Floral Homeotic Selectors

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Introduction

Homeotic genes, those genes whose ectopic expression or loss-of-function mutation results in abnormal organ identity, have been found to play critical roles in both animal and plant development programs (Meyerowitz, 1997b). The determination of cell fate during ontogeny depends on successful assimilation of both spatial and temporal information and the correct transformation of this information into downstream effects. While characterisation of homeotic elements such as the *Drosophila melanogaster Hox* genes and the *Arabidopsis thaliana* ABC floral identity genes has elucidated novel genetic mechanisms, there is still much to be learnt of the nature of their upstream regulation and their downstream mode of action. Homeotic genes in plants appear to determine organ identity by selecting specific cell-cycle programs and inducing the expression of proteins required for individual organ function.

Homeotic Selectors

Although different gene families perform comparable tasks in plant and animal development, it appears that multicellular life, constrained by a specific common unicellular background, may have required similar logic during its evolution in both kingdoms (Meyerowitz, 1997b; Ma, 1997). Homeotic genes are factors which regulate developmental pathways in both plants and animals. These factors are transcriptionally regulated genes encoding putative transcription factors (TFs) which integrate spatial and temporal information to determine and stably maintain the correct regulatory state of downstream target genes (Meyerowitz, 1997b). In general, spatial and temporal signals can be maintained either non-autonomously through such mechanisms as cell-cell interactions and hormone level attenuation or autonomously through an intra-cellular mechanism which crosses cell division (Goodrich, Puangsomlee, Martin, Long, Meyerowitz and Coupland, 1997).

Both plants and animals have MADS domain and homeobox proteins, but they fulfill converse functions. In plants, homeobox genes regulate cell division specifics and MADS box proteins act as homeotic selectors, whereas in animals, homeobox genes determine organ fate and MADS box proteins appear to play a more general role (Meyerowitz, 1997b). Homeotic selectors in both *Drosophila* and *Arabidopsis* share common features. Both are expressed and required for an extended duration during organogenesis, although some functions require transient early expression. Also, these genes are expressed before morphological differentiation. But whereas *Drosophila* homeotic genes are found in clusters, those of *Arabidopsis* tend to be scattered throughout the genome (Weigel and Meyerowitz, 1994).

Plant homeotic factors may be subject to such control mechanisms as spatial and temporal transcriptional regulation, post-transcriptional modification, autoregulation, specific protein-protein interactions, and accessory factor interactions (Davies, 1996, see below). Exactly how the regulation of homeotic elements is effected and what the downstream targets of these elements are is currently undergoing intensive investigation and will aid in understanding both basic genetic mechanisms and specific plant organ patterning. In general, it is thought that the mode of action of homeotic elements is determined either by DNA-binding specificity or by specific cofactor interaction (Krizek and Meyerowitz, 1996).

In *Drosophila*, homeotic elements are activated by the transient expression of certain genes. While this early expression is sufficient to establish the initial patterns of activation, these patterns are maintained by the activity of two antagonistic groups of genes. Together the Polycomb group (Pc-g) genes, which maintain repression, and Trithorax group (Tr-g) genes, which maintain expression, fix the states of their target gene activities across cell divisions. A Pc-g homolog in *Arabidopsis* also fixes a homeotic element state (see below). Thus, another parallel can possibly be drawn between the (suppression of) homeotic selectors in plants and animals. Whether other plant homeotic elements are regulated by alternative mechanisms or by further Pc-g/Tr-g homologs remains an open question (Goodrich et al., 1997).

Floral Development

In contrast to the dynamic, motile nature of cell positioning in animal development, plant organogenesis is almost completely a result of the timing and patterning of the cell life cycle. Changes in relative rates of cell growth and in relative spatial and temporal alignments of cell division determine plant cell fate (Meyerowitz, 1997a). Plant development has typically been viewed as a flexible, environmentally aware program in comparison with the autonomous mode of *Drosophila* cell fate determination. However, it now appears that at least some developmental programs in plants become cell-autonomous (Jürgens, 1997).

A normal plant embryo develops two meristems, the root meristem and the shoot apical meristem (SAM), which lead to the root system and entire above ground plant, respectively. During vegetative growth the SAM forms leaves and new meristems. These secondary SAMs appear at the junctions of leaf primordia and stems and reproduce SAM behaviour. SAMs can be induced to form inflorescence meristems (IMs). These IMs sprout nodes which develop into floral meristems (FMs). SAMs and IMs both follow a spatially and temporally indeterminate growth pattern, thus may form ever-branching structures (Meyerowitz, 1997a).

In contrast, the wild-type FM follows a determinate growth pattern with a finite duration. Each FM leads to one flower, developed from four concentric rings (whorls) of organ primordia in the FM. The four whorls, from the first (outer) whorl, develop into mature sepals, petals, stamens, and carpels, respectively (Meyerowitz, 1997a; Ma, 1997). The ABC Model [of Floral Organ Identity] (see below) successfully predicts the pattern of organ identity genes leading to these organ types. Following organ identity determination in the four whorls, carpals enter a further cycle of organogenesis to form mature ovules and their surrounding structures (Ray, Robinson-Beer, Ray, Baker, Lang, Preuss, Milligan and Gasser, 1994).

Reproductive development in *Arabidopsis* is (partially) dependent upon day length and age of the plant (Okamura, den Boer, Lotys-Prass, Szeto and Jofuku, 1996; Ma, 1997). In order to develop successfully, cells must have accurate information as to their positions in developmental space and time (Gustafson-Brown, Savidge and Yanofsky, 1994). Often, in plants, cell fate is position dependent and maintained through neighbour interactions. However, SAMs may become stably altered around the time of floral induction (Goodrich et al., 1997).

Plant cell growth is typified by a period of cellular expansion following periods of cell division (Sablowski and Meyerowitz, 1998). Cell division patterns in whorls or whorl boundaries are affected, either directly or indirectly, by the organ identity genes, although the positions in which the floral primoridial cells appear seems to be set independently of these identity genes. The shapes of the organs are also determined by the tuning of cell divisions, although, except for ovule development, not much is known of shape-specifying mechanisms (Meyerowitz, 1997a).

The ABC Model

Floral organ identity is determined by the combinatorial interaction of three classes (A, B, and C) of homeotic function. Class B activity is independent of A and C activity, which are mutually exclusive. Class A is expressed in the outer two whorls, class B in the middle whorls, and class C in the inner two whorls of the developing flower. Expression of class A factors leads to sepals, classes A and B to petals, classes B and C to stamens, and class C to carpels.

Alterations to the expression patterns in plants carrying mutations to selector genes of various classes mirror homeotic organ transformations. The three single mutants provide an indication of these phenotypic effects. Loss of A function transforms whorl 1 sepals into carpels and whorl 2 petals into stamens, loss of B function transforms whorl 2 petals into sepals and whorl 3 stamens into carpels, and loss of C function transforms whorl 3 stamens into petals and whorl 4 carpels into sepals. The background state is that of vegetative growth, as indicated by the phenotype of triple loss-of-function mutants. This model successfully predicts organ identity based on homeotic gene activities, which are both necessary and sufficient for organ identity in a floral primordia background (Weigel and Meyerowitz, 1994).

Floral Homeotic Genes

CONSTANS (CO), which encodes two zinc-finger motifs, is known to regulate flowering time and is sufficient to activate LFY expression, even under SD conditions. APETELA1 (AP1) expression follows LFY expression, but involves additional pathways. CO also activates TERMINAL FLOWER 1 (TFL1) which acts to repress LFY expression in the central FM. Under long day (LD) conditions a CO-independent timer is required for floral triggering. Some genes from the regulatory pathway linking LD conditions to floral organogenesis have been characterised at the genetic level (Ma, 1997).

Floral organ development is under the control of a photoperiod-monitoring system and an age-dependent mechanism in at least some (short day (SD)) environments, as well an additional independent mechanism. Phytochrome activity suppresses gibberellin (plant hormone) levels, which leads to reversion of the FM to a SAM. Gibberellin prevents FM reversion either by directly or indirectly promoting AGAMOUS (AG) and LEAFY (LFY) or (some of) their downstream target activities (Okamura et al., 1996; Ma, 1997).

Arabidopsis loss-of-function mutant SHOOT MERISTEMLESS (STM), which prevents initial SAM formation, is a maize homeobox gene KNOTTED1 (KN1) homolog. Petunia NO APICAL MERISTEM (NAM) is phenotypically related to STM and KN1. NAM expression is found in rings around SAMs and FMs and appears to act non-autonomously to promote SAM location and enforce a cell division boundary between SAMs and surrounding tissue (Meyerowitz, 1997a). In the development of floral organs, early-acting genes are required to establish and maintain FM identity and late-acting genes establish organ identity (Mandel, Gustafson-Brown, Savidge and Yanofsky, 1992).

Floral Meristem Identity

The first step on the path leading to mature floral organs is the activation and maintenace of FM identity. At least APETELA1 (AP1) and LEAFY (LFY) are required for transformation of an IM into a FM. LFY is initially expressed in young flower primordia (Mandel et al., 1992). This gene encodes a nuclear protein which is not a member of any known families of TF

(Weigel and Meyerowitz, 1993). AP1, a putative TF, contains a MADS box — a conserved DNA binding motif [MCM1, AG and ARG80, DEFICIENS A, and SRF (Gustafson-Brown et al., 1994)]. It is uniformly expressed in young flower primordia, and is later localised to sepals and petals. Thus AP1 specifies floral meristem identity and acts a homeotic selector of sepal and petal identity (Mandel et al., 1992). AP1 is repressed by TFL in the IM (Gustafson-Brown et al., 1994).

Floral Organ Identity

Spatial expression is achieved through the negative regulation of homeotic elements. The three classes of homeotic organ identity genes are represented in *Arabidopsis* by four genes, class *A* by AP1, *B* by APETELA3 (AP3) and PISTALLATA (PI), and class *C* by AG. AP1 activity in whorls 3 and 4 is repressed by AG, in whorl 4 SUPERMAN (SUP) represses AP3 and PI expression, and AP1 was the first negative regulator of AG activity found in whorls 1 and 2. Expression of AP3, PI, and AG require AP1 and LFY, but none are affected strongly by the loss of AP1. The effects of AP1 and LFY on AG overlap, since only elimination of both removes the normal pattern of AG RNA expression. LFY strongly induces AP3 and PI, whereas AP1 activation of these two is only obvious in the absence of LFY(Weigel and Meyerowitz, 1993; Weigel and Meyerowitz, 1994; Gustafson-Brown et al., 1994).

APETELA2 (AP2), a homeotic gene encoding a novel DNA binding motif, is involved in the establishment of the FM, the specification of organ identity, and the regulation of homeotic gene activity (Okamuro, Caster, Villarroel, van Montagu and Jofuku, 1997). Although many functions of AP2 appear to be redundant, AP1 is known to be required for its organ specification function (Weigel and Meyerowitz, 1994). AP2 activity has shown to not be needed beyond the initial stages of floral development (Goodrich et al., 1997).

Studies of constitutively expressed AP3, which determines petal and stamen identity in concert with PI, show that RNA is found in both flower primordia and stems, but protein is only found in whorls 2, 3, and 4, which leads to the conclusion that it undergoes post-translational modification. AP3 and PI have two phases of expression, an early establishment period, probably under the control of transiently expressed TFs, including LFY, and a late maintenance period, possibly involving autoregulation (see below) (Jack, Fox and Meyerowitz, 1994).

LFY and AP1, FM identity genes, are involved in accurate activation of AG expression and have overlapping roles as positive regulators of AG (Sieburth and Meyerowitz, 1997). Late AG expression may play a role in stamen or pollen development (Sieburth, Running and Meyerowitz, 1995). The LEUNIG (LUG) gene, a cadastral gene — a spatial regulator with no organ identity function, and AP2 negatively regulate AG in whorls 1 and 2, while AG is supressed by CURLED LEAF (CLF) in vegetative tissues (Weigel and Meyerowitz, 1994; Sieburth and Meyerowitz, 1997).

CLF, which represses AG transcription in leaves, IM, and flowers and bears structural homology to a *Drosophila* Polycomb gene, is not required for initial specification of AG pattern, but rather is required later, to maintain repression. While CLF and AP2 act in the same pathway as each other, LUG acts independently of CLF (Goodrich et al., 1997). To further complicate AG regulation, day length affects the epistatic relationship between AG and CLF — under SD conditions CLF might affect an AG-independent function (possibly a MADS box protein) (Ma, 1997).

AG is also potentially involved in ovule development, in which expression is restricted to subcompartments of carpels and ovules. Two other known genes BELL (BEL1) and SIN1 are specific to ovule development. BEL1 negatively regulates AG in ovule integuments, the expression of which leads to homeotic transformations in these tissues (Ray et al., 1994).

Regulation

The initial activation in region specific patterns of homeotic factors cannot be accounted for solely by the mechanisms described above (reviewed in Ma (1998)). There are two classes of upstream regulators, FM identity genes and cadastral genes.

The inactivation of FM identity genes transforms flowers into leaf-bearing shoots. LFY by itself activates *B* function, but can be mostly subsituted for by AP1 in AG regulation, and AP1 has roles in both FM identity and organ identity. But studies have not revealed whether FM genes act directly on homeotic elements. In the snapdragon, *Antirrhinum majus*, it is thought that there is at least one intermediary step between FM and organ identity genes (Weigel and Meyerowitz, 1994). Either each organ identity gene performs the same function in each whorl and combinations of activated genes determine fate or genes have different activities in different whorls (Sieburth et al., 1995).

Cadastral genes set up the initial spatial specificity of organ identity genes. Although some identity genes double as cadastral genes, at least two purely cadastral genes have been found, SUP, which represses *B* in whorl 4, and LUG, which represses the *C* gene AG in whorls 1 and 2. However, how regional identity is established is still unknown (Weigel and Meyerowitz, 1994).

AP2-like Proteins

The AP2 locus is involved in establishment of FM identity, specification of flower organ identity and regulation of floral organogenesis, and spatial and temporal regulation of flower homeotic gene activity. It is also required for normal ovule and seed development (Okamuro et al., 1997). Most AP2 functions appear to be redundant, raising the possibility that AP2 has recently acquired its current functions, and has the potential to assume a more defined, novel role in plant development.

AP2 encodes a putative TF distinguished by a novel DNA binding motif, the AP2 domain. The AP2 domain is a 68 $\overline{aa}$ repeated motif, essential for AP2 function, which contains an 18 $\overline{aa}$ sequence likely to form an amphipathic α-helix. Tobacco AP2 domain homologs are known to bind DNA, providing support for its role as a TF.

At least 12 *Arabidopsis* genes encode RELATED TO AP2 (RAP2) proteins, and of these, at least three are under the control of AP2 in vegetative tissue. Thus AP2, unlike other floral homeotic genes, is also active during vegetative growth. Two genes of known function, AINTEGUMENTA, involved in floral development and ovule formation, and TINY, a suppressor of cell proliferation, encode AP2 domains (Okamuro et al., 1997).

Plant MADS box Proteins

All known organ identity genes (barring AP2) have homologs in *Antirrhinum* and encode MADS domain containing proteins. The MADS domain is a DNA binding and dimerisation motif shared across plant, animal, and fungæ kingdoms (Weigel and Meyerowitz, 1994). At least 20, probably orthologous, genes, scattered throughout the *Arabidopsis* genome, are thought to contain MADS boxes (Rounsley, Ditta and Yanofsky, 1995). Two different MADS box gene homologs have been found in ferns, which predate angiosperm divergence. It is proposed that these genes have been recruited to perform new tasks during floral organ evolution (Münster, Pahnke, di Rosa, Kim, Martin and Saedler, 1997).

The MADS domain is a conserved domain of 56 $\overline{aa}$ which binds the CArG motif [CC(A/T)$_6$GG] (Riechmann, Krizek and Meyerowitz, 1996). All *Arabidopsis* MADS domain proteins contain a MADS domain, involved in DNA binding and dimerisation; an I (or L) domain linking MADS and K, which affects dimerisation properties; a K domain, similar to the coiled-coil keratin structure and thought to form amphipathic α-helices involved in protein-protein interactions; and a C domain of unknown function. The K domain is unique to the plant MADS proteins. The I and K domains define the functional specificity of AP3 and PI, while the I and MADS domains determine AP1 and AG action. The MADS domain and N-terminal portion of the I domain are possibly used for cofactor interaction (Krizek and Meyerowitz, 1996).

Dimerisation

Intrinsic DNA-binding specificities (*in vivo* studies) are quite similar, suggesting that function is not determined by DNA-binding specificities, but by accesory protein interaction (Krizek and Meyerowitz, 1996). Only AP1 homodimers, AG homodimers, and AP3/PI heterodimers bind the CArG motif *in vivo*, thus it appears that the combinatorial ABC organ identity program is not a result of direct interaction between the homeotic proteins and specific DNA sequences (Riechmann, Krizek and Meyerowitz, 1996).

DNA Binding

MADS domain proteins in floral development have different regulatory functions, yet they share a highly conserved region which binds very similar sequences. Also, the $\overline{aa}$s required for direct DNA recognition do not necessarily vary across proteins (Huang, Tudor, Su, Zhang, Hu and Ma, 1996). In fact, functional specificities do not appear to correspond to intrinsic DNA-binding sequence specificities (Krizek and Meyerowitz, 1996).

DNA Conformational Changes

The CArG motif contains an AT-rich centre, similar to other DNA sequences known to bend. Many SRF (a mammalian MADS protein) residues interact with the unique physical DNA structure of slightly different CArG sequences. The binding of MADS proteins to CArG boxes causes bending in DNA. Also, differing $\overline{aa}$ sequences lead to different protein conformations, which affect dimer compactness, which could affect affinities to particular DNA conformations. So, intrinsic physical DNA structure could be differentially recognised because of the degree of bend *at* a CArG box, and the degree of bend and distance *between* CArG boxes (Huang et al., 1996).

However, AP1, AG, and AP3/PI induce similar conformational changes in DNA, ruling out regulatory specificity as a function of particular conformational change. Also, in some chimeric dimers, MADS domains can be swapped without affecting the specific function of that chimeric protein, thus DNA-binding specificity of the three dimers can be changed without affecting functions *in vivo* (Riechmann, Wang and Meyerowitz, 1996).

Nuclear Localisation

AP3 and PI are co-dependent for nuclear localisation, which may be independent of their DNA binding. This function relies on the MADS domain, although the C terminus in PI appears to enhance nuclear localisation over a that of a C-terminal deletion protein. Successful nuclear localisation could be brought about by a bipartite signal, formed between AP3/PI heterodimers; or through a revealed signal, uncovered by a conformational change upon heterodimerisation. An alternative is that heterodimerisation and/or DNA binding is (are) required for nuclear retention (Davies, 1996).

AG Characterisation

Examination of specific *ag*-mutants has separated AG function into stamen specification, carpel specification, and FM determinacy, suggesting that the functions result from different AG activities. The K domain motif is found in a variety of structural and regulatory elements. The *ag*-mutant alleles studied had changes in their K domain, changing the hydophobic interaction face, thus preventing the cofactor-interaction required for determinacy and carpel specification. In line with this reasoning, yeast MCM1, a MADS domain protein, is also regulated by cofactor interactions (Sieburth et al., 1995).

The *Arabidopsis* AG locus contains a 3.8 kb intron between the first and second exons. Using the known regulatory proteins encoded by AP2, LUG, and CLF, deletion of this intragenic section results in loss of negative control over spatial expression, both vegetative and floral, and loss of positive regulation, especially in FMs. Interestingly, the AG introns are highly AT rich (Sieburth and Meyerowitz, 1997), which could lead to DNA bending and indicates that this locus may not subject to regulation by methylation, and thus may require permanent, active, repression for non-expression.

Regulation

Homeotic proteins must act in conjunction with cofactors to regulate downstream genes. MADS domain proteins could bind DNA, altering DNA conformation, either allowing cofactors to occupy adjacent sequences or aiding the recognition of target sites by accessory proteins. Support is found in the *Drosophila Hox* genes which act via protein-protein interactions *in vivo*. Examples of interactions between MADS domain proteins and cofactors also exist in animals and fungi. Some interactions result in the modulation of MADS domain protein activity and cell-specific gene expression, leading to cell specialisation or differing developmental pathways. The K domain is a likely site of interaction between MADS box proteins and various cofactors (Riechmann, Wang and Meyerowitz, 1996).

Methylation

Cytosine residue methylation is implicated in gene regulation and chromatin structure determination, *inter alia*. *Arabidopsis*with reduced cytosine methylation in CG dinucleotides show phenotypic and developmental abnormalities. Their floral organs exhibit homeotic transformations associated with ectopic expression of AG and AP3 in leaf tissue and *clf*-loss-of-function mutants. These abnormalities may be a result of loss of negative regulation of gene expression (Finnegan, Peacock and Dennis, 1996). However, it is not known whether hypomethylation affects homeotic expression directly, or whether the upstream elements are dysregulated.

Polycomb-group Genes

In *Drosophila*, Polycomb group (Pc-G) genes act in conjunction with Trithorax group (Tr-G) genes to maintain stable expression patterns of homeotic elements across cell divisions by inducing particular high order chromatin structures. Pc-G genes contain a conserved SET motif, and act to suppress homeotic function, whereas Tr-G genes act to maintain stable homeotic expression (Goodrich et al., 1997; Ma, 1997)

It is apparent that chromatin structure is important in plant gene expression (Finnegan et al., 1996). There are a number of *Arabidopsis* genes which contain SET domains, including CLF. CLF, which encodes a protein with homology to the Pc-G gene *Enhancer of zeste* (*e(z)*), is required for stable repression of AG function. CLF also contains a nuclear localising signal (NLS) motif in a position consistent with the putative *e(z)* NLS location. It may act by packaging AG to prevent TF access, considering methylation of AG may be required for repression by CLF (Goodrich et al., 1997).

Targets of Homeotic Genes

The AP3/PI heterodimer is required during organogenesis for petal and stamen formation. NAC-LIKE, ACTIVATED BY AP3/PI (NAP) carries the first strong evidence of a direct target of a homeotic gene. NAP functions in the transition between growth by cell division and cell expansion and is homologous to the Petunia gene NO APICAL MERISTEM, (NAM). NAM and *Arabidopsis* homolog, CUC2 (CUP-SHAPED COTYLEDONS 2), are involved in the development of SAMs and the separation of cotyledons and floral organs (Sablowski and Meyerowitz, 1998).

The AP3/PI heterodimer binds the first intron of NAP at a CArG box. NAP encodes a 268 $\overline{aa}$ protein containing a NAC domain and is a late acting developmental target gene. It has been shown that late activation of NAP by homeotic selectors does not require recapitulation, indicating that homeotic selector action is not merely a case of sequential target gene activation. It is possible that time clues may play a role. Dividing cells, the precursors to new tissue, at some point switch to an expansion phase. NAP inhibits this cell expansion, possibly in a strictly transient manner, as cells require NAP to enter elongation mode, and require the decay of NAP levels to finish the transition. It may be that floral cofactors are required for NAP stability or function. NAP is not exclusively a target of AP3/PI. which reflects the possibility that the genes may have been recruited to additional functions by new regulatory mechanisms (Sablowski and Meyerowitz, 1998).

Development Programs

It is clear that the homeotic factors described are involved in a complex regulatory logic. They are subject to both well-characterised *cis*-element regulation and to a separate mechanism which appears to depend on particular properties of DNA methylation and chromatin structure. An acceptable model of this new physical regulatory system must be consistent with certain constraints. The homeotic factors have been shown to bind CArG boxes, but insufficient target sequence specificity precludes an account which relies on sequence motifs. The ABC Model dimers show partner-specificity, one result of which is that different organ identities do not arise from combinations of dimer partners. Also, the three dimers all induce similar conformational changes in DNA. Thus, no specificity is imparted through base-pair level DNA structure.

Plant development depends on the ability to modulate cell division timing and orientation, and cell growth characteristics, as well as trigger organ-specific protein production. Normal vegetative growth can be treated as the ground state from which organs are derived. Each organ can be viewed as the mechanistic variation of specific cell life-cycle parameters. Different programs will give rise to different cell subpopulation characteristics. Given our understanding of the normal regulatory mechanisms, it is trivially true that these programs would be capable of triggering temporally independent organ-specific proteins, such as those responsible for petal pigmentation. Less clear is how particular developmental programs are specified.

Working from the fact that chromatin arranges DNA in cyclic structures, could various homeotic genes anchor themselves at CArG boxes and then induce fixed period conformational changes in the surrounding DNA by interaction through proteins bridges with other dimers or by the nature of the hydrophobic face alone? Within the ABC model, two kinds of reasoning could explain the result that different combinations of selector genes produce qualitatively different organs. First, each selector could act as both a positive and a negative regulator of expression, and each combination of regulatory states defines a unique organ program. This mechanism is limiting in that changes in one organ program will affect (at least) one other, and thus the states are not mutually exclusive. Alternatively, the (possibly mediated) interaction between different homeotic dimers results in the selection of a specific state from a set whose members are mutually exclusive. This specific state could be realised as the expression of a particular gene. However, this does not appear to be the case, and another mechanism must be found.

Genes which suppress and maintain homeotic expression by manipulating chromatin structure have been found in animals and, at least suppressors, in plants. If dimer-pair combinations, possibly interacting through proteins, can create reproducible chromatin conformations, then a means of specifying unique program identities has been found. Different programs, or sets of (at least) regulatory genes, are contained on stacks which cannot all be accessed concurrently. To activate a new program, the relative conformation of the stacks must be altered by some (relatively simple) rule.

Changes in cell-cycle programs are potentially terminal, and thus this novel mechanism may simply provide an additional dimension of security. It is a possibility that genes which are protected by chromatin are less prone to mutation. This, in a single-celled organism, which is both germ-line and soma, could greatly enhance gene stability. On the other hand, the specific requirement of multicellular development for fundamentally different frames of identity could have independently lead to the homeotic mechanism in both plants and animals. Whatever the explanation, it is clear that a regulatory mechanism independent of direct *cis*-interaction operates in development in plants.

Conclusion

The research into organ specification in plants has resulted in the ABC model, which successfully predicts floral organ identity based on the combinatorial action of MADS domain proteins. These homeotic elements are regulated by and operate through a poorly understood regulatory mechanism. The targets of the homeotic selectors were predicted to alter cell-growth features, and specify organ specific products. Currently, the sole known, strictly downstream, target does indeed control an element of cell growth.

Clearly, there is much to be uncovered before multicellular development can even be half-understood. A key step in unfolding the answer will be the successful characterisation of the structural mechanism of control, which may require improvements in technologies. Homeotic selector genes are critical, for both development and its characterisation.

References

Davies, B. (1996). Two is company: the complex arrangements of floral homeotic factors, *BioEssays* **18**(11): 863–866. Review.

Finnegan, E. J., Peacock, W. J. and Dennis, E. S. (1996). Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development, *Proceeding of the National Academy of the Sciences USA* **93**: 8449–8454.

Goodrich, J., Puangsomlee, P., Martin, M., Long, D., Meyerowitz, E. M. and Coupland, G. (1997). A Polycomb-group gene regulates homeotic gene expression in *Arabidopsis*, *Nature* **386**: 44–51.

Gustafson-Brown, C., Savidge, B. and Yanofsky, M. F. (1994). Regulation of the *Arabidopsis* floral homeotic gene APETELA1, *Cell* **76**: 131–143.

Huang, H., Tudor, M., Su, T., Zhang, Y., Hu, Y. and Ma, H. (1996). DNA binding properties of two *Arabidopsis* MADS domain proteins: binding consensus and dimer formation, *The Plant Cell* **8**: 81–94.

Jack, T., Fox, G. L. and Meyerowitz, E. M. (1994). *Arabidopsis* homeotic gene APETELA3 ectopic expression: transcriptional and posttranscriptional regulation determine floral organ identity, *Cell* **76**: 703–716.

Jürgens, G. (1997). Memorizing the floral ABC, *Nature* **386**: 17. View.

Krizek, B. A. and Meyerowitz, E. M. (1996). Mapping the protein regions responsible for the functional specificities of the *Arabidopsis* MADS domain organ-identity proteins, *Proceeding of the National Academy of the Sciences USA* **93**: 4063–4070.

Ma, H. (1997). The on and off of floral regulatory genes, *Cell* **89**: 821–824. Minireview.

Ma, H. (1998). To be, or not to be, a flower — control of floral meristem identity, *Trends in Genetics* **14**(1): 26–32. Review.

Mandel, M. A., Gustafson-Brown, G., Savidge, B. and Yanofsky, M. F. (1992). Molecular characterisation of the *Arabidopsis* floral homeotic gene APETELA1, *Nature* **360**: 273–277.

Meyerowitz, E. M. (1997a). Genetic control of cell division patterns in developing plants, *Cell* **88**: 299–308. Review.

Meyerowitz, E. M. (1997b). Plants and the logic of development, *Genetics* **145**: 5–9. Essay.

Münster, T., Pahnke, J., di Rosa, A., Kim, J. T., Martin, W. and Saedler, H. (1997). Floral homeotic genes were recruited from homologous MADS-box genes preexisting in the common ancestor of fern and seed plants, *Proceeding of the National Academy of the Sciences USA* **94**: 2415–2420.

Okamura, J. K., den Boer, B. G. W., Lotys-Prass, C., Szeto, W. and Jofuku, K. D. (1996). Flowers into shoots: photo and hormonal control of a meristem identity switch in *Arabidopsis*, *Proceeding of the National Academy of the Sciences USA* **93**: 13831–13836.

Okamuro, J. K., Caster, B., Villarroel, R., van Montagu, M. and Jofuku, K. D. (1997). The AP2 domain of APETELA2 defines a large new family of DNA binding proteins in *Arabidopsis*, *Proceeding of the National Academy of the Sciences USA* **94**: 7076–7081.

Ray, A., Robinson-Beer, K., Ray, S., Baker, S. C., Lang, J. D., Preuss, D., Milligan, S. B. and Gasser, C. S. (1994). *Arabidopsis* floral homeotic gene BELL (BEL1) controls ovule development through negative regulation of AGAMOUS gene (AG), *Proceeding of the National Academy of the Sciences USA* **91**: 5761–5765.

Riechmann, J. L., Krizek, B. A. and Meyerowitz, E. M. (1996). Dimerization specificity of *Arabidopsis* MADS domain homeotic proteins APETELA1, APETELA3, PISTILLATA, and AGAMOUS, *Proceeding of the National Academy of the Sciences USA* **93**: 4793–4798.

Riechmann, J. L., Wang, M. and Meyerowitz, E. M. (1996). DNA-binding properties of *Arabidopsis* MADS domain homeotic proteins APETELA1, APETELA3, PISTILLATA, and AGAMOUS, *Nucleic Acids Research* **24**(16): 3134–3141.

Rounsley, S. D., Ditta, G. S. and Yanofsky, M. F. (1995). Diverse roles for MADS box genes in *Arabidopsis* development, *The Plant Cell* **7**: 1259–1269.

Sablowski, R. W. M. and Meyerowitz, E. M. (1998). A homolog of NO APICAL MERISTEM is an immediate target of the floral homeotic gnes APETELA3/PISTILLATA, *Cell* **92**: 93–103.

Sieburth, L. E. and Meyerowitz, E. M. (1997). Molecular dissection of the AGAMOUS control region shows that *cis* elements for spatial regulation are located intragenically, *The Plant Cell* **9**: 355–365.

Sieburth, L. E., Running, M. P. and Meyerowitz, E. M. (1995). Genetic separation of third and fourth whorl functions of AGAMOUS, *The Plant Cell* **7**: 1249–1258.

Weigel, D. and Meyerowitz, E. M. (1993). Activation of floral homeotic genes in *Arabidopsis*, *Science* **261**: 1723–1726.

Weigel, D. and Meyerowitz, E. M. (1994). The ABCs of floral homeotic genes, *Cell* **78**: 203–209. Review.