Cell, Vol. 101, 3–8, March 31, 2000, Copyright ©2000 by Cell Press

The ABCs of Floral Evolution

Minireview

Hong Ma* and Claude dePamphilis
Department of Biology and
The Life Sciences Consortium
Penn State University
University Park, Pennsylvania 16802

The emergence of angiosperms (flowering plants) at least 130 million years ago (Crane et al., 1995) is an evolutionary event of staggering importance to life on earth. Some 250,000 flowering plant species exist today, from which we obtain food, clothing, shelter, medicines, and other biochemical products. Despite recent advances in our understanding of the phylogenetic relationships between angiosperms and other seed plants (e.g., Qiu et al., 1999; Soltis et al., 1999), the sudden appearance of many major lineages in the fossil record by 90–100 million years ago remains almost as puzzling as it was over 100 years ago, when Charles Darwin called this "abominable mystery" to the attention of his friend, the eminent botanist Joseph Hooker (Darwin, 1903). One key to the angiosperms' diversity (and perhaps their sudden success) may have been the evolution of a stable, yet highly flexible, developmental system for determining flower structure. The major features of this developmental system are now well understood in several derived dicot plants (with two embryonic leaves, or cotyledons). The "ABC model" involved describes the activities of a set of homeotic genes whose interactive function establishes the identity of the basic flower organs (Coen and Meyerowitz, 1991; Ma, 1994). Now, an elegant paper by Ambrose et al. in the March issue of *Molecular Cell* (Ambrose et al., 2000) combines genetic and molecular experiments to provide the strongest evidence yet for extending the ABC model to maize, a monocot species of the grass family.

Angiosperm Diversity and the ABC Model of Floral Organ Identity

Based on recent molecular analyses, angiosperm phylogeny contains two large monophyletic groups, monocots and eudicots (including most dicot species), plus several much smaller basal groups (Figure 1A) (Qiu et al., 1999; Soltis et al., 1999). Most familiar angiosperms such as rose, *Arabidopsis*, and *Petunia* are derived eudicots. The monocots include the cereals and other grasses, orchids, and lilies. In most angiosperms, flowers have four types of organs arranged in concentric rings or whorls. The outermost whorl consists of sepals; the next whorl contains petals. In some species, there is no clear distinction between sepals and petals, and these organs are called tepals (Bowman, 1997). Tepaloid flowers are common in basal angiosperms and some monocots, suggesting that distinct sepals and petals are the derived flower form. The male reproductive organs, stamens, are just inside the petals (or tepals), and the female reproductive structure, composed of one or more carpels, occupies the center of the flower.

*To whom correspondence should be addressed (e-mail: hxm16@psu.edu).

Genetic and molecular analyses in two eudicot plants, *Arabidopsis thaliana* and *Antirrhinum majus*, have identified several genes that specify floral organ identity. These studies led to the proposal of the ABC model (Coen and Meyerowitz, 1991; Ma, 1994). In essence, this model states that A, B, and C functions are each active in two adjacent whorls of organs: A in whorls 1 and 2, B in whorls 2 and 3, and C in whorls 3 and 4 (Figure 2A). Thus, A function alone controls the sepal identity, A and B determine petal identity, B plus C specify the identity of stamens, and C alone directs carpel identity. Furthermore, A and C functions antagonize each other, and in the absence of one the other expands to occupy the entire flower. In *Arabidopsis*, the known A function genes are *APETALA1* (*AP1*), *APETALA2* (*AP2*), and *LEUNIG* (*LUG*), B function genes are *APETALA3* (*AP3*) and *PISTILLATA* (*PI*), and the only known C function gene is *AGAMOUS* (Figure 2B).

AP1, *AP3*, *PI*, and *AG* are members of the MADS box multigene family (Riechmann and Meyerowitz, 1997) (Figure 1B). The name MADS box was derived from the four founding members, MCM1 (from yeast), AG, DEFICIENS (*DEF*, an *Antirrhinum* floral gene), and SRF (from human). Both the SRF and MCM1 proteins are transcription factors. Expression analyses indicate that the *AP1*, *AP3*, *PI*, and *AG* genes are each expressed in an area of the floral primordium consistent with their functions. Furthermore, ectopic expression studies showed that *AP3* and *PI* together are sufficient for B function and *AG* is sufficient for C function.

On the basis of genetic and molecular results, the *Antirrhinum* genes *SQUAMOSA* (*SQUA*), *DEF*, *GLOBOSA* (*GLO*), and *PLENA* (*PLE*) are considered the orthologs (here we use this term loosely to mean evolutionary and functional homologs) of *AP1*, *AP3*, *PI*, and *AG*, respectively (Ma, 1994). Therefore, molecular analysis of the ABC genes provides an excellent opportunity to understand the evolutionary conservation and divergence of floral development in angiosperms.

Conservation of Gene Functions in Higher Eudicots

MADS box genes from *Arabidopsis* and *Antirrhinum* with the same function (e.g., A function) are more similar to each other than they are to other MADS box genes of different functions in the same species (Purugganan, 1997) (Figure 1B). Therefore, newly isolated MADS box genes from diverse eudicot species can be assigned a putative function based on sequence similarity (Riechmann and Meyerowitz, 1997; Kramer et al., 1998). This identification by sequence similarity requires caution, however, because additional paralogs with very similar sequences exist in *Arabidopsis* for both *AP1* (e.g., *AGL2*) and *AG* (e.g., *AGL1*) (Purugganan, 1997). Therefore, it is often difficult to predict which gene(s) might be orthologs of *AP1* or *AG* based on sequence information alone.

Another criterion for an orthologous relationship is similarity in expression pattern. Both *AP1* and *AG* have distinctive temporal and spatial expression patterns consistent with their functions, but their paralogs have

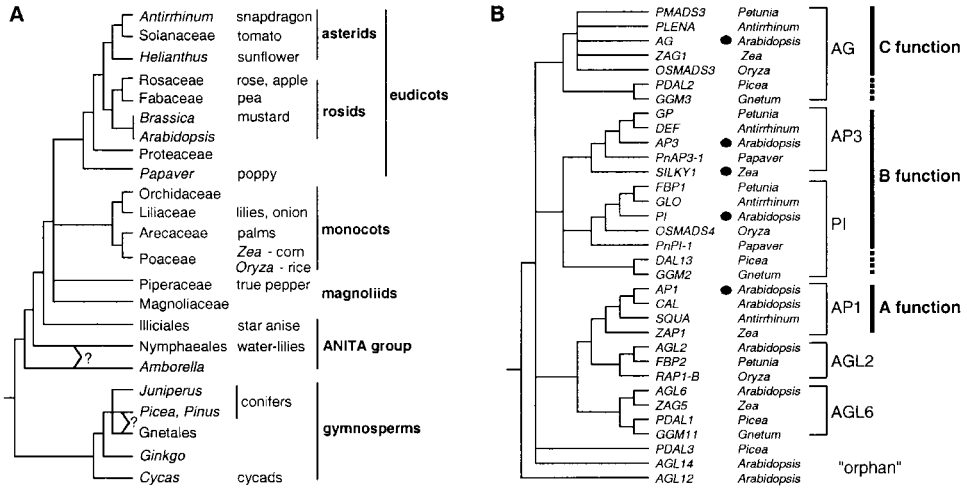

Figure 1. Phylogenetic Trees of Representative Seed Plants and MADS Box Genes

(A) A schematic representation of seed plant relationships drawn based on several recent studies (Qiu et al., 1999; Soltis et al., 1999; Bowe et al., 2000; Chaw et al., 2000). Only a few species of eudicots and monocots are shown. The common names are shown in the center column, and the major groups are listed on the right. The two question marks represent a possible relationship closer than indicated here (references above and T. Barkman and C. D., unpublished data).

(B) An illustration of the MADS box gene family in seed plants, based on recent analyses (Purugganan, 1997; Kramer and Irish, 1999; Winter et al., 1999). Four subfamilies of ABC genes are shown with the *Arabidopsis* genes highlighted. Also shown are two other groups (*AGL2*, *AGL6*) very similar to the *AP1* group, and several so-called "orphan" genes. The dashes next to the gymnosperm genes indicate that they are putative B and C function genes.

different expression patterns. For example, *AG* is expressed in both stamens and carpels, whereas the closely related *AGL1* gene in *Arabidopsis* is expressed only in carpels (Flanagan et al., 1996). Even stronger evidence for orthology came from experimental studies using transgenic plants (Ma, 1994; Riechmann and Meyerowitz, 1997). Because *AG* is both necessary and sufficient for C function, both loss-of-function and gain-of-function analyses of putative *AG* orthologs in transgenic

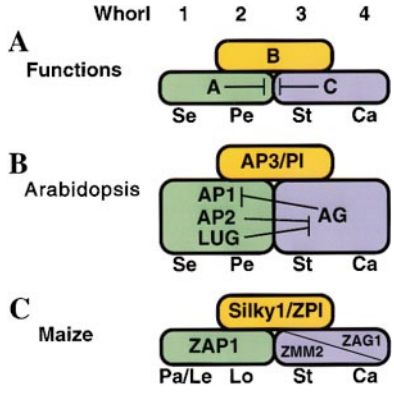

Figure 2. The ABC Model

(A) A cartoon of the model, with boxes indicating the floral region where the function is active. The lines with a short bar indicate a mutually antagonistic relationship between A and C functions and respective genes.

(B) *Arabidopsis* genes.

(C) Maize genes, showing *Silky1* and presence of a putative *PI* ortholog *ZPI* (Ambrose et al., 2000). The diagonal line in the C function box indicates functional divergence of two *AG* orthologs (Mena et al., 1996). Mutant analysis is not yet available to support the role of *ZAP1* as an A function gene required for lemma/palea and lodicule identities. Therefore, this part of the model is rather tentative.

plants indicate that these genes indeed have conserved functions. Similar functional tests were performed for putative B function genes in *Petunia*.

In some plants, one or more ABC genes are duplicated (Purugganan, 1997; Kramer et al., 1998). Often the duplicated genes have overlapping patterns of expression, but one plays a more critical functional role than the other. For example, the *Arabidopsis AP1* and *CAL* genes have similar sequences and expression patterns; they are functionally redundant and *cal* mutations have no visible defects in the presence of normal *AP1* function, but the converse is not true. In *Petunia*, one of two putative *PI* orthologs, *FBP1*, is required for specifying both petal and stamen identity (Riechmann and Meyerowitz, 1997), indicating that the second *PI*-like gene (*pMADS2*) is not sufficient for B function. Therefore, when there is gene duplication, one or both of the duplicated genes may be required for one of the ABC functions.

Evolution of MADS Box Genes in Basal Eudicots

Basal eudicots are distant from the genetically tractable species used to elucidate the ABC model. To test whether the model applies, Kramer and colleagues isolated *AP3* and *PI* homologs from several species of Ranunculales, including Iceland poppy (Kramer et al., 1998), which diverged from other eudicots prior to the lineage leading to *Arabidopsis* and *Antirrhinum* (Figure 1A). Each of these basal eudicots has homologs of the B function genes that are sufficiently similar in sequence to be assigned as putative orthologs.

However, RNA and/or protein expression patterns of some of these genes cast some doubt about strict conservation of these genes (Kramer and Irish, 1999). Expression of the putative B function genes is fairly uniform and constant throughout the stamen primordia. Although they are also expressed in the petal primordia,

the level is not uniform. In Iceland poppy, for example, the *PnAP3-1* and *PnPI-1* genes are expressed in initial petal primordia but become restricted to the tip of petal primordia at later stages. Therefore, putative *AP3* and *PI* orthologs in these basal eudicot plants may serve the role of B function genes in regulating the identity of stamens, but they may diverge to a degree from their counterparts in higher eudicots in controlling petal identity. In the absence of additional studies with transgenic plants or mutants, both conservation and divergence need to be regarded as tentative, albeit intriguing.

Regulation of Floral Organ Identity in Monocots

Monocots are distinct from eudicots, forming a separate clade in modern molecular phylogenies (Figure 1A) (Soltis et al., 1999; Qiu et al., 1999). In particular, the grass family (Poaceae) contains a large number of species that have flowers with highly derived structures. Although the grass reproductive organs (stamens and carpels) are conserved, their sterile floral organs (lemma, palea, and lodicules) are different from the sepals and petals found in flowers of eudicots and many other monocots. It is unclear what the relationships are between the grass sterile floral organs and those of most eudicot or monocot species. Do ABC genes specify even the highly derived floral organs of grasses, and can the function of ABC genes help to determine the relationship between sterile floral organs of grasses and eudicots?

Maize (*Zea mays*) is a grass species that has separate male and female flowers in the tassel and ear, respectively. It is an excellent species for developmental and evolutionary comparisons because of its rich genetics and available molecular tools, including the ability to generate transposon insertional mutants and their use to clone the corresponding genes. Furthermore, a number of maize MADS box genes have been isolated, including putative A and C function genes (Mena et al., 1995, 1996). However, until now, no known maize B function gene has been isolated. Now, in the paper published in *Molecular Cell*, Ambrose et al. (2000) have used genetic and molecular approaches to demonstrate that the maize *Silky1* gene is an ortholog of the eudicot B function genes.

Although the male and female flowers initially have the same set of floral organs, at maturity the two types of maize flowers have distinctive organs. The male flowers have a lemma and a palea at opposing positions that surround the lodicules and stamens (Figure 3A). Two lodicules are interior to the lemma and near the base of three stamens, whereas the pistil aborts during early development. In the female flower, there is a central pistil that has a long silk (long style), while the three stamens abort. Two lodicules, a lemma and a palea, are present but reduced in size. The *silky1* mutant flower retains the lemma and palea, but the lodicules are replaced by organs resembling lemma and palea. In addition, the positions of stamens are occupied by organs with silk-like protrusions (Figure 3B). In the *silky1* female flower, there are three additional silks surrounding the normal central silk, indicating that the stamens, which would normally abort, have transformed into pistils with silks.

The transformation of stamens into pistils in *silky1* mutants is reminiscent of eudicot mutants defective in B function. Therefore, the *silky1* mutant might be defective in a B function gene. In eudicots, defects in B function also cause the conversion of petals into sepals. Thus, the homeotic transformations observed in *silky1* mutants provide strong genetic evidence that the maize lodicule is a modified petal and lemma/palea may be related to sepals. In other words, the *silky1* mutant phenotypes provide a rare opportunity to gain new insights into the evolutionary relationship between the sterile organs of grasses and sepals/petals of other angiosperms.

Figure 3. The *silky1* Male Flower Phenotype

(A) A wild-type maize tassel, showing stamens with red anthers extending outside the male flowers.

(B) A *silky1* mutant tassel, showing silks in place of stamens, indicating a homeotic transformation of stamens to pistils.

Although the *silky1* mutant floral phenotypes are consistent with a defect in a B function gene, DNA sequencing of *Silky1* was required to determine whether it is a MADS box gene. New mutant alleles were generated using the maize transposon *Mu* and were used to clone the *Silky1* gene. The predicted SILKY1 protein sequence is very similar to the B function proteins AP3 and DEF. In situ hybridization indicates that the *Silky1* transcripts appear first in early floral meristems, with subsequent localization to lodicule and stamen primordia. In contrast, no expression was detected in palea, lemma, or pistil. It is known that eudicot B function genes *AP3* and *DEF* are expressed in petal and stamen primordia, but not in sepals or pistils. Therefore, Ambrose and colleagues concluded that *Silky1* is indeed an ortholog of *AP3* and *DEF* (Ambrose et al., 2000).

The maize *ZAG1* gene was shown to be a C function gene (Mena et al., 1996). Therefore, the loss of both *Silky1* and *ZAG1* gene functions should result in the production of flowers with only sepal-like organs, and, indeed, the *silky zag1* double mutant produced flowers with only palea/lemma-like organs. Therefore, the genetic, morphological, sequence, and expression data presented by Ambrose et al. (2000) all strongly support the idea that *Silky1* is a B function gene similar to *AP3* and *DEF* and that lodicules are homologous organs to petals and lemma/palea are possibly homologous to sepals.

The conservation of B and C functions in grasses is also supported by the analysis of putative rice *PI* and *AG* orthologs (*OsMADS4* and *OsMADS3*, respectively) in transgenic rice plants (Kang et al., 1998). Using cosuppression to reduce the expression of *OsMADS4*, they

found that these plants exhibited conversion of lodicules and stamens to palea/lemma-like organs and carpels, respectively, consistent with the loss of B function. In addition, plants expressing an antisense RNA of the *OsMADS3* produced flowers with anthers that are abnormal and resemble lodicules. This further supports the idea that lodicules are homologous to petals. The results from two different grass species strongly support the idea that the ABC model is conserved in grasses.

Summary and Perspectives

Because eudicots and monocots are on separate deep branches of the angiosperm phylogenetic tree, the strong genetic and molecular evidence supporting the conservation of the ABC model suggests that this model represents an ancient regulatory network and is likely to be generally applicable to most, if not all, angiosperms. This does not mean, however, that various modifications of specific components of the model cannot occur in different angiosperm lineages at different times, as we have discussed for some of the minor divergence of gene functions when duplicate genes have arisen to take the place of a single gene. In these cases, the sum of the duplicate genes can be equivalent of the single-copy ABC genes, and the general framework of the model remains intact.

It is likely that the ABC MADS box genes have their origins in plants long before the appearance of angiosperms, on the basis of molecular clock estimates and phylogenetic analysis (Purugganan, 1997). MADS box genes homologous to eudicot B and C function genes (and expressed in patterns consistent with these functions) have been isolated from the closest relatives of flowering plants, the nonflowering seed plants, the gymnosperms, including conifers (Figure 1) (Rutledge et al., 1998) and Gnetales (Winter et al., 1999). Among all gymnosperms, Gnetales have until recently been considered the closest relatives of angiosperms; along with several extinct lineages, these plants have become known as the "Anthophytes" because of similarities in reproductive structure and development (Crane et al., 1995). However, recent molecular studies indicate that all gymnosperms form a monophyletic group and that Gnetales are closest to conifers (Figure 1A) (Qiu et al., 1999; Winter et al., 1999; Bowe et al., 2000; Chaw et al., 2000). This implies that the morphologically similar structures in angiosperms and Gnetales have evolved independently. The fact that gymnosperms have no flowers suggests that the ancestral B function homologs may regulate only male reproductive organs, and C function homologs may control both male and female reproductive organs. If this is indeed true, then the portion of the ABC model for reproductive organs may have been established and conserved since the time when gymnosperms and angiosperms shared a common ancestor, perhaps 300 million years ago or more (Bowe et al., 2000; Chaw et al., 2000).

The isolation of angiosperm and gymnosperm MADS box genes that exhibit strong sequence similarities to the ABC genes (Winter et al., 1999) provides powerful tools to test evolutionary relationships of not only regulatory gene functions but also developmental structures. The study by Ambrose et al. (2000) makes it clear that the ABC model may have predictive use for interpretation of relationship between floral organs of diverse angiosperms (see also Bowman, 1997). Several angiosperm lineages (*Amborella*, Nymphaeales, Illiciales) are now known to branch earlier than the common ancestor of monocots and eudicots (Figure 1A). These include plants that produce flowers with tepals or "petaloid sepals"; therefore, although it may now be predicted that C function will be conserved, it is not yet clear whether distinct A and B functions are established in these basal groups. Perhaps a portion of Darwin's mystery may be solved by learning whether these basal angiosperms possess A and B functions and how angiosperms evolved distinct sepals and petals (Kramer et al., 1998). Further studies of the MADS box gene family in these basal angiosperms, other gymnosperms, and nonseed plants promise to generate new insights into floral evolution and development.

Selected Reading

Ambrose, B.A., Lerner, D.R., Ciceri, P., Padilla, C.M., Yanofsky, M.F., and Schmidt, R.J. (2000). Mol. Cell *5*, 569–579.

Bowe, L.M., Coat, G., and dePamphilis, C.W. (2000). Proc. Natl. Acad. Sci. USA *97*, in press.

Bowman, J.L. (1997). J. Biosci. *22*, 515–527.

Chaw, S.-M., Parkinson, C.L., Cheng, Y., Vincent, T.M., and Palmer, J.D. (2000). Proc. Natl. Acad. Sci. USA *97*, in press.

Coen, E.S., and Meyerowitz, E.M. (1991). Nature *353*, 31–37.

Crane, P.R., Friis, E.M., and Pedersen, K.R. (1995). Nature *374*, 27–33.

Darwin, C. (1903). In More Letters of Charles Darwin: A Record of His Work in a Series of Hitherto Unpublished Letters, Vol. 2, F. Darwin and A.C. Seward, eds. (London: John Murray).

Flanagan, C.A., Hu, Y., and Ma, H. (1996). Plant J. *10*, 343–353.

Kang, H.G., Jeon, J.S., Lee, S., and An, G. (1998). Plant Mol. Biol. *38*, 1021–1029.

Kramer, E.M., Dorit, R.L., and Irish, V.F. (1998). Genetics *149*, 765–783.

Kramer, E.M., and Irish, V.F. (1999). Nature *399*, 144–148.

Ma, H. (1994). Genes Dev. *8*, 745–756.

Mena, M., Mandel, M.A., Lerner, D.R., Yanofsky, M.F., and Schmidt, R.J. (1995). Plant J. *8*, 845–854.

Mena, M., Ambrose, B.A., Meeley, R.B., Briggs, S.P., Yanofsky, M.F., and Schmidt, R.J. (1996). Science *274*, 1537–1540.

Purugganan, M.D. (1997). Mol. Evol. *45*, 392–396.

Qiu, Y.L., Lee, J., Bernasconi-Quadroni, F., Soltis, D.E., Soltis, P.S., Zanis, M., Zimmer, E.A., Chen, Z., Savolainen, V., and Chase, M.W. (1999). Nature *402*, 404–407.

Riechmann, J.L., and Meyerowitz, E.M. (1997). Biol. Chem. *378*, 1079–1101.

Rutledge, R., Regan, S., Nicolas, O., Fobert, P., Cote, C., Bosnich, W., Kauffeldt, C., Sunohara, G., Seguin, A., and Stewart, D. (1998). Plant J. *15*, 625–634.

Soltis, P.S., Soltis, D.E., and Chase, M.W. (1999). Nature *402*, 402–404.

Winter, K.U., Becker, A., Munster, T., Kim, J.T., Saedler, H., and Theissen, G. (1999). Proc. Natl. Acad. Sci. USA *96*, 7342–7347.