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Genetic analyses of signalling in flower development using *Arabidopsis*

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Abstract

Flower development can be divided into four major steps: phase transition from vegetative to reproductive growth, formation of inflorescence meristem, formation and identity determination of floral organs, and growth and maturation of floral organs. Intercellular and intracellular signalling mechanisms must have important roles in each step of flower development, because it requires cell division, cell growth, and cell differentiation in a concerted fashion. Molecular genetic analysis of the process has started by isolation of a series of mutants with unusual flowering time, with aberrant structure in inflorescence and in flowers, and with no self-fertilization. At present more than 60 genes are identified from *Arabidopsis thaliana* and some of them have cloned. Although the information is still limited, several types of signalling systems are revealed. In this review, we summarize the present genetic aspects of the signalling network underlying the processes of flower development.

Introduction

Distinct from animals, the basic plan of the plant body is the continuous repetitive growth of a structural unit. The unit of aerial portion is a shoot which is composed of a stem, a meristem at the top of stem, and leaves continuously formed at the meristem. Flowers are considered to be specifically differentiated shoots, because they are composed of a short stem and four kinds of floral organs, sepals, petals, stamens and carpels. Floral organs are considered to be homologous organs of leaves, because their anatomy and developmental process are quite similar. We can easily notice that they are flat and laterally symmetric, and have the same branching pattern of major vascular bundles. The process of flower development can be divided into four major steps: phase transition from vegetative to reproductive growth, formation of inflorescence meristem, formation and identity determination of floral organs, and growth and maturation of floral organs. Several different types of signalling mechanism, between or within cells, must have important roles in each step of flower development, because each step requires cell division, cell growth and cell differentiation in a concerted fashion. Without any signalling system, it will be impossible to show the drastic changes of cells such as formation of organ primordia at the symmetrical positions. In

order to unveil the signalling mechanism and its genetic regulatory systems, genetic, biochemical, anatomical and physiological analyses of the processes of flower development are being undertaken using *Arabidopsis thaliana* [52, 56, 63, 69], *Antirrhinum majus* [20, 21, 84], *Petunia hybrida* [103], *Zea mays* [45, 106] and other plant species. In this review, we summarize the present aspects of the genetic network underlying the flower development of *Arabidopsis*. Genes and mutants of *Arabidopsis* involved in the flower development are listed in Table 1. Results of studies on other plant species are almost consistent with those of *Arabidopsis*; therefore, it is strongly suggested that the signalling systems in flower development concluded from *Arabidopsis* studies are fundamental and common to other dicot plants, possibly to all angiosperms.

Transition from vegetative to reproductive growth

Floral initiation of *Arabidopsis* is known to be induced by several environmental factors: photoperiod, cold treatment (vernalization), gibberellin treatment, and nutrients. When wild-type plants are grown at 22 °C under continuous illumination or long-day conditions, floral buds appear after forming 6–8 rosette leaves. The shape of the apical meristem was observed with microscopes to examine the timing of transition from vegetative to reproductive growth. Swelling of the apical dome increases at about 10 days after imbibition, and the first flower primordium appears at about 15 days after imbibition [4, 58]. In the short-day condition, the timing of flowering delays to more than 40 days. When the seeds are sown in pots and incubated at 4 °C in low light, flowering time is reduced [55].

By characterizing the responses of the late-flowering mutants to photoperiod and vernalization, more than 40 mutants of 12 different loci were classified into 3 phenotypic classes [42, 46, 55]. The *fca*, *fpa*, *fve*, *fy* and *ld* mutants are sensitive to both photoperiod and vernalization, whereas the *co* (new name of *fg*) and *gi* (new name of *fb*) mutants are insensitive to both environmental conditions. The rest of the mutants, *fe*, *fd*, *fhc*, *ft* and *fwa*, are sensitive to daylength, but less sensitive or insensitive to cold treatment. In addition to the mutagen-induced mutants, additional late-flowering genes were identified by analyzing other ecotypes of *Arabidopsis*. Late-flowering ecotypes from Europe carry a late-flowering gene, named *FLA* [45]. Two late-flowering genes, *FRI* and *KRY*, are found in Scandinavian natural populations [18]. These mutants are responsive to vernalization. Based on the characterization of the mutants, a model of flowering pathways with genetic and environmental factors is proposed [42]. Attempts to clone the late-flowering genes are being made by chromosome walking or by tagging [4, 75]. Recently, the LUMINIDEPENDENS (LD) gene has been cloned [46]. The gene product is suggested to be a transcriptional factor because predicted amino acid sequence contains two nuclear localization signals and a glutamine-rich region which is known to work as transcription activation domain.

Another set of genes controlling the flowering time have been identified as mutants which initiate flowers earlier than wild type. *elf1* (for *early flowering 1*) and *elf2* mutants are sensitive to photoperiod, but *elf3* is not [112]. The *tfl* (for *terminal flower*) mutant shows early flowering and sensitivity to photoperiod as well as morphological deficiency in the inflorescence meristem identity (see next section) [88, 87, 112]. A mutant of a putative protein kinase gene, *tousled* (*tsl*), shows a late-flowering phenotype, as well as pleiotropic morphological defects (see next section). Another mutant, *embryonic flower* (*emf*), generates flowers immediately after germination bypassing vegetative growth [95]. It is suggested that the *EMF* gene activates vegetative growth and represses flower initiation.

Studies of *Arabidopsis* mutants have revealed the involvement of other factors in the process of flower initiation. First, a plant hormone, gibberellin, is crucial to flower initiation [110]. A gibberellin-insensitive mutant, *gai*, flowers early in a long-day condition, and shows a late-flowering phenotype in a short-day condition.

Table 1. Genes involved in the regulation of flower development in *Arabidopsis thaliana*.

Genes	Mutant phenotype	Proposed function of gene product	References
1. Genes regulating transition from vegetative to reproductive growth			
Late-flowering genes (*CO, FCA, FD, FE, FHA, FLA, FPA, FRI, FT, FVE, FY, GI*)	delayed flowering		4, 18, 42, 45, 55, 75
LUMINIDEPENDENS (*LD*)	delayed flowering	contains nuclear-localization signals and glutamine-rich region/transcription factor	46
EARLY FLOWERING1.2.3 (*ELF1,2,3*)	early flowering		112
TERMINAL FLOWER (*TFL*)	early flowering/conversion of inflorescence meristems to floral meristems		3, 86, 87, 113
TOUSLED (*TSL*)	delayed flowering/reduced number of floral organs/curling of cauline leaves	protein kinase	80
EMBRYONIC FLOWER (*EMF*)	generation of inflorescence meristem without vegetative growth		95
GIBBERELLIN DEFICIENT (*GA1*)	late flowering/defective in gibberellin biosynthesis	*ent*-kaurene synthase	94, 110
GIBBERELLIN INSENSITIVE (*GAI*)	late flowering/insensitive to gibberellin		110
PHYTOCHROME A (*FRE/HY8/PHY2/PHYA*)	long hypocotyl in continuous far-red light/less sensitive to night break	phytochrome A	64, 70, 76, 109
LONG HYPOCOTYL3 (*HY3/PHYB*)	early flowering/long hypocotyl	phytochrome B	29, 32, 76
LONG HYPOCOTYL2 (*HY2*)	early flowering/long hypocotyl	biosynthesis of chromophore	29, 32
PHOSPHOGLUCOMUTASE (*PGM*)	late flowering/deficient of starch synthesis	phosphoglucomutase	13
STARCH OVER-PRODUCTION (*SOP*)	late flowering/deficient of starch degradation		14
2. Genes involved in formation of inflorescence meristem			
TERMINAL FLOWER (*TFL*)	conversion of inflorescence meristems to floral meristems		see above
LEAFY (*LFY*)	partial conversion of floral meristems to inflorescence meristems	transcription factor	35, 81, 107
APETALA1 (*AP1*)	production of axillary flowers in flowers/homeotic conversion of sepals to leaves/absense of petals in strong mutants	MADS box protein transcription factor	10, 31, 38, 53, 54

Table 1. (Continued)

Genes	Mutant phenotype	Proposed function of gene product	References
APETALA2 (*AP2*)	homeotic conversion of sepals to leaves and petals to stamens in weak mutants/homeotic conversion of sepals to carpels/petals and stamens are mostly absent in strong mutants	negative regulator of AGAMOUS	6, 8, 41, 43, 66, 69
CAULIFLOWER (*CAL*)	single mutant is phenotypically wild/conversion of flower meristems to inflorescence meristems in combination with *ap1* mutations		10
CLAVATA1 (*CLV1*)	large meristem/more carpels		17, 23, 47, 66
PIN-FORMED (*PIN*)	forming no floral buds or deformed flowers	a component of auxin polar transport system?	28, 30, 67
FILAMENTOUS FLOWER (*FIL*, *Fl*-54)	after forming several flowers, development of floral meristem stops immaturely, but recovers later/few floral organs/lack of anthers/partial homeotic conversions of sepals to petals, stamens to petals, stamens to carpels		41
ACAULIS1 (*ACL1*)	few flowers/short inflorescence axis		12
AUXIN RESISTANCE1 (*AXR1*)	short inflorescence axis/bushy inflorescence/resistant to auxin/no root gravitropism		27, 48
AUXIN RESISTANCE2 (*AXR2*)	short inflorescence/resistant to auxin		99
3. Genes regulating formation and identity determination of floral organs			
APETALA1 (*AP1*)	see above		see above
APETALA2 (*AP2*)	see above		see above
APETALA3 (*AP3*)	homeotic conversion of petals to sepals and stamens to carpels	MADS box protein transcription factor	6, 8, 38, 39, 68
PISTILLATA (*PI*)	homeotic conversion of petals to sepals and stamens to carpels	MADS box protein transcription factor	6, 8, 28, 34, 66
AGAMOUS (*AG*)	homeotic conversion of stamens to petals/indeterminate floral meristem/no pistil	MADS box protein transcription factor	6–8, 26, 59, 111
SUPERMAN (*SUP*)	more stamens/small pistil	regulator of PI and AP3 expression	9, 82
FILAMENTOUS FLOWER (*FIL*, *Fl*-54)	see above		see above
TOUSLED (*TSL*)	see above		see above
FASCIATA1, 2 (*FAS1, 2*)	fewer petals & stamens/narrow sepals & petals/stem flattening		47
CLAVATA1 (*CLV1*)	see above		see above

Table 1. (Continued)

Genes	Mutant phenotype	Proposed function of gene product	References
4. Genes regulating floral organ growth and maturation			
BICAUDAL (*BIC*, *Fl-89*)	two clumps of stigmatic papillae and two horny structures at the top of pistils/narrow sepals & petals		41
FIDDLEHEAD (*FDH*)	fusion of buds, floral organs and leaves		50, 51
FILAMENTOUS FLOWER (*FIL*, *Fl-54*)	stamens lacking anthers		see above
ANTHERLESS (*AT*)	stamens lacking anthers or with anthers converted to sepals		15, 16
male-sterile mutants (*MS1*, *MS2*, *MS3*, *MS4*, *MS5*, *MS7*, *MS8*, *MS15*, *MSK*, *MSW*, *MSX*, *MSZ*, *BM3*)	deficient pollen development		1, 16, 25, 77
MALE-STERILE H (*MSH*)	no dehiscence of anthers		25
APT (*BM3*)	abortive pollen development after meiosis/lack of APRT activity	adenine phosphoribosyl-transferase (APRT)	61, 77
POP1 (POLLEN-PISTIL INTERACTIONS)	lack of extracellular pollen coat, tryphine		73
QUARTET 1, 2 (*QRT1, 2*)	pollen grains released in tetrads		74
BELL1 (*BEL1*)	abortive ovules/conversion of ovules to carpel-like structures		60, 79
SHORT INTEGUMENTS1 (*SIN1*)	abortive ovules		79
OVULE MUTANT-2,3 (*OVM2, 3*)	defective integument development		78

Flowering is promoted by cold treatment, whereas gibberellin-deficient mutants, *ga1–3*, flower late in a long-day condition, is unable to flower in a short-day condition, and is insensitive to vernalization. The *ga1* mutant dramatically responds to sprayed GA_3 and produces flowers as promptly as GA-treated wild-type plants. These results indicate that the process of flowering requires a certain level of GA and GA-mediated signal transduction systems. Second, phytochromes A and B are involved in the response to daylength. Phytochrome A-deficient mutants, *phyA*, are insensitive to photoperiod [76], whereas phytochrome B-deficient mutants, *phyB* and *hy3*, and a chromophore-defective mutant, *hy2*, are responsive to photoperiod and initiate flowers earlier than wild-type plants [29, 32, 76]. Interestingly, *hy3* and *hy2* mutations compensate for the late-flowering phenotype when combined with some of the late-flowering mutations, because flowering time becomes shorter in double mutants of *hy3* or *hy2* with one of the later-flowering mutants, *fca*, *fwa* or *co*, under continuous light [32]. Third, starch degradation and movement in plant body is considered to be responsible to flower initiation, because retardation of flowering is observed in starchless mutant, phosphoglucomutase-defective (*pgm*), and in starch degradation-deficient mutant, starch overproducer (*sop*) [13, 14].

Control of flowering initiation is a multifactorial process including many genes which may regulate a network of signalling pathways. The search of key factors such as florigen and antiflorigen has not been successful so far. Although more detailed physiological, genetic and molecular studies are required to clarify the nature of

signalling pathways, a recent hypothesis postulates at least two promotive pathways and two inhibitory pathways in the process [5].

Formation of the inflorescence meristem

Immediately after reproductive growth has started, the vegetative shoot is converted to an inflorescence. Floral buds are formed one by one at the apex, and the axis elongates. The inflorescence meristem has three functions: (1) formation of floral meristem on its flanks; (2) formation of axis tissues at its bottom; and (3) maintenance of the inflorescence meristem at the top. Recent mutational studies have revealed that different sets of genes regulate the three functions. The early-flowering gene, *TFL* (for *terminal flower*), is known to govern the 2nd and the 3rd functions, because *tfl* mutant shows conversion of the indeterminate inflorescence meristem to a flower, and stop of the growth of inflorescence [3, 86]. In several cases, a large flower with an excess number of floral organs are formed at the terminal part. Lateral branches also terminate in a flower. The first function of the inflorescence meristem is regulated by several genes. In *leafy* mutants (*lfy*), floral meristem develops into an inflorescence carrying aberrant flowers without petals and stamens [35, 81, 107]. The cloned *LFY* gene shows extensive sequence homology with the *Antirrhinum majus* gene, *FLORICAULA* (*FLO*), which has the same mutant phenotype to *LFY* [19, 107]. LFY and FLO are suggested to be transcription factors, because they have a proline-rich domain and an acidic domain characteristic of transcriptional activating domains. It is suggested that the *LFY* gene is involved in the decision of identity and maintenance of the floral meristem, because *LFY* is expressed in young flower primordia, but not in inflorescence meristems [107]. Double mutant analysis with *lfy* and other flower mutants have revealed that (1) LFY interacts with APETALA1 (AP1) and APETALA2 (AP2); and (2) the *lfy* mutation is epistatic to *pistillata* (*pi*) and *agamous* (*ag*) mutations [35]. Further genetic analysis of multiple mutants has led to draw a simple model of inflorescence development [87]. According to this model, three genes, AP1, AP2 and LFY, promote growth of the floral meristem and repress development of the inflorescence meristem. Instead, TFL promotes inflorescence meristem and suppresses floral meristem. TFL may repress expression of *AP1*, *AP2* and *LFY* genes, whereas the three genes may negatively control *TFL* expression (Fig. 1). Mutual activation of *AP1*, *AP2* and *LFY* is also proposed by other groups [10, 83]. The model of the positive and negative interactions is supported by the observations of *in situ* hybridization. Expression of *LFY* and *AP1* genes normally observed in floral buds of early

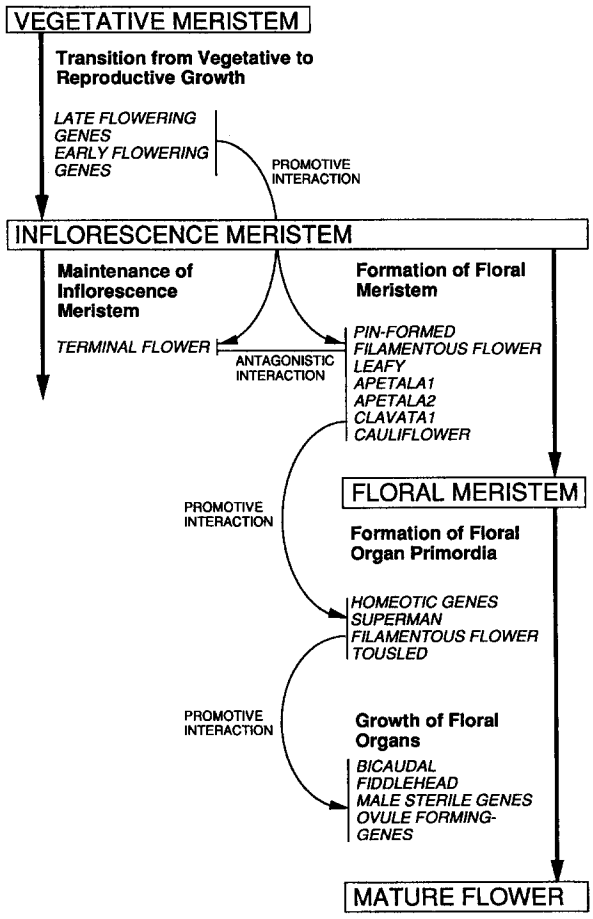


Fig. 1. Genetic regulatory network in the process of flower development. Genes involved in each step are listed in italics. Thick vertical arrows indicate temporal change of the fate of meristemic cells.

stages is also observed in the converted floral meristem at the top of inflorescence axis of the *tfl* mutant [10, 31, 107].

Two genes, *CAULIFLOWER* (*CAL*) and *CLAVATA1* (*CLV1*), are also involved in establishing and maintenance of floral meristem. Although strains carrying a single mutation in *CAL* gene have normal inflorescence, double mutants having mutations in both *CAL* and *AP1* in homozygous state convert a floral meristem to an inflorescence meristem with highly branched flowers, just like the famous vegetable cauliflower [10]. Both *AP1* and *LFY* are positively regulated by *CAL*. Interestingly, the flower morphology of the triple mutant, *ap1 cal tfl*, and that of another triple mutant, *ap1 cal lfy*, are indistinguishable from that of the *ap1 tfl* double mutant and that of the *ap1 lfy* double mutant, respectively [10]. The epistasis of *tfl* and *lfy* mutations to *cal* in an *ap1* mutant background indicates the close interaction of the gene products in the process of floral meristem establishment. *CLV1* is known to have a function that determines the size of vegetative, inflorescence and floral meristems. The large floral meristem of the *clv1* mutant generates a flower with a big pistil of 4 carpels [17, 23, 47, 66]. When *clv1* is combined with the *lfy*, *ap1* or *tfl* mutant, the *clv1* mutation enhances the phenotypes of single mutants, indicating that *CLV1* is involved in determining meristem identity by interacting with *LFY*, *AP1* and possibly with *TFL* [10, 87].

In addition to the five genes described above, we would like to emphasize that two more genes, *PIN-FORMED* (*PIN*) and *FILAMENTOUS FLOWER* (*FIL*), are playing important roles in establishing floral meristems. Phenotype of *pin* mutants are pleiotropic: they show structural abnormalities in embryo, phyllotaxis, leaves, inflorescence and flowers [30, 67]. The typical inflorescence of the *pin* mutant looks like the tip of a pin because it fails to generate floral buds (Fig. 2A). The naked apex often shows segments which may correspond to internodes between flowers. Because the inflorescence axes continuously grow at a normal rate, it is suggested that the *pin* mutant is missing the first of the three functions of the inflorescence meristem described above, formation of floral meristem, but is still keeping the other two functions, formation of axis tissues and maintenance of the meristem. In some cases, however, deformed flowers are formed at the top of axes (Fig. 2B). The flowers often lack stamens, but have a few wide petals or many narrow petals, and usually have a pistil at the center. In extreme cases, a pistil-like structure with no other organs is formed. The pistils are sterile, because ovules are not fully developed. In addition to the structural defects of the inflorescence, the *pin* mutants have aberrant phenotypes in other organs. The position of the two cotyledons are not symmetric like that of wild type. In some cases two cotyledons are fused to one. Rosette leaves are often wide with a vein branched at the base. Phyllotaxis on the inflorescence axes is also abnormal; two lateral shoots are often formed in 'opposite' positions in the mutant, whereas lateral shoots are formed in 'alternate' positions in wild type [30, 67].

We found wild-type plants grown on agar medium containing an auxin polar transport inhibitor, 9-hydroxyfluorene-9-carboxylic acid (HFCA) or N-(1-naphthyl)phthalamic acid (NPA), to be a phenocopy of the *pin* mutant [67]. Because almost all of the pleiotropic phenotypes of the mutant were observed in the drug-treated plants, we assumed that the activity of auxin polar transport is reduced in the *pin* mutant, and that reduction of the activity is the cause of structural abnormalities. This assumption was supported by the result of direct measurement of the auxin transport activity. The activity of the inflorescence of the mutant is about 10% of that of wild type [66]. Further supporting evidence for the assumption was obtained from *in vitro* embryo culture of Indian mustard, *Brassica juncea* [49]. Abnormal embryos with fused cotyledons similar to these of the *pin* mutant were induced by auxin polar transport inhibitors. These results indicate that the auxin polar transport system plays an important role in the process of floral meristem establishment. In order to examine at which stage(s) of flower bud development the normal auxin transport system is required, a series of shift experiments have been done (Junichi Ueda *et al.*,

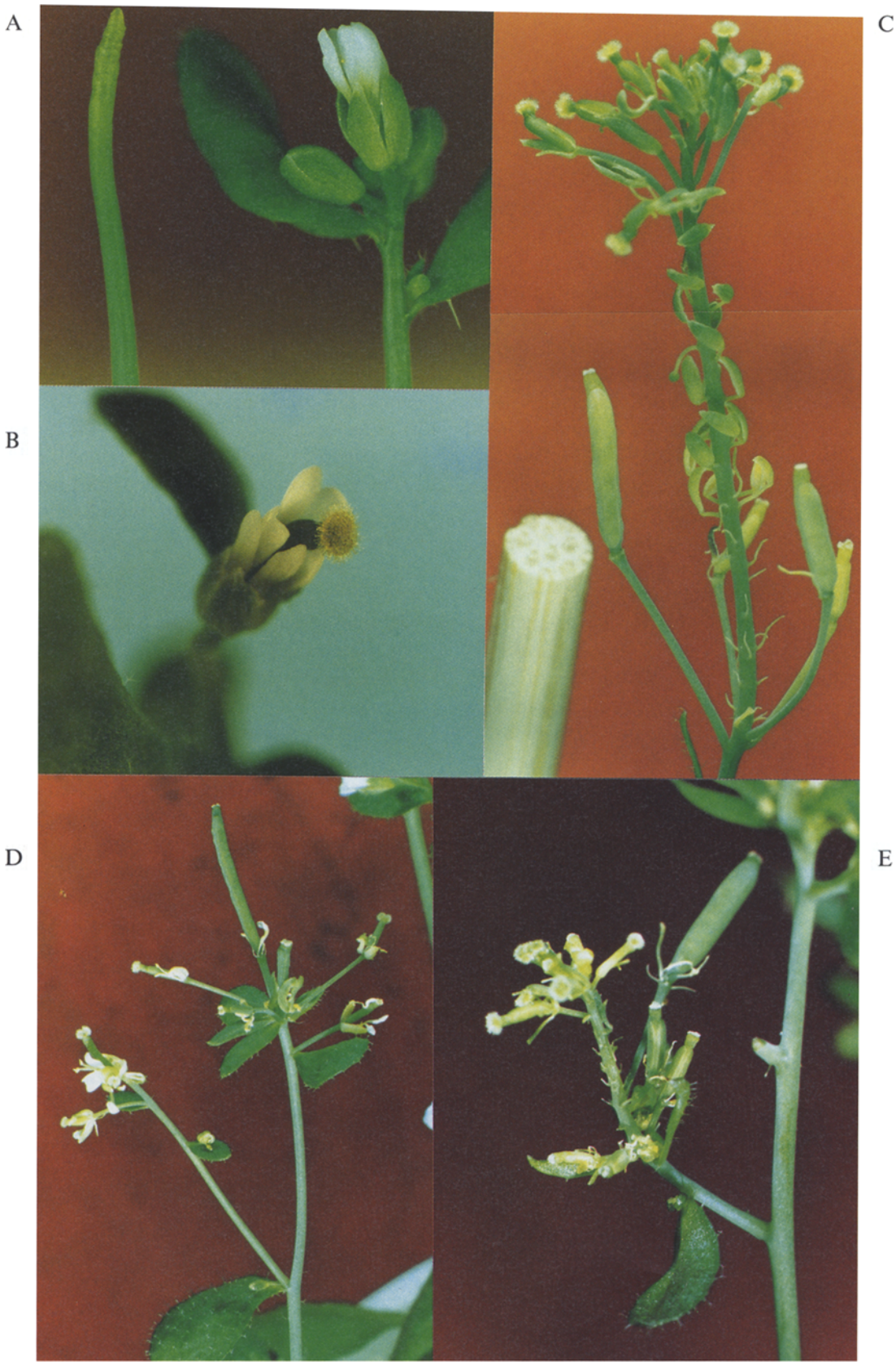
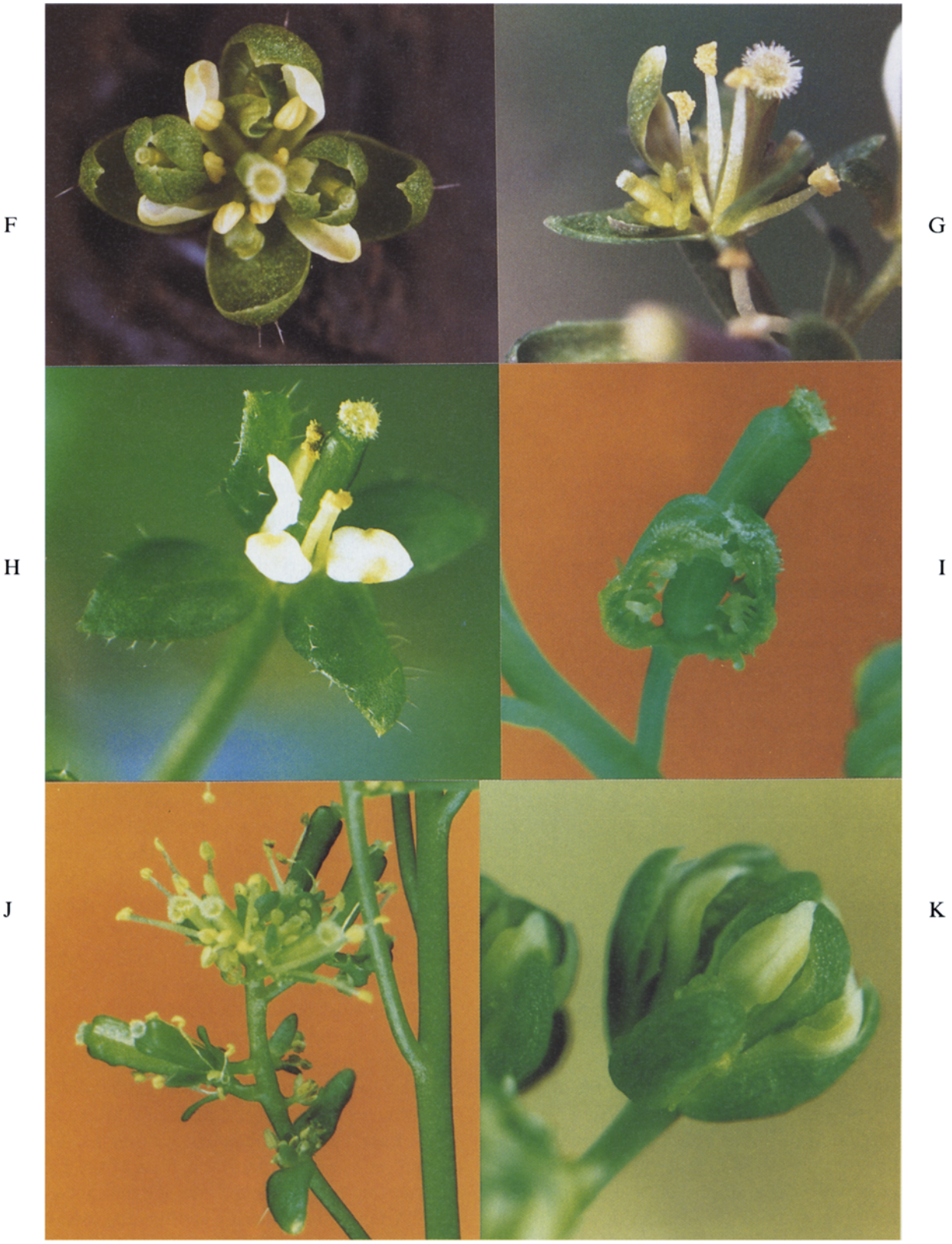


Fig. 2. Phenotypes of wild type and mutant *Arabidopsis* flowers. A. Inflorescence of the wild type (right) and of the *pin-formed* (*pin*) mutant (left). B. A deformed flower of the *pin* mutant. C. Inflorescence of the *filamentous flower* (*fil*) mutant. Phases 1, 2, 3 represent the four fertilized siliques at the bottom, filaments and sepal-like structures in the middle region, and a cluster of flowers at the top, respectively. D. Inflorescence of the *fil tfr* (*terminal flower*) double mutant. Only the phase 1 flowers are formed. E.



A flower of the *fil ap1* (*apetala1*) double mutant. A flower is converted to an inflorescence. F. A flower of the weak allele of the *ap1* mutant, the *Fl-1* strain. Four normal petals are formed. G. A flower of the strong allele of the *ap1* mutant, *ap1–1*. No petals are formed. H. A flower of the weak allele of the *ap2* (*apetala2*) mutant, *ap2–1*. Four sepals and four petals are converted to leaf-like structures and staminoid petals, respectively. I. A flower of the strong allele of the *ap2* mutant, *ap2–3*. The flower has two sepals converted to carpels, lacks petals and stamens. J. A flower of the weak *ap1*/weak *ap2* double mutant. A flower is converted to an inflorescence with flowers lacking petals. K. A flower of the heavy *ap2 ag* double mutant. Staminoid petals are formed.

manuscript in preparation). Wild-type plants germinated and grown for several days under continuous light on agar medium containing HFCA were transferred onto agar medium without the drug. After incubation on the medium for 3 weeks, the structure of the first flower was examined. More than 80% of the first flower was normal when the plants were transferred at 4 days after germination. However, when transferred at 6 days after germination, nearly 80% of the plants developed an inflorescence of a complex form composed of two regions; the lower region shows *pin* mutant-specific segments with no floral buds, but the upper region bears normal floral buds. This structure indicates that development of the first flower was repressed, but the repression did not work for the development of later flowers. A series of experiments indicated that the presence of transport inhibitor before 4 days after germination do not influence floral bud formation, but that the presence of the drug at 6 days or more after germination represses the normal development of the first floral meristem. Shift experiments in the reverse direction help to determine the period that the drug is effective for development of the first flower. When wild-type plants germinated and grown on agar medium without the drug were transferred onto medium containing the drug at 10 days after germination, nearly 70% of plants showed pin-formed inflorescence; however, when transferred at 14 days after germination, all of the first flowers were normal. Therefore, by combining the results of shift experiments, it is strongly indicated that formation of the first floral meristem is inhibited by auxin polar transport inhibitors if the drug is supplied from 5 to 12 days after germination. This 'critical 7 days' includes the timing of transition from vegetative to reproductive growth, and precedes the timing of formation of the first floral meristem of visible size. Therefore, reduction of auxin polar transport activity may have crucial effects on floral meristem formation at very early stages, even if considering that the drug remains for several days in plant body after removal of the drug from the medium.

We have also noticed that *FILAMENTOUS FLOWER* (*FIL*) gene is regulating establishment and development of floral meristem. A *fil* mutant (former name is *Fl-54*) shows several structural defects in flowers and inflorescences [44]. Although wild-type plants bear flowers in a spiral arrangement at more or less constant intervals along the inflorescence axis, the mutant has three different phases of flower formation under growth conditions of constant temperature, illumination, nutrients and water supply (Fig. 2C). After flower initiation, the *fil* mutant elongates an inflorescence axis normally, and forms about 10 flowers (phase 1). The flowers show several structural abnormalities in shape, number and position of floral organs and have long peduncles. Numbers of sepals, petals and stamens are decreased. Most of the stamens lack anther sacs. In some flowers, the top of filaments is white and flat like a petal, or covered by stigmatic papillae. Homeotic conversion is also observed in petals and sepals. Some petals carry pollen sacs. Margins of sepals are sometimes white, showing partial conversion to petals. In flowers lacking some floral organs, the remaining organs are not positioned symmetrically. Pistils are less affected by the mutation and set seeds. After forming the phase 1 flowers, formation of floral buds is stopped at the inflorescence meristem, and instead, more than 10 filaments and more than 10 sepal-like structures are formed (phase 2). Because the sepal-like structure is facing the inflorescence axis as the abaxial sepal, and sits on a peduncle-like short stem, we suppose this structure corresponds to a floral bud whose development is stopped at very early stages after forming only the abaxial sepal. It would be worth noting that the abaxial sepal is located at the outermost on the floral meristem, and develops first in the 16 floral organs in a flower [63, 89]. The filament would be a peduncle without flowers, because in a rare case, a small floral bud is formed at the top of a filament. After forming the phase 2 cluster, a cluster of flowers is formed again (phase 3). Structural defects of the phase 3 flowers are heavier than those of the phase 1 flowers. A sepal-like structure is often attached to the long peduncle, suggesting that the phase 3 flowers originate from the sepal-like structure, but the developmental process is not stopped. Usually,

petals are missing, and sepals and stamens are converted to filaments with no extra organs at the top. Pistils look normal and fertile. The phase shift of inflorescence is also observed in lateral shoots as well as in the main stem. Phases are changed in an order of 1–2–3, but phase 1 is omitted in some lateral shoots coming later. In summary, the *FIL* gene may have two different functions: one in early stages of floral bud development and the other in formation and growth of floral organ primordia. A lack of the first function results in the formation of filaments or sepal-like structures instead of flowers. A defect of the second function causes a decreased number of floral organs and misdifferentiation of the organs. The phase shift observed in the mutant inflorescence strongly suggests involvement of a cell-cell communication system in the early stage of floral meristem development. Although synchronization of the phases in main and side shoots of individual plant is not clear, one can postulate that some diffusible factor(s) are participating in the communication. One possible explanation of the phase shift is that the amount of the factor(s) or the amount of receptor molecules of the factor(s) changes to high-low-high as plants grow, and phase 2 results when the amount decreases to a level lower than a threshold level enough to support the development of floral meristem to a mature flower. In order to examine the interactions with products of other genes regulating establishment and growth of the floral meristem, a series of double mutants were constructed (Komaki *et al.* and Okada *et al.*, manuscripts in preparation). Changes of the three phases are observed when combined with *lfy*, *ap1*, *ap2* and *clv1* mutants. The *fil tfl* double mutant has only the phase 1 flowers, because growth of the inflorescence meristem terminates before shifting to phase 2 (Fig. 2D). In combination with *fil* and a strong allele of the *ap1* mutant, *ap1–1*, phase 1 flowers are converted to inflorescence meristem bearing many flowers (Fig. 2E). When combined with weak alleles of *ap1* or *ap2* mutant, the flower structure of double mutants resembles that of a heavy allele of the *ap1* or *ap2* mutant, respectively. *FIL* also interacts with *CLV1*, because inflorescences of the *fil clv1* double mutant are fasciated and form a clump of meristemic tissue covered by stigmatic papillae when aged more than 6 weeks. These results show that *FIL* controls the process of floral meristem formation in combination with *AP1*, *AP2* and *CLV1*.

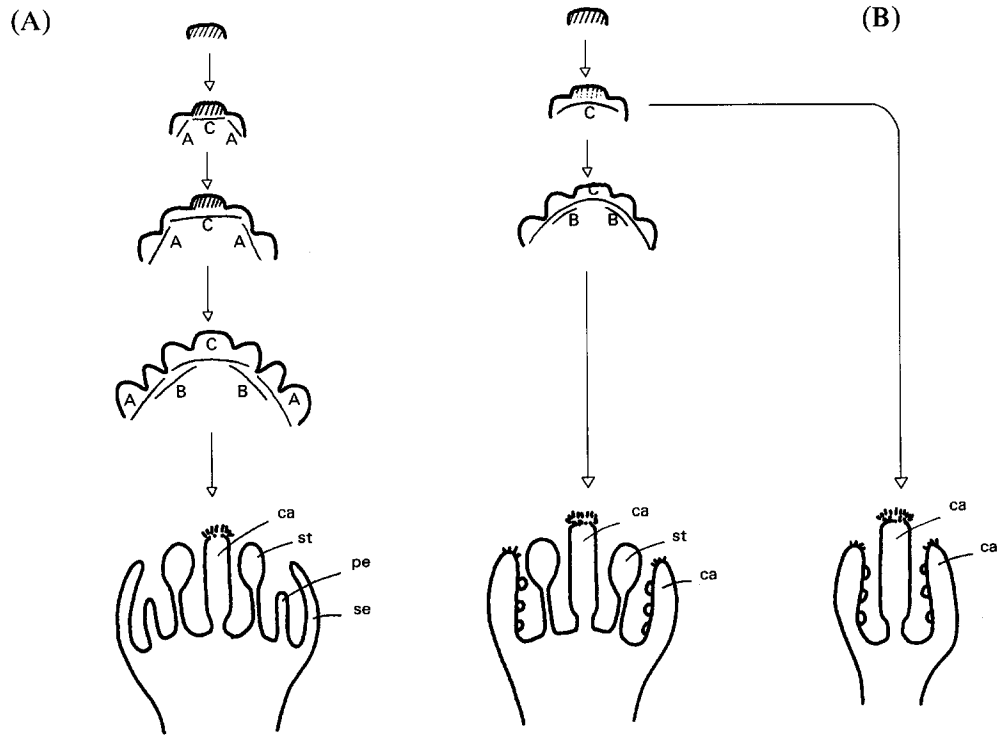
Several other genes are reported to control growth of inflorescence axis. *ACAULIS1* (*ACL1*) has a very short inflorescence axis and bears less than 10 flowers. Because cells of the mutant at the internode are small but the number of cells is not changed, a short axis is considered to result from the defect in cell elongation process. The size of the inflorescence meristem of the *acl1* mutant is reduced to about half of that of wild type [102]. A defect in internode elongation is also observed in two auxin-resistant mutants, *axr1* and *axr2*. The short inflorescence of the *axr1* mutant is caused by a decreased number of cells [48]. However, in the case of the *axr2* mutant, cell elongation is repressed [99]. The heavy allele of the *axr1* mutant is bushy. It generates about 30 thin-stemmed primary inflorescences [27], indicating that auxin has a role in activation of branching and growth of lateral shoots.

Formation and identity determination of floral organs

A flower originates from a floral meristem which is composed of three layers of undifferentiated cells (L1, L2 and L3) [104, 105]. Analysis of chimeras between tomato strains differing in number of carpels has shown that number and size of carpels are not determined by the genotype of cells in the outer cell layers, L1 and L2, but by the genotype of L3 cells [96]. Because coordinated cell division and expansion between cells of the three layers are necessary to generate floral organs, this result indicates the importance of cell-cell communication in the vertical direction in the floral meristem. Intercellular communication in the horizontal direction within the meristem is also known to be indispensable for organizing the floral organs in a flower. If an immature floral meristem is cut along the median line and has

been incubated for several days, partly or completely divided flowers are produced [24, 33, 90]. The structure of the resulting flower is dependent on the stage of flower development at which the microsurgery is done. When the floral meristem is bisected before forming sepals, two almost complete flowers are regenerated. However, if bisected when sepal primordia are already formed but primordia of other organs are not formed yet, two flowers with normal numbers of petals, stamens and carpels are formed, but the number of sepals is not compensated. A series of median bisection experiments suggests that the identity of the single flower is based on cell-cell communication between the center and peripheral regions of meristem, and that the position of floral organs is fixed on the basis of the cell-cell communication system. The molecular nature of the communication system in either vertical or horizontal direction in the floral meristem is not clarified yet. Although similar experiments have not been repeated in *Arabidopsis*, the same communication system is undoubtedly involved in the process of floral bud formation in this plant. Further investigations are necessary to identify genes controlling the system.

Recent genetic and molecular studies of homeotic mutants of *Arabidopsis* and *Antirrhinum* have provided a beautiful model explaining how the floral organ identity is determined [8, 10, 22, 31]. This model, designated the ABC model [22, 52], is based on three assumptions: (1) the floral meristem is simplified to be composed of four concentric regions, named whorl 1, 2, 3 and 4 from outside to inside; (2) three groups of homeotic genes are expressed in different whorls, namely group A genes in whorls 1 and 2, group B genes in whorls 2 and 3, and group C genes in whorls 3 and 4; (3) the fate of organ primordia is determined by the combination of homeotic genes expressed in the whorl where the primordia are located, namely expression of group A genes leads to sepals, expression of group A and B genes to petals, expression of group B and C genes to stamens, and expression of group C genes to carpels. *Arabidopsis* genes identified to correspond to



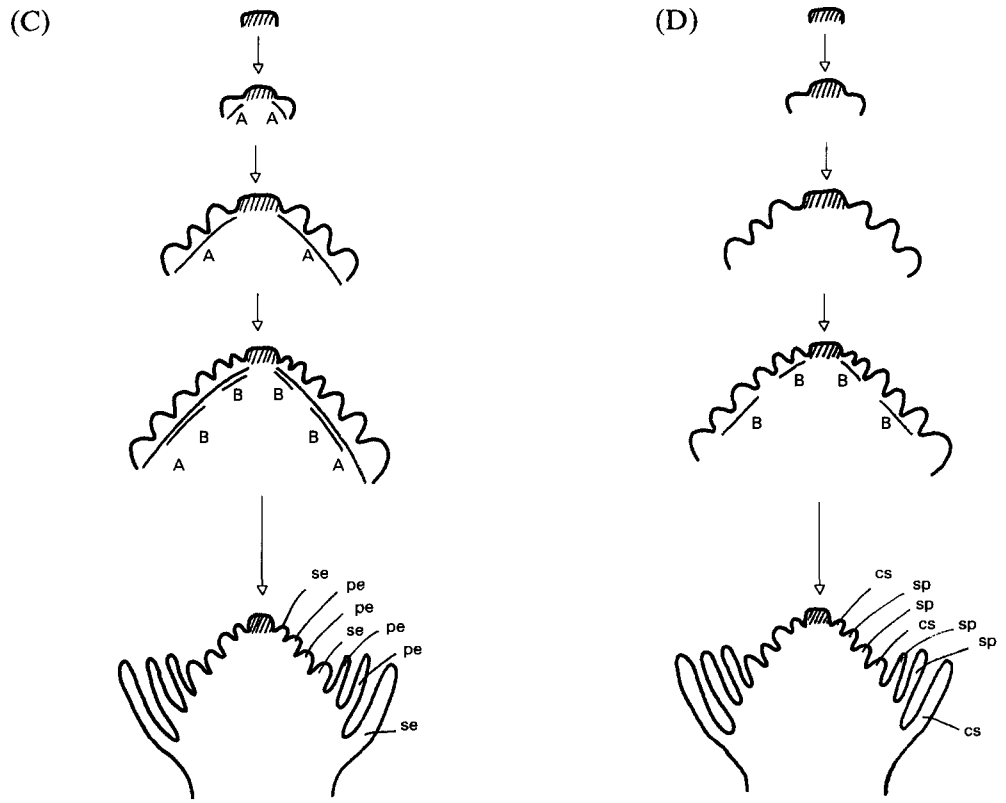


Fig. 3. Schematic representations of the modified model of whorl formation and floral organ identity. Bars A, B and C in the figures indicate the region where groups A, B and C of organ identity genes are expressed, respectively. Hatched regions indicate meristemic cells. Figures at the bottom represent the final structure of flowers. se, sepal; pe, petal; st, stamen; ca, carpel; sp, staminoid petal; cs, carpeloid sepal. (A). Development of wild-type flowers. At early stages, group A genes are expressed at the marginal region, and group C genes are expressed at the central region of the floral meristem. When the amount of group C gene products exceeds the threshold level, proliferation of meristemic cells is halted, and the carpel primordia are formed at the top of meristem. At a later stage, group B genes are expressed in the 2nd and 3rd whorls. The combination of group A, B and C genes determines the identity of organ primordia. (B). A flower of a group A gene mutant. In case of heavy mutants of group A genes, a heavy allele of *ap1* or a heavy allele of *ap2*, expression of group C genes is enhanced and distributed to the whole regions of meristem. Therefore, the amount of group C gene products increases faster and reaches the threshold level before the meristemic cells proliferate to form 4 whorls. If proliferation of the meristemic cells stops after 2 whorls have been formed, organ primordia develop to carpels. If 3 whorls are formed, group B genes are expressed at the intermediate whorl. Organ primordia on the intermediate whorl develop to stamens. The same situation is expected in the weak *ap1*/weak *qp2* double mutant. (C). A flower of a group C gene mutant. Lack of group C gene expression leads the meristemic cells to proliferate eternally, and to make many whorls indeterminately. Expression of group A genes also continues. If it is assumed that group B genes are expressed discontinuously, floral organs repeat the pattern of sepals-petals-petals. (D). A flower of a heavy allele of an *ap2-ag* double mutant. Many whorls are formed owing to the lack of group C genes. If group B genes are expressed in the same discontinuous pattern as that of C, organs repeat the pattern of carpeloid sepals-staminoid petals-staminoid petals.

the A, B, and C groups are *AP1* and *AP2* (group A), *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) (group B), and *AGAMOUS* (*AG*) (group C), which are known to cause several different types of homeotic conversion of floral organs [7, 8, 22, 31]. A similar set of genes have also been studied in *Antirrhinum majus*, namely *SQUAMOSA* (*AP1* homologue), *DEFICIENS* (*AP3* homologue), *GLOBOSA* (*PI* homologue) and *PLENA* (*AG* homologue) [11, 12, 20, 22, 37, 84, 91, 101]. Interestingly, all of the homeotic genes listed above except *AP2* encode a protein containing a con-

served motif, the MADS box [11, 37, 39, 40, 53, 54, 68, 84, 91, 92, 101]. The MADS-box-containing proteins are considered to be transcription factors, because well-characterized MADS-box proteins of human (SRF) and yeast (MCM1) promote expression of other genes as transcription factors [65, 71], and because some of the MADS-box genes, SRF, MCM1, *DEFICIENS* and *AG*, are known to bind to a DNA sequences containing a consensus sequence, 5′-$CC(A/T)_6GG$ – 3′ (termed CArG box) [36, 62, 65, 72, 85, 88]. *AP2* gene product, however, does not contain the MADS box, but encodes a putative transcription factor, because this protein contains a nuclear localization signal and a highly acidic serine-rich domain which is found in several DNA-binding proteins [69].

In situ hybridization experiments using probes of the homeotic genes, *AP1*, *AP3*, *PI* and *AG*, showed a unique pattern of localized expression as expected from the model [7, 28, 31, 39, 108, 111]. However, expression of *AP2* is not localized in whorls 1 and 2 as predicted from the model but in all regions of floral meristem, even in leaf, stem and root at low levels [68]. It is suggested that *AP2* behaves as group A genes only in combination with some other gene product(s); a possible candidate is *AP1*. The localized expression of the homeotic genes is explained by negative and positive regulatory interactions between the genes, namely (1) *AP3*, *PI* and *AG* are activated by *AP1* and *LFY*; (2) *AP1* is repressed by *AG*; (3) *AP3* and *PI* are repressed by another gene, *SUPERMAN* (*SUP*) [9, 82]. The regulatory circuit is supported by the ectopic expression experiments using transgenic plants [40, 54, 59].

These lines of results are consistent and strongly support the ABC model, at least assumptions (2) and (3). However, as for assumption (1), the model does not explain how the number of whorls is determined. In addition, flower structure of some homeotic mutants is not fully explained from the model. As mentioned in Bowman *et al.* [7, 8], it is not easy to understand why strong alleles of *ap2* mutants lack organs in whorls 2 and 3 (petals and stamens) (Fig. 2I) and nevertheless the missed whorls are recovered in the strong *ap2 ag* double mutant (Fig. 2K). The recovery of the second whorl organs in the *ap2 ag* double mutant is explained as a result of ectopic expression of AG, but its mechanism is not shown [7, 8, 30]. In the case of the strong *ap1 ag* double mutant, petals are recovered in flowers of aged plants; nevertheless, the strong *ap1* allele usually lacks flowers [30]. In addition, it is also difficult to explain why petals are missing in the weak *ap1*/weak *ap2* double mutant, although both weak *ap1* and *ap2* mutants have four petals (Fig. 2F, H, J) [10, 38, 41, 43].

Figure 3 shows a modified version of the organ identity model which can explain the difficulties described above. This model is based on three additional assumptions. First, full expression of group C genes stops proliferation of undifferentiated meristemic cells at the top of the floral meristem, and converts the meristemic cells to carpel primordia. Second, because expression of group A and C genes (or activation of the gene products) is mutually repressed, expression level of group C genes as well as distribution of cells expressing group C genes are controlled by a balance of expression between group A and group C genes. Third, whorls are formed from the meristemic cells from the marginal to the central region of floral meristem. Following to the model of wild-type flower development, group A genes are expressed at the marginal region, whereas group C genes are expressed at the central region of floral meristem at very early stages of flower development (Fig. 3A). As floral meristem grows, whorls are formed one by one from the marginal region of the meristem. The expression level of group C genes increases at the central region, and finally reaches a threshold level high enough to stop further proliferation of floral meristemic cells. At this stage, normal proliferation of meristemic cells results in the formation of four whorls. Group B genes express at later stages than group A and C genes. The identity of primordia formed on each whorl is determined by a combination of the expression of group A, B, and C genes. In case of a heavy allele of group A mutants, expression of group C genes is not restricted to the inner region of meristem. The enhanced expression of

group C genes will stop the proliferation of meristemic cells at an earlier stage than that of wild-type flowers, so that the meristem stops its growth before it has formed four whorls. As a result, only two or three whorls are formed (Fig. 2I). In case of flowers of three whorls, expression of group B may be permitted at the intermediate whorl. Primordia formed on both the outermost and the innermost whorl differentiate to carpels, and primordia on the middle whorl to stamens (Fig. 3B). A similar mechanism will work in case of strong alleles of the *ap1* mutant and weak *ap1*/weak *ap2* double mutants (Fig. 2). This model indicates that whorls 2 and 3 present in wild-type flowers are not deleted in the mutant flowers, but only two or three whorls are formed, and the whorls differentiate as whorls 1, 3 (in case of flowers of three whorls) and 4 of wild type. On the contrary, in the *ag* mutant, a lack of group C gene function leads to continuous proliferation of the meristemic cells (Fig. 3C). Indeterminate growth of the meristem results in formation of many whorls. Assuming that group B genes will be expressed at every second and third whorls following to a mechanism somewhat similar to that of wild type, the reiterated pattern of sepals-petals-petals is formed. In strong *ap2 ag* double mutants, a lack of group C gene function may lead to the formation of many whorls as shown in the *ag* mutant. If it is assumed that group B genes are expressed at the same regions as those postulated for the *ag* mutant, floral organs are formed in the reiterated pattern of carpeloid sepals-staminoid petals-staminoid petals (Fig. 3D). In case of a strong *ap1* (*ap1-1*) *ag* double mutant, petals are recovered in flowers of aged plants, although flowers of young plants do not have petals or petal-like organs, but instead bracts with an axillary shoot are repeatedly formed [31, 38]. The failure of recovery of petal-related organs in the young plants of the double mutant can be explained as that the initial activation of *LFY* by the *AP1* gene product is abolished by the *ap1* mutation, and that floral meristem is partially converted to leafy inflorescence. The modified model (Fig. 3) shows that the number of whorls is determined by the balance of group A and C homeotic genes, and that mutations of the genes affect the number of whorls as well as the identity of organ primordia. We believe this model to explain the architecture of flowers more easily than the original ABC model. It would be worthy of further evaluation.

Several other genes are reported to control the number of floral organs. As described in the preceding section, flowers of the *fil* mutant have decreased numbers of sepals, petals and/or stamens [41]. The position of remaining organs deviates from the symmetric arrangement observed in wild-type flowers. FIL is possibly required for correct arrangement of floral organ primordia. Mutants of the putative protein kinase gene, *TOUSLED* (*TSL*), form flowers of decreased number of sepals, petals, and stamens [80]. Electron microscopic analysis revealed that the symmetric pattern of initiation of sepal primordia is altered. Pleiotropic phenotypes of *tsl* and *fil* are somewhat similar, but the two genes are not allelic, because *TSL* is mapped on chromosome 5 [80] whereas *FIL* is on chromosome 2 (Komaki *et al.*, unpublished results). Reduction of floral organs is also reported in stem-fasciation mutants, *fasciata1* (*fas1*), and *fasciata2* (*fas2*) [47]. Mutations of *CLV1* cause enlargement of apical and floral meristems, and form four carpel primordia and an extra meristemic tissue at the innermost whorl [17, 23, 47, 66].

Floral organ growth and differentiation

The organ identity genes activate new sets of genes that promote growth and differentiation of the floral organ primordia. This step is most likely mediated by cascades of transcriptional regulation, because the organ identity genes encode proteins having characteristics of transcriptional factors. Searches of the target genes are being done using several different procedures, such as screening of organ-specific cDNA clones, differential screening of cDNA libraries of wild-type and homeotic mutant flowers, and direct biochemical or immunochemical isolation of the DNA fragment-MADS box protein complex. It is still difficult to have a general scope of the regulatory cascade,

because only a few putative target genes have been isolated from several plant species. An extracellular leucine-rich repeat protein gene, *FIL2*, is specifically expressed in stamens and carpels of *Antirrhinum majus* [93]. Because the *FIL2* gene is not expressed in a homeotic mutant, *deficiens*, and has a CArG box, the binding motif of MADS box proteins, in its promoter region, it is strongly suggested that *FIL2* is a target of the *DEFICIENS* gene product. The ascorbate oxidase gene of *Brassica napus* has a CArG box in its promoter [2]. This gene is specifically expressed in pollens at later stages of the maturation process. In *Petunia hybrida*, a family of DNA-binding protein containing two repeats of a zinc finger motif is expressed specifically in floral organs; namely, *EPF1* is mainly expressed in petals, and *EPF2–5* is transcribed in petals and stamens, whereas *EPF2–7* is expressed in sepals and petals at high level but in other floral organs at low level [97, 98]. The organ-specific pattern of gene expression and the presence of the CArG box in the promoter region of *EPF1* and *EPF2–5* genes strongly suggest that the *EPF* genes are positively regulated by the MADS-box-containing floral organ identity genes. Because EPF1 has a DNA-binding activity in the promoter region of *EPSPS*, the 5-enolpyruvylshikimate-3-phosphate synthase gene, which is a key enzyme in the pigment synthesis pathway in petals, it is postulated that a transcription regulatory cascade, MADS-box-containing petal identity genes (groups A and B) – *EPF1* – *EPSPS*, is working in petals of *Petunia* [97]. DNA-binding analyses of SRF and MCM1, MADS-box proteins of human and yeast, demonstrate that the proteins bind to the target genes as ternary complex with accessory proteins [62, 100]. The MADS-box-containing plant genes are also postulated to act as transcription regulators in the form of a multicomponent protein complex.

There are several other genes that control growth and maturation of floral organs. *BICAUDAL* (*BIC*, a new name of *Fl*-89) has a function in pistil development [41]. Flowers of the *bic* mutant have a pistil with two clumps of stigmatic papillae and two horn-shaped structures at the top. Sepals and petals of the mutant are narrower than those of the wild type. In the process of normal growth of pistils, two carpel primordia develop to a cylinder-formed pistil as a result of coordinate growth and fusion of the carpels. The opening at the top of young pistils is closed at the later stage [66, 89]. The *bic* mutant is defective in the fusion of the two carpels, and in the closing at the top of pistils. The loss of fusion process of carpels is drastically enhanced in the *bic ap1* double mutant, indicating an interaction of the two gene products in pistil development (Komaki *et al.*, unpublished results). Another interesting gene, *FIDDLEHEAD* (*FDH*), is reported to be possibly involved in the ontogenetic fusion process [50]. Floral organs and leaves of the *fdh* mutant fuse and form structures reminiscent of fern fiddleheads, by altering epidermal competence to adhere to cell walls. *FDH* appears to be a regulatory gene controlling the carpel-specific fusion program.

Another important process in floral organ maturation is development of gametes. Male-sterile mutants are classified into four groups: defective to form functional stamens, deficient in microsporogenesis, lacking tryphine in pollens, and deficient in dehiscence of anthers [16]. Two genes are reported to generate stamens with deformed anthers, *ANTHERLESS* [15, 16] and *FIL* [41], mutant flowers of these genes have stamens with no anthers or anthers converted to sepals. A number of male-sterile (MS) genes control microsporogenesis [16, 25]. One of the MS genes, *MS2*, has been shown to have nucleotide sequences homologous to an open reading frame found in the wheat mitochondrial genome, though the relationship between *MS2* and the cytoplasmic male sterility remains unknown [1]. Abortion of pollen development also results from a mutation of adenine phosphoribosyl transferase, a key enzyme in the salvage pathway of purine synthesis [61, 77]. Two genes are known to control formation of outer walls of pollen grains. One gene, *POP1*, is responsible for the synthesis of extracellular pollen coat, lipoidic tryphine [73]. Failing germination of the mutant pollens on the stigma demonstrates that tryphine is required for

the cell signalling in the pollen-stigma interactions. Other genes, *QRT1 & 2*, cause cell wall fusion of four pollen grains originated from the single mother cell [74]. This mutation will permit tetrad analysis to be available in *Arabidopsis*. In addition, *MSH* is involved in the dehiscence of anthers [25]. Mutants defective in ovule development are also studied. Four genes are reported to be involved in the control of integument development [78, 79]. The *ovm3* mutant lacks both outer and inner integuments, whereas the *bell* mutant lacks inner integument. The structure of ovules is abnormal in the *sin1* mutant because of aberrant cell divisions in the integuments. Embryo sac of *ovm2* mutant looks to be replaced by nuclear cells. At the later stages of pistil development, cells of the outer integuments of *bell* mutant sometimes develop to a carpel-like structure [60]. Similar homeotic conversion of ovules to carpels is observed in an *ap2* mutant, *ap2–6* [60].

Summary and future perspectives

Although they are a scattered set of observations, the *Arabidopsis* mutants summarized in this review demonstrate a flow of genetic information leading the process of flower development. As shown in Fig. 1, the flow branches after inflorescence meristem has been formed. One branch aims to maintain the inflorescence meristem, and the other one supports formation and development of floral meristem. The floral meristem develops to a floral bud bearing a set of floral organs. Because the concerted patterns of cell division, cell elongation and cell differentiation are observed in every step of the process, intercellular and intracellular signalling systems are undoubtedly underlying the regulatory network of the genes controlling the process. Although the information is still limited, at least four different types of signalling systems are revealed. First, cascades of transcriptional regulation play major roles in the steps of formation of floral meristem and of formation and development of floral organ primordia, because many putative transcription factors are involved in the steps. Second, plant hormones may function as signals of intercellular communication in flower initiation, and in floral bud formation. Gibberellin has been shown to be involved in the control of flowering time. The auxin transport system is required in the step of formation of floral meristem. Third, as shown in a mutant of a protein kinase gene, *TOUSLED*, protein kinase is involved in the determination of floral organ primordia, as well as in the growth of leaves and roots. Fourth, the regulatory pathway of flower initiation may share some steps with the signalling pathways of photomorphogenesis, because several phytochrome mutants show a delayed-flowering phenotype.

As shown in Table 1, more than sixty genes have been identified to be involved in flower development. However, the molecular function of the genes mostly remain to be clarified. There would be four different approaches for the future study of signalling pathways. The first is cloning of the mutated genes using efficient procedures such as gene tagging, subtraction, or orthodox chromosome walking. Functions of the isolated genes will be examined *in vivo* by analyzing the phenotype of transgenic plants which overexpress or ectopically express the genes. The second is isolation of new types of mutants or new alleles of known genes. A wide spectrum of mutations is required to identify the genetic regulatory networks of signalling pathways. The third is the examination of the phenocopy of the mutants. Treating wild-type plants with drugs of known action or with artificial conditions will help us to postulate the molecular function of mutated genes. The fourth is integration of the accumulated data of genetic, physiological, anatomical, biochemical and molecular studies on flower development. Close exchange of information between researchers of different approaches is required.

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