Arabidopsis* plants so that gene products accumulated in the plastids. Up to 20% of the dry weight of these plants was due to PHB which merits studies for commercial usage.

 *Arabidopsis* is resistant to microbial pathogens. Studying and manipulating both the pathogen and the plant can show the interactions occurring and the knowledge can be used for genetically engineered resistance of crop plants to agricultural pathogens. A resistant gene in *Arabidopsis*, RPS2, was found by mutagenizing *Arabidopsis* in the laboratory rather than by finding a resistant plant in the field. RPS2 encodes a novel protein containing a motif made of 14 imperfect leucine-rich repeats which is involved in protein dimerization, and a motif that binds adenosine triphosphate. Significantly, this protein likely serves as a receptor for a specific molecular ligand from the pathogen. This gene has been implicated to resist infection by *Pseudomonas syringae*, a bacterial pathogen important to crops. RPS2 is similar to the RPP5 gene of *Arabidopsis* which confers resistance to the fungal pathogen *Peronospora parasitica*.

 When a vegetative meristem develops into an inflorescence meristem floral growth begins. The vegetative stage is characterized by a rosette. There is a juvenile rosette and then a mature rosette (Medford et al., 1992). An inflorescence meristem may branch into several floral meristems which will each develop into a flower (Koornneef et al., 1998). The formation of floral organs including petals, sepals, and stamen are under developmental controls and these have been well studied. Floral transition is marked by the establishment of a floral fate in these meristems and by the suppression of leaf production. In this transition there is a bi-directional development with flowers being initiated acropetally and leaf primordia being suppressed basipetally (Hempel and Feldman, 1994). There are two distinct inflorescence phases. Early inflorescence is when there are nodes bearing coflorescences or paraclades, which are the axillary buds of the leaf primordia developing into secondary shoots, and leaves. Internode elongation or bolting occurs and the inflorescence then bears flowers and this is known as late inflorenscence (Schultz and Haughn, 1993). The best quantitative way to monitor flowering initiation is the total leaf number together with time to flowering. This phase change has both gradual and precipitous changes. There is a gradual build-up of trichomes at the abaxial or lower side of the leaf while they slowly disappear from the adaxial side (Telfer et al., 1997). There is a floral initiation process (FLIP) that is controlled by the Floral Meristem Identity or FLIP genes, which include LEAFY (LFY), APETALA1 (AP1), APETALA2 (AP2), CAULIFLOWER (CAL), and UNUSUAL FLORAL ORGANS (UFO) (Haughn et al., 1995).

 Transition to flowering is controlled by large numbers of genes that have been identified in *Arabidopsis* by mutant analysis (Peeters and Koornneef, 1996). Other aspects of phase change also affect mutants. Some things that could affect them are changes in leaf shape and trichome density. Although there are genes called flowering time genes the thinking is that they don’t control only the change to flowering (Telfer et al., 1997). To initiate the change to late inflorescence the FLIP genes are needed. TERMINAL FLOWER1 (TLF1) is a gene product that is part of controlling the timing of phase transition and appears to regulate timing of expression of LFY, AP1, and AP2 (Schultz and Haughn, 1993). LFY, AP1, and AP2 have partly redundant functions but LFY is the only to be expressed in the vegetative phase also. If any of these genes are absent there is a gradual change from coflorescence to inflorescent shoots. These three products are required in combination in the reproductive stage to ensure the rapid and complete start of the floral program. In the absence of 1 of these the others activate very slowly to the mechanism that controls the timing of phase switching.

 There are environmental and endogenous controls for flowering within *Arabidopsis*. One of the obvious controls is that Arabidopsis is a facultative long-day (LD) plant so it flowers earlier under LDs than short days (SDs). When plants are to a sufficient age one LD will induce flowering (Mozley and Thomas, 1995). This characteristic may be used to monitor the morphological and molecular changes involved within the plant. Interactions of photoreceptors like phytochrome and cryptochrome and a circadian rhythm or clock mechanism mediate photoperiodic control of flowering (Carre, 2001).

 Photoreceptors are part of setting the phase of the circadian rhythm but they also directly affect the flowering. This means that the quality of light the plant receives can impact flowering time. Blue light and far-red light more effectively promote flowering than red light (Eskins, 1992). Light is not an absolute requirement for flowering to occur since if plants were provided with sufficient carbohydrates for the growing shoot meristem rapid then flowering would occur in complete darkness (Madueno et al., 1996). New studies indicate that EARLY FLOWERING3 (ELF3) gene is required for photoperiodic flowering and normal regulation of circadian rhythms within *Arabidopsis* (Hicks et al., 2001). Another treatment to promote flowering is vernalization or transient exposure to low temperatures. The effectiveness of this treatment depends upon the stage of the plant in development, the length of the exposure, and the temperature to which the plants were exposed (Napp-Zinn, 1985). The leaf number and flowering time are also impacted by the growing temperature. Vernalization, photoperiods, and light quality affect the flowering phenotype of *Arabidopsis* plants. FRIGIDA (FRI) is a dominant allele that produces a large delay in flowering time. Vernalization reduces the responsiveness to photoperiod but even after 80 days of vernalization plants still showed a slight photoperiod response. After 30-40 days of vernalization the affect of FRI was eliminated equally in SD and LD plants of 2 ecotypes (Lee and Amasino, 1995). Far-red light increased the petiole length moreso in FRI containing plants than in the ones that the effect of FRI was eliminated by vernalization (Lee and Amasino, 1995).

 The sensitivity for the environmental factors depends upon genotype but these factors are thought to modulate certain endogenous components. Certain chemical treatments have been shown to promote flowering. Gibberellins and base analogues reveal relatively large effects on flowering. A severe reduction of endogenous gibberellins delays LD plant flowering and stops SD flowering (Blazquez et al., 1998). The failure of gibberellin-deficient ga1-3 mutants to flower in short days was paralleled by the absence of LEAFY promoter induction. The causal connection of gibberellins and LEAFY, a floral meristem identity gene, was confirmed by a constitutively expressed LEAFY transgene that restored function to the gal-3 mutants in SD plants (Blazquez et al., 1998). The impairment of gibberellin biosynthesis only revealed a decrease in LEAFY expression in LD plants and plants treated with sucrose in the dark.

 Flower meristem identity genes play a specific role in the initiation of phase change to flowering. If these genes are inactivated the transition will occur but individual flowers and leaves may be replaced. LFY and AP1 are 2 primary genes expressed in young flower primordia. The RNA of LFY is found in young leaf primordia within the vegetative phase. These occur in homologous positions on the shoot meristem to where later flower primordia will be found (Blazquez et al, 1997). LFY transcription plays a critical role in the change to flowering because LFY RNA increases in level with floral induction so there is an increase in copy number of alleles or rather constitutive expression of LFY results in early flowering (Weigel and Nilsson, 1995; Blazquez et al., 1997). Flower-meristem identity genes partially mediate the effects of flowering-time genes on the transition to flowering but double mutant studies along with the observation of an attenuation of the 35S::LFY phenotype in SD have shown that there are other pathways that act parallel to of downstream of LFY transcription (Weigel and Nilsson, 1995; Ruiz-Garcia et al., 1997).

 Genetics and the variations occurring within ecotypes and those induced by mutagenic treatments play an important role in studying flowering time in *Arabidopsis*. Early- or late-flowering mutants affect genes within the plant that control for both environmental and endogenous factors. Naturally occurring genetic variations has been seen since the earliest research with *Arabidopsis*. The earliest known report of a mutant was in 1873 by A. Braun. To illustrate this variation 32 ecotypes were analyzed under SD and LD light conditions, with and without vernalization treatment (Karlsson et al., 1993). Interactions were found between ecotype, photoperiod, and vernalization (Carre, 2001).

 Some late-flowering mutants are *constans* (*co*), *gigantea* (*gi*), and *luminidependens* (*ld*). There are more mutant alleles at these loci as well as at 11 other loci in the Landsberg *erecta* (L*er*) allele (Koornneef et al., 1991). Late flowering mutants can be put into at least two phenotypic groups dependent upon their response to environmental cues like photoperiod and vernalization (Martinez-Zapater et al., 1994). One group, including mutants like *fca, fve, fpa,* and *fy,* shows a strong response to photoperiod and vernalization but under LD and SD photoperiods show a delayed-flowering phenotype (Koornneef et al., 1991). The other group would include *constans* (*co*), *gigantea* (*gi*), *fwa*, and *ft.* These and other mutants in this group show a much lower responsiveness to environmental conditions. *co* and *gi* don’t respond to photoperiod whatsoever while *ft* and *fwa* still show a photoperiodic response (Koornneef et al., 1991). The CONSTANS gene has been shown to promote early flowering in *Arabidopsis*. Double mutants were constructed containing *co* and mutations affecting gibberellic acid responses and the results suggest that *co* does indeed play some part in early flowering (Putterill et al., 1995). FWA is a loss-of-function mutation in normally flowering revertants of the *fwa* mutant. In these plants the transition to flowering is delayed (Soppe et al., 2000).

 Early-flowering mutants were described after late-flowering mutants. The most dramatic phenotypes are exhibited with mutants of *embryonic flower 1*  and *2* (*emf*1 and *emf*2). After germination occurs instead of a normal rosette the plant immediately makes cauline leaves followed by floral buds. The flowers of these mutants tend to be abnormal and incomplete (Aubert et al., 2001). The normal vegetative phase is bypassed and EMF genes seem to play a central role in this. Altering EMF1 expression in transgenic plants can cause changes in several occurrences of the plant. There are changes in flowering time, shoot determinacy, and inflorescence architecture (Aubert et al., 2001).

 Although only briefly described in this paper, there is evidence supplied that control of flowering is complex and multigenic. A recent hypothesis on this is that the transition to flowering is the default state of development (Haughn et al., 1995; Martinez-Zapater et al., 1994). Backing this hypothesis is the idea that *Arabidopsis* can flower with few leaves in complete darkness with sufficient sucrose provided to the shoot meristem (Madueno et al., 1996). Also, there have never been any mutants described that are completely without flower-like structures while *emf*1 and *emf*2 mutants have little to no vegetative development (Aubert, 2001).

 Researchers have really just started really looking into *Arabidopsis thaliana* within the last 10 years and are now gathering more genetic, molecular, and physiological data. We now know some of the molecular elements involved in the circadian clock and some of the other initial steps, the cloned flowering genes in the intermediate steps, and the target genes of floral induction are known as well. This knowledge brings with it several questions like how light and clock signals are integrated and how they interact with the flowering genes. Vernalization affects the transition to flowering but molecularly we don’t know what is going on. One other thing that further studies could be based on would be the affects of hormones on flowering. For example, we know that gibberellins have a strong role in the transition to flowering but the function is still not clear.

LITERATURE CITED

Aubert, D., Chen, L., Moon, Y., Martin, D., Castle, L.A., Yang, C., and Sung, Z.R. 2001. EMF1, a novel protein involved in the control of shoot architecture and flowering in *Arabidopsis*. *Plant Cell* 13: 1865-1875.

Blazquez, M., Soowal, L., Lee, I., and Weigel, D. 1997. LEAFY expression and flower initiation in *Arabidopsis*. *Development* 124: 3835-3844.

Blazquez, M.A., Green, R., Nilsson, O., Sussman, M.R., and Weigel, D. 1998. Gibberellins promote flowering of *Arabidopsis* by activating the LEAFY promoter. *Plant Cell* 10: 791- 800.

Carre, I. 2001. Day-length perception and the photoperiodic regulation of flowering in *Arabidopsis. J. Biol. Rhythms* 16: 415-423.

Eskins, K. 1992. Light-quality effects on *Arabidopsis* development. Red, blue and far-red regulation of flowering and morphology. *Physiol. Plant.* 86: 439-444.

Haughn, G.W., Schultz, E.A., and Martinez-Zapater, J.M. 1995. The regulation of flowering in *Arabidopsis thaliana*: meristems, morphogenesis, and mutants. *Can. J. Bot.* 73: 959-981.

Hempel, F.D. and Feldman, L.J. 1994. Bi-directional inflorescence development in *Arabidopsis thaliana*: acropetal initiation of flowers and basipetal initiation of paraclades. *Planta* 192: 276-286.

Hicks, K.A., Albertson, T.M., and Wagner, D. R. 2001. EARLY FLOWERING3 Encodes a novel protein that regulates circadian clock function and flowering in *Arabidopsis*. *Plant cell* 13: 1281-1292.

Karlsson B.H., Sills, B.R., and Nienhuis, J. 1993. Effects of photoperiod and vernalization on the number of leaves at flowering in 32 *Arabidopsis thaliana* (*Brassicaceae*) ecotypes. *Am. J. Bot.* 80: 646-648.

Koornneef, M., Alonso-Blanco, D., Peeters, A.J., and Soppe, W. 1998. Genetic control of flowering time in *Arabidopsis*. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49**: 345-370.

Koornneef, M., Hanhart, C.J., and Van der Veen, J.H. 1991. A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 229: 57-66.

Lee, I. and Amasino, R.M. 1995. Effect of vernalization, photoperiod, and light quality on the flowering phenotype of *Arabidopsis* plants containing the FRIGIDA gene. *Plant Physiol.* 108: 157-162.

Madueno, F., Ruiz-Garcia, L., Salinas, J., and Martinez-Zapater, J.M. 1996. Genetic interactions that promote the floral transition in *Arabidopsis*. *Semin. Cell Dev. Biol.* 7: 401-407.

Martinez-Zapater, J.M., Coupland, G., Dean, C., and Koornneef, M. 1994. The transition to flowering in *Arabidopsis*. In *Arabidopsis*, E.M. Meyerowitz and C.R. Somerville, eds (Cold Spring Harbor Laboratory Press), pp. 403-433.

Medford, J.I., Behringer, F.J., Callos, J.D., and Feldman, K.A. 1992. Normal and abnormal development in the *Arabidopsis* vegetative shoot apex. *Plant cell* 4: 631-643.

Mozley, D. and Thomas B. 1995. Developmental and photobiological factors affecting photoperiodic induction in *Arabidopsis thaliana. J. Exp. Bot.* 46: 173-179.

Napp-Zinn, K. 1985. *Arabidopsis thaliana.* In *CRC Handbook of Flowering* (*ed.* H.S. Halevy), pp. 492-503. CRC Press, Boca Raton, FL.

Peeters, A.J.M. and Koornneef, M. 1996. Genetic variation of flowering time in *Arabidopsis thaliana. Semin. Cell Dev. Biol.* 7: 381-389.

Putterill, J., Robson, F., Lee, K., Simon, R., and Coupland, G. 1995. The CONSTANS gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell* 80: 847-857.

Ruiz-Garcia, L., Madueno, F., Wilkinson, M., Haughn, G., Salinas, J., and Martinez-Zapater, J.M. 1997. Different roles of flowering-time genes in activation of floral initiation genes in *Arabidopsis. Plant cell* 9: 1921-1934.

Schultz, E.A. and Haughn, G.W. 1993. Genetic analysis of the floral initiation process (FLIP) in *Arabidopsis. Development* **119**: 745-765.

Soppe, W.J.J., Jacobsen, S.E., Alonso-Blanco, C., Jackson, J.P., Kakutani, T., Koornneef, M., and Peeters, A.J.M. 2000. The late flowering phenotype of *fwa* mutants is caused by gain- of-function epigenetic alleles of a homeodomain gene. *Mol. Cell* 6: 791-802.

Telfer, A., Bollman, K.M., and Poethig, R.S. 1997. Phase change and the regulation of trichome distribution in *Arabidopsis thaliana. Development* **124**: 637-644.

Weigel, D. and Nilsson, O. 1995. A developmental switch sufficient for flower initiation in diverse plants. *Nature* 377: 495-500.