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How floral meristems are built

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Abstract

The formation of flowers involves the activity of a genetic network that acts in meristems to specify floral identity. The main output of this network is the initiation of a developmental patterning program for the generation of floral organs. The first characteristic of meristem identity genes is their capacity to integrate the environmental and endogenous cues that regulate the onset of flowering. This mechanism synchronizes temporal and spatial information, ensuring that flowers arise in the correct location at the appropriate time. The second characteristic of this network is the mutual regulatory interactions established between meristem identity genes. These interactions provide flexibility and robustness against environmental noise and prevent reversion once the decision to flower has been made. Finally, the third feature is the overlap between the meristem identity and other developmental programs that operate simultaneously to regulate different aspects of the construction of flowers.

Reprogramming the identity

The decision to flower triggers a new developmental program that ends with the formation of reproductive structures. The transition to reproductive development is accompanied by a number of changes in the physiology of the plant. In Arabidopsis, as in many other plants, these changes include an acceleration of cell division at the apex, elongation of the stem, and also the formation of flowers at the flanks of the shoot apical meristem (Steeves and Sussex, 1989). Thus, the acquisition of floral identity by a meristem is only a subprogram of reproductive development.

The idea that flowers consist of modified leaves was first registered in Goethe's texts (Goethe, 1790), but physiological and genetic support for this concept has been found only recently (Bowman *et al.*, 1991). For instance, careful morphological analysis in Arabidopsis has shown that flower primordia arise from the equivalent primordia that form leaves during vegetative growth, *i.e.* phyllotaxy is not altered in the shoot apical meristem during the transition to reproductive development (Figure 1). Under the appropriate inductive conditions, vegetative primordia can indeed be turned into flower primordia, and even produce chimeric organs (Hempel and Feldman, 1995; Hempel *et al.*, 1998; Long and Barton, 2000). As we will see later, further evidence comes from the observation that a mutant defective in floral meristem identity (FMI) keeps producing leaves with associated shoots after the transition to reproductive development has occurred. This review deals with the molecular mechanisms that cause the *reprogramming* of primordia during this transition. Most of the discussion will be focused on Arabidopsis, but there will be reference to work

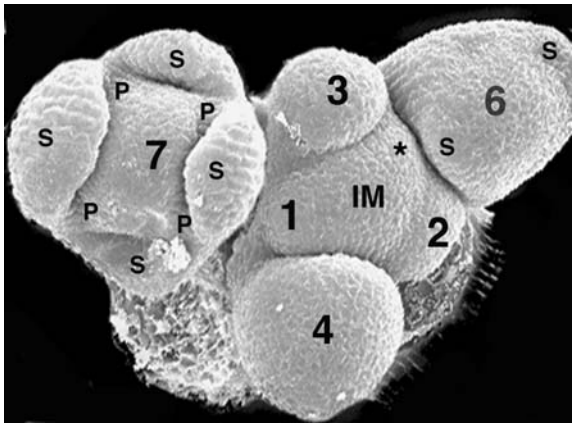

Figure 1. The Arabidopsis inflorescence. After floral transition, the shoot apical meristem acquires inflorescence meristem identity (IM). The IM gives rise to floral meristems (FM) at its flanks in a spiral phyllotaxy. An asterisk marks the position of floral anlagen, while numbers identify the developing FM. In FM at later stages (6 and 7), floral organ primordia are already differentiating (S, sepal; P, petal).

with other plant species when available evidence exists that either extends or challenges models established in Arabidopsis.

FMI presents a set of features that we would first like to emphasize. The formation of flowers comprises at least four processes: determining the correct location of newly emerging primordia, deciding the correct timing for the formation of flowers, conferring floral identity to the primordia, and allowing outgrowth of the flower with the correct patterning (Weigel, 1995). Contrary to what could be expected considering that these four processes occur sequentially, there is increasing evidence that there is functional interaction between the different programs, that confer robustness and versatility to the whole process of making flowers. The integration of temporal and spatial cues by FMI genes, and how the architecture of the network allows the conversion of a sum of gradual inputs into a discrete output, is a good example and has been thoroughly reviewed elsewhere (Simpson and Dean, 2002; Komeda, 2004; Parcy, 2005). We will also discuss interactions between the program that establishes meristem identity with the programs that establish organ polarity or outgrowth. Furthermore, mutual interactions between members of the FMI class of genes is a key feature that allows a sharp transition between vegetative and reproductive development, and guarantees that no reversion will occur once the decision to flower has been taken, thereby providing robustness to the gene regulatory network.

The divas: meristem identity genes

The identification of the key elements that control meristem identity has been a direct consequence of mutant analysis in Arabidopsis and Antirrhinum. In other words, the main evidence for the involvement of these proteins in the determination of floral identity is based on loss- and gain-of-function studies with the corresponding genes. As shown in Table 1, similarities between loss-of-function mutants in orthologous genes in different species supports the idea that there is a common mechanism for the establishment of FMI. As we will see below, the genes presented in this section are not the only important actors; however, they appear to form the backbone of the regulatory network controlling floral meristem formation.

LEAFY/FLORICAULA LFY/FLO

Mutants defective in *LEAFY/FLORICAULA* (*LFY/FLO*) are available in various angiosperms, including tomato, pea, maize, snapdragon and Arabidopsis, and all show severe defects in flower development (Table 1). For instance, in snapdragon *flo* mutants, flowers are replaced by inflorescences (Coen *et al*., 1990). The same phenotype is observed in Arabidopsis *lfy* mutants, in which the first flowers on the bolting shoot are replaced by leaves with their associated axillary meristems that develop into secondary shoots (Schultz and Haughn, 1991; Weigel *et al*., 1992). These axillary meristems progressively acquire an increasing degree of floral identity because the *APETALA1* (*AP1*) meristem identity gene (see below) becomes activated independently of *LFY* (Huala and Sussex, 1992; Bowman *et al*., 1993).

Consistent with the floral defects observed in model species, *LFY/FLO* genes are expressed throughout the young floral meristems of a very large variety of angiosperms (Figure 2). However, a low level of *LFY/FLO* expression is not always confined to floral tissues. In Arabidopsis, *LFY* expression is detectable in leaf primordia during the vegetative phase of growth, and increases as the time for floral transition approaches (Blázquez

Table 1. Meristem-identity genes from different plant species. Only those have been included, for which there is genetic evidence with respect to their involvement in FMI.

Species	Gene	Phenotype of loss-of-function mutant	References
Arabidopsis	*LFY*	Conversion of flowers into leaves with associated inflorescence shoots. Aberrant carpeloid flowers subtended by bracts develop in apical positions	Schultz and Haugh (1991) and Weigel *et al*. (1992)
Antirrhinum	*FLO*	Conversion of flowers into inflorescence shoots	Coen *et al*. (1990)
Pea	*UNI*	Partial loss of floral identity. Flowers lack petals and stamens and axillary flowers arise from sepal axils Extreme reduction in leaf complexity: compound leaves are transformed into unifoliated leaves	Hofer *et al*. (1997)
Petunia	*ALF*	Conversion of flowers into inflorescence shoots Occasionally, aberrant carpeloid flowers develop in apical positions	Souer *et al*. (1998)
Tomato	*FA*	Conversion of flowers into indeterminate vegetative shoots Late flowering	Molinero-Rosales *et al*. (1999)
Maize	*ZFL1*/*ZFL2*	Incomplete vegetative-reproductive transition Reduced number of tassel branches and formation of ''tassel ears''	Bomblies *et al*. (2003)
Arabidopsis	*AP1*/*CAL*	*ap1*: branched flowers. Sepal-to-bract conversion, new flowers forming in the axils. Petals usually absent *ap1 cal*: Flower-to-inflorescence conversion in a highly reiterative pattern, forming cauliflower heads composed of undifferentiated meritems. *ap1*-like flowers develop at later stages	Bowman *et al*. (1993), Mandel *et al*. (1992), and Kempin *et al*. (1995)
Antirrhinum	*SQUA*	Conversion of flowers into inflorescence shoots. Occasionally flowers develop with no defects in floral organ identity	Huijser *et al*. (1992)
Pea	*PIM*	Floral meristems behave as secondary inflorescence meristems producing shoots that eventually form aberrant flowers with leaf-like sepals and reduced petals and stamens	Taylor *et al*. (2002)
Arabidopsis	*TFL1*	Inflorescence meristem converted to floral meristem (indeterminate-to-determinate inflorescence) Early flowering	Alvarez *et al*. (1992)
Antirrhinum	*CEN*	Inflorescence meristem converted to floral meristem (indeterminate-to-determinate inflorescence)	Bradley *et al*. (1996)
Pea	*DET*	Conversion of the primary inflorescence meristem into a secondary inflorescence meristem, which in turn, produces a flower and a determinate inflorescence	Singer *et al*. (1990) and Foucher *et al*. (2003)
	LF	Early flowering	Murfet (1975)
Tomato	*SP*	Premature conversion of the sympodial vegetative apex into a terminal determinate inflorescence shoot	Pnueli *et al*. (1998)

et al., 1997). The expression of the pea *LFY* ortholog, *UNIFOLIATA* (*UNI*), is visible at high levels during early stages of leaf development, which suggests a role for *LFY*/*FLO* genes in leaf development, at least in legumes (Hofer *et al*., 1997). This is particularly evident in pea *uni* mutants, which show a clear defect in leaf shape consisting of a dramatic reduction in its complexity. In this role, *UNI* interacts synergistically with *STAMINA PISTILLOIDA* (Taylor *et al*., 2001), the pea ortholog of the Arabidopsis gene *UNUSUAL FLORAL ORGANS* (*UFO*). Interestingly, constitutive expression of *UFO* causes curling of leaf margins that is abolished in *lfy* mutants (Lee *et al*., 1997). More importantly, *LFY* is not only necessary, but also sufficient to confer floral

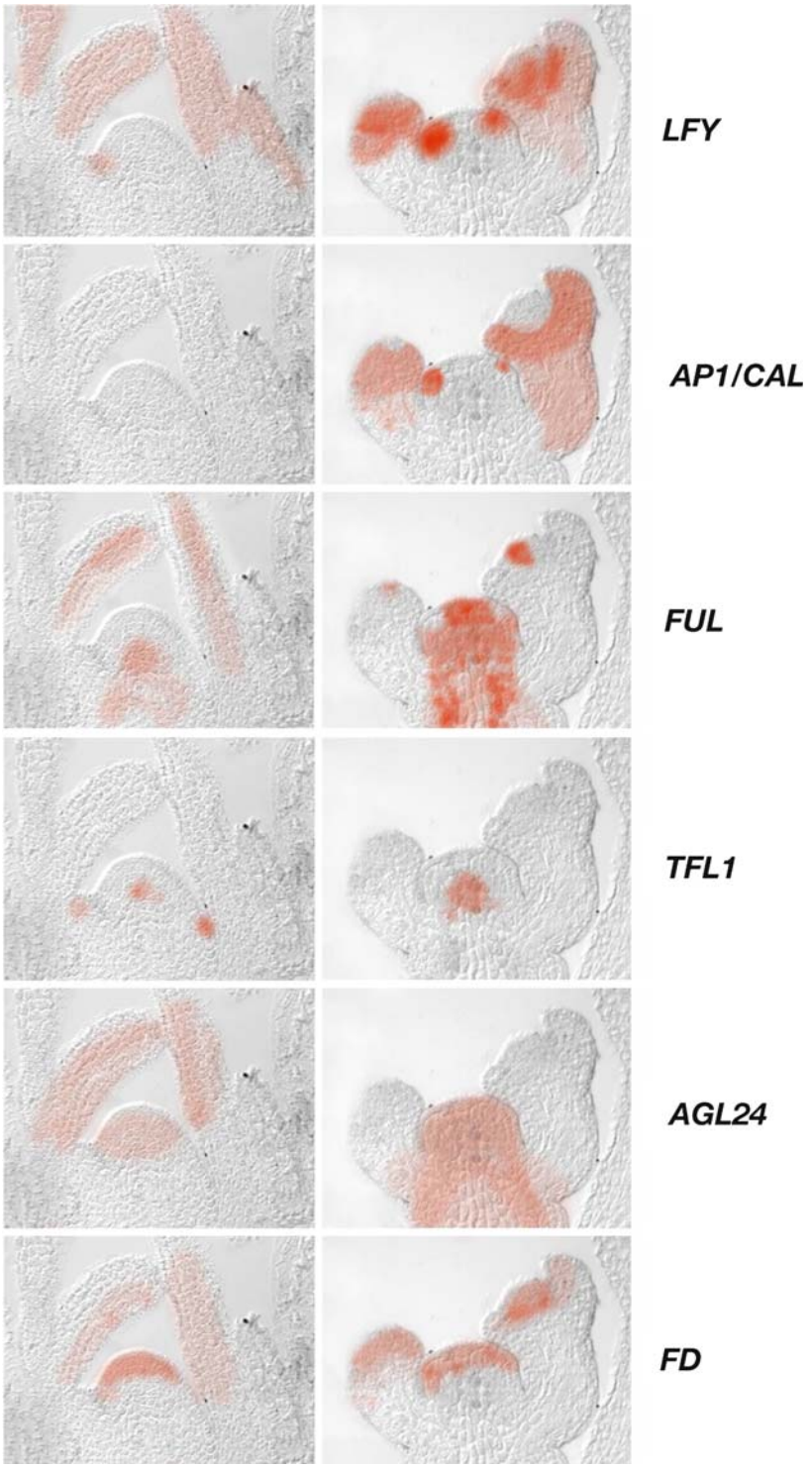

Figure 2. Expression pattern of the main meristem identity genes in Arabidopsis. Domains of RNA expression have been shadowed in red color in a longitudinal section of the shoot apical meristem before (left column) and after (right column) floral transition.

identity to emerging primordia, because ectopic and constitutive expression of *LFY* in Arabidopsis results in the precocious transformation of leaves and axillary shoots into wild-type-looking flowers (Weigel and Nilsson, 1995). Again, the pivotal role for *LFY*/*FLO* genes in the initiation of floral development in all angiosperms is confirmed by the ability of the Arabidopsis *LFY* gene to produce ectopic flowers in other plant species, including woody plants such as poplar and citrus trees (Figure 3) (Weigel and Nilsson, 1995; Peña *et al*., 2001).

LFY encodes a transcription factor that has so far been found only in the plant kingdom (from moss to core eudicots) (Maizel *et al*., 2005). In moss, *LFY* is required for the first division of the embryo (Tanahashi *et al*., 2005). In gymnosperms and angiosperms, *LFY* seems to be associated with the development of reproductive structures. As opposed to other types of transcription factors, LFY does not belong to a multigenic family. Most gymnosperms are believed to possess two paralogous *LFY*-like genes, termed *LEAFY* and *NEEDLY*, that might in some cases be specifically expressed in male and female reproductive organs,

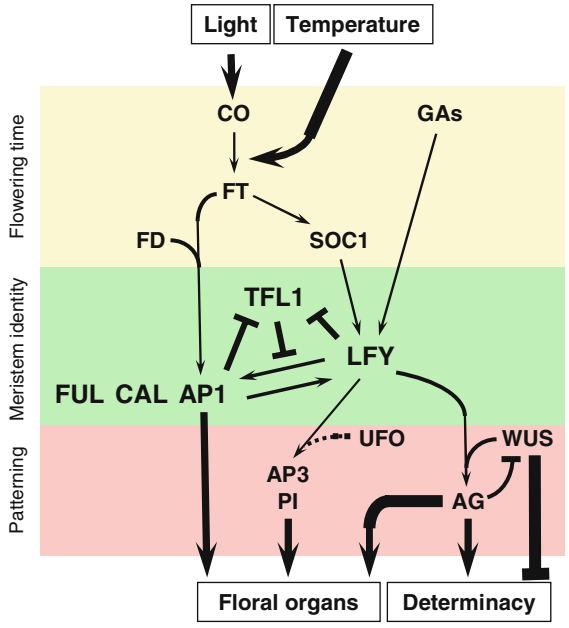

Figure 3. Schematic representation of the interactions involved in the specification of floral meristems. The dotted line refers to the participation of UFO in the activation of B-class genes by LFY, based on genetic interactions. See text for details.

respectively (for a review, see Frohlich and Parker, 2000). *LFY* in angiosperms is orthologous to the *LFY* paralog from gymnosperms, rather than to *NLY*. It therefore appears that the *NLY* paralog was lost from the angiosperm lineage before the last common ancestor of the extant flowering plants. Among the different theories for the evolution of flowers (Albert *et al*., 2002), the *Mostly Male Theory* postulates that flowers have evolved by the ectopic development of ovules on the male reproductive axis of a gymnosperm ancestor (Frohlich and Parker, 2000; Frohlich, 2003). According to this theory, such a change may have involved the transfer of some regulatory activities from *NLY* to *LFY*, and subsequent loss of *NLY*, together with all downstream female development programmes, other than those necessary for ovule development.

LFY has been shown to bind specific sequences present in the regulatory regions of the homeotic genes *AP1* (Parcy *et al*., 1998), *APETALA3* (*AP3*) (Lamb *et al*., 2002) and *AGAMOUS* (*AG*) (Busch *et al*., 1999). The consensus binding site for LFY has not be precisely defined. Sequence comparisons of the sites from *AP1* promoter and *AG* regulatory intron lead to propose the CCANTGG sequence as consensus, but this core sequence is not perfectly conserved in the site present in *AP3* promoter (Lamb *et al*., 1992). The LFY protein appears to be expressed in a fairly uniform manner in early floral buds (Parcy *et al*., 1998; Sessions *et al*., 2000). This uniform expression pattern results from the uniform expression of the *LFY* promoter (Blázquez *et al*., 1997) but probably also from LFY's ability to move between cells. Early work in Antirrhinum, using chimeras in which *FLO* is expressed in single meristem layer, showed that FLO was able to activate downstream genes in a non-cell-autonomous manner (Hantke *et al*., 1995). Consistent with this observation, LFY expressed from a L1 specific promoter (*AtML1*) acts throughout the floral meristem and the LFY protein has been shown to move between cells (Sessions *et al*., 2000; Wu *et al*., 2003).

APETALA1, CAULIFLOWER *and* FRUITFULL

Arabidopsis *AP1* and *CAULIFLOWER* (*CAL*) are MADS-box genes highly related in sequence that also share similar patterns of expression (Mandel *et al*., 1992; Kempin *et al*., 1995). Both

genes are expressed throughout young floral meristems, shortly after *LFY* onset of expression. Later in flower development, their expression becomes restricted to first and second whorls of the flower, and to floral pedicels (Figure 2). *AP1* and *CAL* act redundantly to specify FMI once the floral transition has taken place (Bowman *et al*., 1993). While *cal* mutants have no visible phenotype, *ap1* single mutants only show moderate defects in FMI in addition to defects in floral organ identity of whorls 1 and 2. *ap1* Mutants develop leaf-like organs in place of sepals and in the axils of those, new floral meristems form that can reiterate this pattern a number of times, producing "branched flowers". In fact, the function of *AP1* and *CAL* in FMI is revealed by the striking phenotype observed when both mutations are combined. Simultaneous loss of function of *AP1* and *CAL* causes a complete transformation of floral meristems into inflorescence meristems, which give rise to new meristems that again behave similarly, reiterating this pattern an indefinite number of times to form structures similar to cauliflower heads. Eventually, these high order meristems acquire floral identity and are able to produce stamens and carpels. The IM-to-IM-to-IM- trap in which *ap1 cal* mutants appear to be caught, indicates that *AP1* and *CAL* are primarily required for the IM-to-FM transition, as further supported by the phenotypes of *35S::AP1* or *35S::CAL* plants, both early flowering and showing IM-to-FM conversion (Mandel and Yanofsky, 1995a).

A new partner for *AP1* and *CAL* in the specification of FMI was uncovered when mutations in a third related MADS-box gene, *FRUITFULL* (*FUL*), are combined with *ap1* and *cal*. *FUL* is a close relative of *AP1* and *CAL*, but their expression patterns in the inflorescence are almost complementary (Figure 2) (Mandel and Yanofsky, 1995b). *FUL* is strongly upregulated in the IM after the floral transition, but excluded from FM, where it reappears at later stages of floral development restricted to carpel primordia. The lack of *FUL* expression in FMs is mediated by AP1, since *FUL* RNA is strongly and uniformly detected in both IM and FMs of *ap1* and *ap1 cal* inflorescences. Consistently with *FUL* expression pattern, *ful* single mutants flower slightly late under long day conditions and show severe defects in fruit development, but no FMI phenotypes are observed (Gu *et al*., 1998). However, *ap1 cal ful* triple mutants present a dramatic defect in FMI. The inflorescence meristem of these mutants produces, after floral induction, reiterative structures resembling those of *ap1 cal*, but with stronger vegetative traits, since a high-proportion of the newly formed meristems are subtended by leaf-like organs and *ap1 cal ful* meristems are unable to acquire floral identity (Ferrándiz *et al*., 2000). While it is unlikely that *FUL* has a prominent role in the specification of FMI in wild-type plants, sequence homology with *AP1* and *CAL* and the strong ectopic expression of *FUL* in *ap1 cal* inflorescences could explain that it is able to partially replace AP1 and CAL function.

AP1-like genes have only been found in other angiosperms (Litt and Irish, 2003). There are few other *AP1*-like genes functionally characterized in detail, and, while comparative analysis support their role in FMI, important differences in mutant phenotypes are found, perhaps reflecting different degrees of functional redundancy or the effect of particular inflorescence architectures. In Antirrhinum, the MADS-box gene closest to *AP1* is *SQUAMOSA* (*SQUA*) (Hujser *et al*., 1992). Its expression in floral meristems and the two outer whorls of the flowers basically follows the same pattern as that of *AP1* in Arabidopsis. However, FMs of the *squa* mutant are replaced by vegetative shoots and only rarely form flowers, which otherwise do not show defects in floral organ identity. In pea, mutations in the *AP1*-like *gene PROLIFERATING INFLORESCENCE MERISTEM* (*PIM*) cause partial conversion of FMs into inflorescence-like structures and also defects in floral organ identity, with leaf-like sepals and absent or aberrant petals and stamens (Taylor *et al*., 2002).

TERMINAL FLOWER1

TFL1 belongs to a third group that also contributes to the regulation of the identity of floral meristems in Arabidopsis. The phenotype caused by mutations in *TFL1* indicates that the role of this gene in FMI is opposite to that of *LFY* or *AP1* and *CAL*. In *tfl1* mutants the inflorescences are replaced by flowers; cauline leaves subtend solitary flowers, rather than shoots; and the meristems of the inflorescence shoots are converted into floral meristems and form terminal flowers (Shannon

and Meeks-Wagner, 1991). Therefore, while *LFY*, *AP1* and *CAL* specify FMI, *TFL1* would specify inflorescence shoot identity. Another difference between FMI genes and *TFL1* is that *tfl1* mutations also affect flowering time (Shannon and Meeks-Wagner, 1991; Bradley *et al*., 1997). *tfl1* Mutants flower earlier than the wild type, indicating a second function of TFL1 as a repressor of the floral transition.

Plants constitutively expressing *TFL1* have an enlarged rosette and a highly branched inflorescence which eventually produces normal flowers (Ratcliffe *et al*., 1998). This effect has been interpreted as all developmental phases of the shoot apex being prolonged: vegetative, early inflorescence and late inflorescence, opposite to the phenotype of the *tfl1* mutant, where the phase transitions would occur faster than in the wild type. From this point of view, TFL1 has been proposed to participate in a common mechanism underlying major developmental transitions at the shoot apex, rather than playing two separate roles repressing flowering and specifying shoot identity (Ratcliffe *et al*., 1998).

Expression of *TFL1* somehow correlates with its proposed role as negative regulator of *LFY*, *AP1* and *CAL*: it is found in the center of the shoot meristems, not in emerging primordia (Figure 2; Bradley *et al*., 1997). Moreover, in *tfl1* mutants *LFY* and *AP1* expression invades the inflorescence apices and they get converted into flowers, as will be discussed below (Weigel *et al*., 1992; Bowman *et al*., 1993; Gustafson-Brown, 1994; Bradley *et al*., 1997).

TFL1 belongs to a small Arabidopsis gene family and, in contrast to *LFY*, *AP1* and *CAL*, it does not encode a transcription factor but a protein with homology to proteins from the phosphatidyl ethanolamine-binding proteins (PEBP) (Bradley *et al*., 1997). The PEBP family has members in bacteria, yeast, and animals, including the mammalian Raf Kinase Inhibitor Protein (RKIP) (Yeung et al., 1999). Suppression of Raf-1 kinase activity and MAP kinase signaling by RKIP has been reported to modulate signaling pathways involved in the control of growth and division in animals. The mechanism of action of these proteins in plants has not been elucidated. However, recent studies indicate that its Arabidopsis homolog FLOWERING LOCUS T (FT) (Kardailsky *et al*., 1999; Kobayashi *et al*., 1999), with an opposite function to that of TFL1, activates flowering by acting in the nucleus, as part of a complex with the bZIP transcription factor *FD* (see below; Abe *et al*., 2005; Wigge *et al*., 2005).

TFL1 and FT share about 59% amino-acid identity, and it has been shown that the substitution of a single aminoacid (His to Tyr in positions 88 or 85) is sufficient to convert TFL1 to FT function and *vice versa* (Hanzawa *et al*., 2005). This suggests that their biochemical function is very similar, and a likely hypothesis is that the identified aminoacid residue is involved in differential binding to interactors.

Interestingly, the His residues are conserved in TFL1 functional homologs from different plant species (Hanzawa *et al*., 2005). However, there are notable differences in the function of these homologs that could be due to the expression patterns in the different plants. In Arabidopsis, *TFL1* is expressed both in vegetative and inflorescence meristems and controls both the length of the vegetative phase (flowering time) and the determination of the inflorescence shoot (Bradley *et al*., 1997). In contrast, *CENTRORADIALIS* (*CEN*), the most likely ortholog in Antirrhinum, is only expressed in the inflorescence meristem and recessive mutations in this gene alter the determination but not flowering time (Bradley *et al*., 1996). Moreover, two *TFL1* homologs are present in pea: *LATE FLOWERING* (*LF*), which acts only as a repressor of flowering, and *DETERMINATE* (*DET*) which exclusively controls inflorescence determination. The spatial expression pattern of these genes has not been analyzed in detail, but RT-PCR data show that *LF* is expressed both in vegetative and inflorescence apices, while *DET* is only expressed in inflorescence apices (Foucher *et al*., 2003).

Start me up: upstream regulators of meristem identity genes

The precise time at which newly emerging primordia switch from a vegetative to a floral fate is regulated by multiple environmental and endogenous factors. As we will see below, the molecular mechanism that integrates this information and triggers the floral developmental program is primarily based in the initial up-regulation of FMI

genes by all these signals that promote flowering. In Arabidopsis wild-type plants, the main integrator, among floral meristem genes, is *LFY*. As mentioned above, *LFY* expression is already detectable in leaf primordia and increases until the floral transition (Blázquez *et al*., 1997; Hempel *et al*., 1997). The actual level of expression of *LFY* is absolutely relevant to trigger the switch to a floral fate. First, the level of *LFY* expression correlates with the efficiency of different signals to promote flowering (*i.e*. long- vs. short days, application of GA_3, etc.). Second, *LFY* expression is delayed in several late-flowering mutants. And third, altering the number of copies of *LFY* – expressed from its own promoter – affects flowering time. Accordingly, *LFY* expression has been shown to be downstream of all pathways that promote flowering time, namely the gibberellin (GA) pathway – that is essential for flowering under short days–, the day-length-dependent pathway, and the autonomous pathway (Blázquez *et al*., 1998; Nilsson *et al*., 1998; Aukerman *et al*. 1999; Blázquez and Weigel, 2000).

The first evidence that *LFY* is involved in the promotion of flowering by the GA pathway came from genetic analyses: mutants deficient in GA biosynthesis show a dramatic reduction in *LFY* expression under short days and do not flower. This defect is largely rescued by *LFY* overexpression, indicating that endogenous GA promotes flowering at least in part by positively regulating *LFY* expression level (Blázquez *et al*., 1998). Regions responsible for the response to GA have been identified in the *LFY* promoter and contain a *cis*-element resembling the binding site for R2R3 MYB transcription factors (Blázquez and Weigel, 2000). One of these factors, *AtMYB33* is a good candidate to upregulate *LFY* in response to GA since its expression increases at the shoot apex upon floral transition and the *At*MYB33 protein binds the GA response element in *LFY* promoter (Gocal *et al*., 2001). The cascade from GA to *LFY* activation might also involve the miR159 microRNA which targets the *AtMYB33* gene and delays flowering when overexpressed (Achard *et al*., 2004).

The regulation of *LFY* expression by day length operates through the interaction between the flowering-time gene *CONSTANS* (*CO*) and the circadian clock but, again, the precise mechanism for this regulation remains to be determined. On one hand, a few studies suggest that the MADS-box transcription factor SUPPRESSOR OF CONSTANS OVEREXPRESSION1 (SOC1) mediates the regulation of *LFY* expression by CO, but this hypothesis remains to be firmly demonstrated (Lee *et al*., 2000; Mouradov *et al*., 2002; Jack, 2004). On the other hand, other studies have implicated a different MADS-box gene, *AGL24*, in the up-regulation of *LFY* expression. *AGL24* expression sharply increases in the apex at floral transition and *agl24* mutants flower late and show reduced *LFY* expression at the time the wild type flowers (Yu *et al*., 2002). Whether *LFY* expression is decreased simply because *agl24* flowers late or whether *LFY* reduced level is responsible for *agl24* late flowering remains to be determined. Nevertheless, *LFY* overexpression is capable of complementing the *agl24* late flowering, suggesting that one of the roles of AGL24 role might be to upregulate LFY (Yu *et al*., 2002).

Despite earlier studies indicated that *LFY* expression was largely independent of FT, two recent reports indicate that the flowering-time integrator FT might actually contribute to *LFY* regulation. Global analysis of gene expression before and after floral transition shows reduced *LFY* expression in the *ft* even before the transition occurs (Schmid *et al*., 2003). Moreover, a pulse of *FT* expression in the leaves induces *LFY* up-regulation at the shoot apex (Huang *et al*., 2005). How FT affects *LFY* expression is not known at present. It might involve interaction between FT and the bZIP transcription factor FD at the shoot apex, as recently demonstrated for *AP1* activation by FT (Abe *et al*., 2005; Wigge *et al*., 2005). Alternatively, since FT has been recently shown to induce *SOC1* expression (Yoo *et al*., 2005), *LFY* up-regulation by FT might use SOC1 as intermediate.

In contrast to *LFY*, expression of *AP1* appears in stage 1 floral meristems only after floral transition has been initiated, being first observed in the stage 1 floral meristems. The main regulators of *AP1* expression are LFY and FT. Direct activation by LFY serves the purpose of allowing irreversible activation of the floral development program, as will be discussed below. Regulation by FT is manifested by the lack of expression of *AP1* in the *lfy ft* double mutant as compared to *lfy* single mutant (Ruiz-García *et al*., 1997). A more recent study, using transcriptional profiling

analysis, has provided further confirmation, showing that up-regulation of *AP1* is severely delayed in *ft* mutants in response to floral inducting conditions (Schmid *et al*., 2003).

An intriguing issue is how does FT activate *AP1* expression considering that it is not expressed in the shoot apex and it does not encode a transcription factor. A first piece of evidence indicates that the *FT* mRNA moves from the leaves (Huang *et al*., 2005), where it is induced by CO (Samach *et al*., 2000; Suárez-López *et al*., 2001; An *et al*., 2004), to the apex. Furthermore, it has been shown that *AP1* up-regulation by FT is mediated by the bZIP transcription factor FD, which is expressed preferentially at the shoot apex, in a domain that overlaps with that of *AP1* (Abe *et al*., 2005; Wigge *et al*., 2005). Observations that support this idea are, for instance, that loss-of-function mutations in *FD* suppress the severe early flowering phenotype caused by *FT* overexpression; furthermore, the phenotype of *fd lfy* resembles that of *ft lfy* in the sense that no flowers or floral organs are produced and *AP1* expression cannot be detected. Interestingly, FT and FD proteins interact physically in the apex, allowing the participation of FT in the transcriptional complex that activates *AP1* (Abe *et al*., 2005; Wigge *et al*., 2005).

Despite the interesting expression pattern of *TFL1*, little is known about the mechanisms through which it is established. As mentioned above, *TFL1* is expressed in the center of the shoot meristems. The fact that its expression is weak in the vegetative meristem but it increases with floral transition (Bradley, 1997) suggests that genes controlling flowering time might contribute to *TFL1* regulation. This is consistent with the observed fast up-regulation of *TFL1* following activation of *CO* in transgenic plants carrying a glucocorticoid-inducible version of *CO* (Simon *et al*., 1996). However, there is no evidence that shows whether *TFL1* is a direct target of CO.

Maintaining a stable switch: interactions between FMI genes

An important requirement in the transition between the vegetative and the reproductive developmental programmes is that once the decision to flower has been made, a mechanism has to ensure that it will successfully reach its goal. As opposed to plants such as *Impatiens balsamina* (Pouteau *et al*., 1997; Tooke *et al*., 2005), proliferous roses (Goethe, 1790) or some Arabidopsis mutants (Okamuro *et al*., 1993, 1996; Parcy *et al*., 2002), wild-type Arabidopsis flowers never revert to other reproductive or vegetative structures. Avoiding reversion is particularly important in annual plants, like Arabidopsis, but this mechanism is important in any case to distinguish between proper inductive signals and environmental noise – such as transient changes in temperature that do not reflect seasonal cues. In Arabidopsis, robustness of the switch is achieved by mutual and feedback regulation between meristem identity genes. The most relevant interactions in this respect are: (i) the induction by LFY of *AP1* and *CAL*, which in turn positively regulate *LFY* expression irrespective of environmental conditions; (ii) the repression of *TFL1* expression by LFY, AP1 and CAL; (iii) the interference of TFL1 in the up-regulation of *AP1* by LFY.

The first known action of LFY after floral induction is the induction of *AP1* and *CAL* transcription. In wild-type plants, *AP1* and *CAL* are expressed just after *LFY*, in stage 1 and 2 flower meristems (Mandel *et al*., 1992; Kempin *et al*., 1995). In an *lfy* mutant, this expression is delayed, it does not occur in the first nodes of the bolting shoot (Ruíz-García *et al*., 1997). Conversely, overexpression of *LFY* is sufficient to induce *AP1* expression in young leaves (Parcy *et al*., 1998). Several lines of evidence demonstrate that this activation is direct. *AP1* and *CAL* expression is upregulated when LFY activity is induced in transgenic plants carrying a glucocorticoid-inducible allele of *LFY*, and this induction does not require any protein synthesis (Wagner *et al*., 1999, William *et al*., 2004). In addition, LFY binds to sequences present in the *AP1* promoter (Parcy *et al*., 1998; Wagner *et al*., 1999; William *et al*., 2004). Together, these data strongly suggest that LFY activates *AP1* and *CAL* by binding their regulatory sequences. Recently, this binding was demonstrated *in vivo* by chromosome immunoprecipitation (William *et al*., 2004). The relevance of the LFY binding site characterized in the *AP1* promoter – which is absent from the *CAL* promoter – remains to be established. Also, the identity of the coregulator that LFY requires to activate these two genes also is unknown but recent data suggests

that it might be downstream of FD/FT (Abe *et al*., 2005; Wigge *et al*., 2005).

Once LFY has activated *AP1* and *CAL*, these two genes in turn are involved in the maintenance of *LFY* expression. Indeed, in the *ap1 cal* double mutant, floral fate of the meristem is lost around stage 3 as the floral meristem starts to develop like an inflorescence meristem (Bowman *et al*., 1993): it generates new floral buds which become inflorescence meristems again, thereby generating the fractal structure of a cauliflower curd. Analysis of *LFY* expression by *in situ* hybridization showed that *LFY* expression is normal in stage 0–1 of *ap1 cal* floral buds but disappears in later stages (Bowman *et al*., 1993).

An important requirement for the network of meristem identity genes to be robust is to ensure that expression of *TFL1* does not occur in floral buds and that FMI genes are not expressed in the inflorescence meristems. Antagonistic interactions between *TFL1* and the FMI genes *LFY*, *AP1* and *CAL*, are responsible for this control (Shannon and Meeks-Wagner, 1993; Liljegren *et al*., 1999). At least two observations indicate that *LFY* represses *TFL1* expression; first, *TFL1* is ectopically expressed at lower nodes of the inflorescence of *lfy* mutants, in nodal positions where flowers would be formed in the wild-type plant (Ratcliffe *et al*., 1999); and second, ectopic expression of *LFY* completely inhibits *TFL1* expression at the shoot apex (Ratcliffe *et al*., 1999).

AP1 and *CAL* also contribute to prevent *TFL1* repression in floral meristems. Constitutive and ectopic expression of *AP1* strongly reduces the expression of *TFL1*, suggesting that *AP1* acts as a repressor of *TFL1* (Liljegren *et al*., 1999; Ratcliffe *et al*., 1999). On the other hand, no ectopic expression of *TFL1* is observed in the shoots that replace flowers in the *ap1* mutant (Ratcliffe *et al*., 1999). This reflects the ability of CAL to repress *TFL1*, because *TFL1* is strongly expressed in young meristems, equivalent to stage 1 floral meristems, formed in the proliferative inflorescence of the *ap1 cal* double mutant (Ratcliffe *et al*., 1999). Given that LFY, AP1 and CAL are transcription factors, it is possible that they directly bind *cis*-elements in the *TFL1* promoter. Indeed, that *LFY* could act as a direct repressor of *TFL1* is suggested by the fact that conversion of LFY into a constitutive activator of transcription by attaching the VP16 transactivation domain causes ectopic expression of *TFL1* in floral primordia (Parcy *et al*., 2002).

Equivalent to the interaction with *TFL1*, the specification of floral meristems also requires the down-regulation of *AGL24* by LFY and AP1. In wild-type plants, *AGL24* expression can be found in the inflorescence meristem and possibly in the peripheric layers of the floral meristem. In *lfy* or *ap1* mutants, *AGL24* invades the inside layers of the floral meristem and contributes to induce inflorescence features to the floral meristem (Yu *et al*., 2004).

On the other side of the same coin, *TFL1* prevents meristems from assuming floral identity by inhibiting the FMI genes at two levels. First, *TFL1* retards the up-regulation of these genes: when high levels of *TFL1* are present, as in plants that overexpress TFL1 constitutively, *LFY* and *AP1* are activated much later than in the wild type (Ratcliffe *et al*., 1998). Conversely, when *TFL1* function is compromised, as in the *tfl1* mutant, *LFY* and *AP1* are prematurely activated and become ectopically expressed in the shoot apex (Weigel *et al*., 1992; Bowman *et al*., 1993; Gustafson-Brown *et al*., 1994; Bradley *et al*., 1997). Second, *TFL1* interferes with the activity of the FMI genes. This is deduced from the phenotype of double transgenic plants constitutively expressing both *TFL1*, and *LFY* or *AP1*. In these plants, the shoot apex and axillary meristems do not immediately develop flowers, but shoots (Ratcliffe *et al*., 1999). This suggests that TFL1 blocks the response to LFY and AP1, and not only their expression.

Who does the job: activation of homeotic genes

Meristem identity genes are not only important to prevent young floral meristem from reverting to inflorescences but also to establish their floral identity (*i.e*. start the floral patterning and differentiation program). We are still far from having identified all the genes involved in this process: although *LFY* and *AP1* play a prominent role in it, most of the molecular evidence has been gathered for the involvement of *LFY*, which we will more thoroughly describe here. We have already discussed in detail how LFY induces *AP1* and *CAL*, which act both as meristem and organ identity genes. In an analogous way, the localized

expression pattern of the B-class genes *AP3* and *PI* is achieved by the combinatorial action of LFY and its coregulator UFO (Lee *et al*., 1997; Parcy *et al*., 1998; Honma and Goto, 2000; Lamb *et al*., 2002): LFY provides the floral specificity whereas UFO provides the spatial information within the floral primordium. The mechanism of *AP3* activation by the combination of LFY and UFO is not understood. LFY is able to bind to a *cis*-element present in *AP3* promoter suggesting part of the activation might be direct (Lamb *et al*., 2002). *UFO* encodes an F-box protein supposed to be involved in protein degradation, thus, the existence of an unidentified repressor of B genes has been postulated but remains to be demonstrated. Interestingly, the LFY-VP16 allele, which is constitutively activated, is not sufficient to induce *AP3* expression throughout the floral meristem, suggesting either that UFO controls binding of LFY to the *AP3* promoter, or that the postulated repressor is strong enough to counteract the VP16 activation domain (Parcy *et al*., 1998). The situation is also complicated by the fact that AP1 also contributes to *AP3* induction (Ng and Yanofsky, 2001). LFY might thus affect *AP3* expression both directly, and indirectly through AP1 activation (Lamb *et al*., 2002)

LFY also participates in C gene induction. However, LFY is not absolutely required for *AG* expression: in an *lfy* mutant, the flowers bear detectable *AG* expression and abnormal carpelloid organs. In the Californian poppy, *LFY* expression coincides with that of the B-class genes, but not with that of C-class genes, suggesting that the activation of C-class genes by LFY might not be a general feature of angiosperms (Becker *et al*., 2005). As for B-class genes, LFY acts together with a locally expressed coregulator, the WUS homeodomain protein, to locally induce *AG*. In *AG* regulatory sequences, LFY and WUS bind adjacent sites, and *AG* activation is thought to be achieved by concomitant binding of the two regulators which would recruit complementary elements of the basal transcription machinery (Lenhard *et al*., 2001; Lohmann *et al*., 2001; Hong *et al*., 2003). Because *WUS* expression domain in the flower is much smaller than that of *AG*, and because *AG* is still expressed in the center of *wus* flowers (when they form), it is possible that other LFY coregulators also contribute to *AG* induction.

As recent analysis suggests, LFY might also be involved in the activation of *SEPALLATA* genes, but whether this activation is direct and which coregulators are required remain to be understood (Schmid *et al*., 2003).

Finally, based on genomic-scale experiments such as comparison between wild type and mutant right after the floral transition or using the LFY allele that can be activated posttranslationally (LFY-GR fusion) (Wagner *et al*., 2004; William *et al*., 2004), a battery of LFY-activated genes have been identified. Understanding to which flower specific features, these genes contribute is a great challenge for the years to come.

Not alone: interactions with patterning programs

The formation of flowers requires the concurrent instructions of several developmental programmes: (i) the specification of the correct position for the new flower; (ii) the patterning of the flower primordium to establish symmetries and polarities in the developing structures; (iii) the control of cell division to determine the actual size of the flower and number of floral organs; and (iv) the actual program that determines the identity of the flower. While these processes seem, at first glance, to operate sequentially (phyllotaxy vs. organ identity) or in parallel to each other (polarity vs. primordium identity), a more accurate view must include the molecular interactions between the elements that execute each program (Figure 4). These interactions very likely reveal the evolutionary dynamics that has led to the invention of flowers.

Differences in local concentration of auxin at the apex are the inductive cue that marks the anlagen where new primordia will arise, both during the vegetative and the reproductive phase (Reinhardt *et al*., 2003; Reinhardt, 2005). The acquisition of floral identity is independent from this other process, given that the level of *LFY* expression is not affected by auxin, but it is important to note that *LFY* is expressed as soon as the anlagen are established, and is confined to this group of cells (Weigel *et al*., 1992). At least formally, *LFY* expression is under the control of auxin, although it is not clear how direct this regulation can be. One possibility is that transcription factors encoded by genes primarily

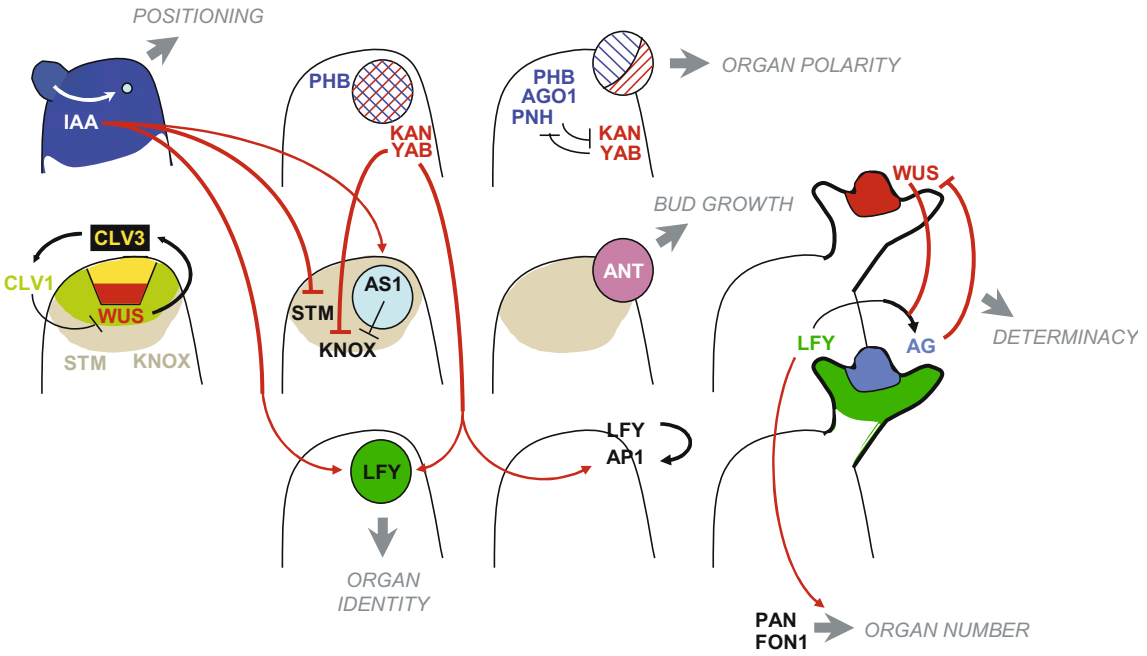

Figure 4. Interactions between developmental processes during the formation of flowers. The sequence of events starts with the positioning of floral anlagen directed by auxin (IAA) gradients in the apex. Organ polarity is established even before primordia are visible, through the action of PHABULOSA (PHB), KANADI (KAN), and several YABBY (YAB) proteins (Sawa *et al*., 1999a, b; Eshed *et al*., 2001), and later involve PINHEAD (PNH) and ARGONAUTE (AGO1) (Lynn *et al*., 1999; Kidner and Martienssen, 2005). Primordium outgrowth depends on the initial down-regulation of *SHOOTMERISTEMLESS* (*STM*) and *KNOX* genes in the anlagen through the activity of ASYMMETRIC LEAVES1 (AS1) (Bowman *et al*., 2002), and involves AINTEGUMENTA (ANT) (Mizukami, 2001). Finally, floral identity is specified by the meristem identity genes *LFY* and *AP1*, and termination of the floral meristem is achieved through the activity of AG, a primary target of LFY. Interactions between overlapping pathways are represented by red arrows, and the expression domains of the genes involved in these processes are depicted in colors.

upregulated in the anlagen, such as those encoded by *ASYMMETRIC LEAVES1* (*AS1*) or the *YABBY* family (Bowman *et al*., 2002) are responsible for the localized expression of *LFY* in those cells. Evidence to support this hypothesis is the altered development of floral meristems in null mutants of the Arabidopsis *YABBY* gene *FILAMENTOUS FLOWER* (*FIL*), and the transformation of flowers into inflorescences in *fil ap1* or *fil ap1 cal* mutants (Sawa *et al*., 1999a, b).

Contrary to the shoot apical meristem, floral meristems are determinate and the central region is consumed in the formation of the ovary. The mechanism by which this meristem terminates involves the interaction between the meristem identity gene *LFY*, and the machinery that maintains meristems undifferentiated, represented by the *CLAVATA* (*CLV*) and *WUSCHEL* (*WUS*) pathway (Sharma *et al*., 2003). As seen above, one of the direct targets of LFY is *AG*, which, apart from its role in the specification of the identity of the two inner whorls in the flower, is also responsible for preventing indeterminate growth of the floral meristem. This additional role is revealed by the phenotype of *ag* mutants, which maintain a stem cell population in the flower and continue to produce organs indefinitely (Lenhard *et al*., 2001). The opposite effect is observed in plants that overexpress *AG* (Mizukami and Ma, 1997), and in *wus* mutants (Laux *et al*., 1996), suggesting that these two genes play antagonistic roles in meristem maintenance. Since *WUS* is permanently expressed in *ag* mutants, even at very late stages of development (Lenhard *et al*., 2001; Lohmann *et al*., 2001), AG can be considered a negative regulator of *WUS*. This negative feedback loop is completely responsible for the arrest of growth in floral meristems, as shown by the restoration of the *wus* phenotype in *ag wus* double mutants.

Conclusions

The last 20 years have been very successful in the application of genetic approaches – mutant analysis and screening for modifiers – to elaborate the cast of characters involved in the determination of FMI. But the script is not fully written. One of the challenges for the years to come will be to bridge the gap between genetic data that point to interactions between players, and the actual molecular mechanisms that underlie these interactions. In this quest, biochemical tools are very likely going to be crucial for understanding the combinatorial action of the different regulators and may help reconstitute in test tubes part of the interactions that take place in intact plants. With this strategy, it is also likely that new characters will be unveiled, particularly those that belong to regulatory complexes but their essential or redundant nature prevents their identification with standard genetic tools.

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