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A new class of mutations in *Arabidopsis thaliana*, *acaulis1*, affecting the development of both inflorescences and leaves

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SUMMARY

We isolated and analyzed mutants of *Arabidopsis thaliana, acaulis,* with flower stalks that are almost absent or are much reduced in length. The mutations are divided between two loci, *acaulis1* (*acl1*) and *acaulis2* (*acl2*). The *acl1-1* mutation has been assigned to linkage group 4 in the vicinity of locus *ap2*. The *acl1-1* mutant showed premature arrest of the inflorescence meristem after the onset of reproductive development, followed by consequent reduction in the number of flower-bearing phytomers and therefore flowers. The apical meristem of the inflorescences was morphologically normal but its radius was about half that of the wild type. The *acl1* mutants are also defective in the development of foliage leaves. Both defects could be rescued by growth at a specific temperature (28°C). The length of the cells in *acl1-3* mutant was less than that in the wild type but the numbers of cells in leaves and internodes of *acl1* mutants were calculated to be the same as those of the wild type. Thus, the defects in inflorescences and leaves were attributed to defects in the process of elongation (maturation) of these cells. Temperature-shift experiments showed that the Acl1$^+$ product was necessary at all developmental stages. A critical stage was shown to exist for recovery from the cessation of development of inflorescence meristems that was caused by the *acl1-1* mutation. Grafting experiments showed that the *acl1-1* mutation does not affect diffusible substances. An analysis of double mutants carrying both *acl1-1* and one of developmental mutations, *ap1, clv1, lfy,* or *tfl1,* showed that *ACL1* is a new class of gene.

Key words: *acaulis1* mutation, *Arabidopsis thaliana*, flower-stalk, bolting process

INTRODUCTION

A current focus of molecular biology is the elucidation of molecular mechanisms of developmental processes in multicellular eucaryotes. In plants, one of the most characteristic features of development is axial development. There is a gradient in terms of age along the axial structure, as there are active meristematic tissues at the apices of the shoot and root. These apical meristems divide continuously to establish the basic patterns of new organs and tissues. There are also lateral primordia, which start to develop when environmental conditions become favorable for the growth of lateral tissues. The main axial structure of a plant is its shoot system, which is a stack of unit segments, each composed of an internode with a leaf and a bud. The unit segment is called a phytomer (Evans, 1940). The ontogeny and morphology of shoots represent the most fundamental aspects of axial development. Shoot systems are divided developmentally into two stages, vegetative and reproductive. The vegetative shoot system encompasses leaves, stems, and buds. The reproductive shoot system is called the inflorescence and is involved in the formation of leaves, flower stalks and flowers. On the basis of their ontogeny, inflorescences of plants are categorized as determinate and indeterminate (reviewed by Weberling, 1989). The arrangement of components can be expected to be under genetic control and to be susceptible to environmental conditions (Hilu, 1983; Marx, 1983).

We are interested in identifying genetic tools with which to analyze the developmental control of reproductive shoots in *Arabidopsis thaliana*, which has indeterminate inflorescences. During the process of flowering in *A. thaliana*, the development of floral organs and the bolting (elongation of internodes of inflorescences) of flower stalks seem to be tightly coupled. At the vegetative stage, a shoot of *A. thaliana* is in a compressed form (rosette) and its internodes do not elongate. The first few flower buds differentiate at the center of the rosette, and then flower stalks begin to elongate, with the developing flower buds remaining at the tips of the stalks (Smyth et al., 1990). Under normal conditions, wild-type plants do not extend the internodes of their flower stalks before the completion of the development of the first few flower buds. Long flower stalks are always observed when these flower buds start to open. Some species

(e.g., *Antirrhinum majus* L. and *Petunia* × *hybrida* Hort. Vilm.) have elongated internodes during both vegetative and reproductive stages, and other species exhibit the rosette arrangement even at the reproductive stage (for example, most species of the genus *Viola*). The wild-type strain of Belgian endive (*Cichorium intybus* L., Compositae) initiates flowers on flower stalks after bolting. However, transgenic plants that had been transformed with the aid of *Agrobacterium rhizogenes* strain A4 produced erect stems (bolting) but did not produce flowers on the elongated stems (Sun et al., 1991). According to the review of Wareing and Phillips (1981), results of many physiological experiments with long-day plants suggested that bolting and differentiation of flower buds are apparently independent processes. *Hyoscyamus niger* L. begins the elongation of internodes (bolting) before the differentiation of flower buds when the plantlets are supplied with gibberellic acid (GA_3) under short-day conditions. Even when AMO-1618, an inhibitor of GA synthesis, which inhibits the elongation of internodes (bolting) completely, is added to cultures of *Silene armeria* L., the differentiation of flower buds progresses normally (Wareing and Phillips 1981). In certain species of Gramineae, including teosinte (*Euchlaena mexicana*), internode elongation is known to begin independently of panicle formation, when a certain number of internodes have differentiated (Takeda, 1977). Taken together, as Wareing and Philips (1981) discussed earlier, these various observations suggest that the processes of development of flower buds and bolting are independent of one another. The apparent coupling of bolting and floral development in *Arabidopsis* may only mean that these two processes are temporally coincidental events. In order to dissect the processes involved in the development of flower stalks, we attempted to isolate flower-stalkless mutants of *A. thaliana*. This paper describes a new mutation of this type, designated *acaulis*.

MATERIALS AND METHODS

Isolation and characterization of mutant lines

Seeds were sown on rockwool moistened with MGRL medium and plants were grown at 22°C under continuous white light as described elsewhere (Tsukaya et al., 1991). For temperature-shift experiments, growth temperatures were 16°C, 22°C and 28°C, as described in the text.

The screening for mutants was performed using M2 populations derived from ethyl methanesulfonate(EMS)-mutagenized seeds of *Arabidopsis thaliana* ecotype Columbia in Tokyo laboratory. The marker lines used for genetic mapping were GPR1 (*an, gl1, tt4, th1*) derived from ecotype Columbia, and MSU22 (*er, hy2, gl1, tt5*), MSU15 (*er, cer2, bp, ap2*) and W100 (*an, ap1, er, py, hy2, gl1, bp, cer2, ms1, tt3*) derived from ecotype Landsberg. We used the following genetic nomenclature, which is based on the proceedings of the Third International Arabidopsis Meeting (East Lansing, MI, USA, 1987) and has been widely used by molecular geneticists who study *Arabidopsis*: wild-type alleles are given in block capitals and italicized; mutant alleles are given in lower-case, italicized letters; and protein products are given in block capitals, without italics.

Morphological characterization

All measurements were made after growth of mutant lines and wild-type plants (Columbia wild) as described above. The measurements were made on several independent plants.

Organs used for histological analysis were obtained from plants grown under the same conditions. For anatomical studies, all organs were fixed overnight in FAA, which contained 5% (v/v) acetic acid, 45% (v/v) ethanol, and 5% (v/v) formaldehyde and they were then dehydrated in a graded ethanol series at room temperature. Completely dehydrated tissues were then preincubated in a solution of 50% (v/v) Technovit 7100 (Kulzer & Co. GmbH, Wehrheim, FRG) and 5% (v/v) glycerol in ethanol for about 1 hour and then washed in 100% Technovit resin. Next, the samples were dipped in 100% resin and incubated for 6 hours in a vacuum to ensure penetration by the resin. The samples were incubated overnight in air. Hardening of resin and embedding in 100% Technovit 7100 resin were achieved by the method described in the manual from the supplier of the Technovit 7100 resin. Sections of 10 μm thickness were cut with Histoknives (Kulzer & Co. GmbH) on an LR-85 microtome (Yamato-Koki, Asaka, Japan), affixed to glass slides, and stained with 0.1% (w/v) toluidine blue in 0.1 M phosphate buffer (pH 7.0) at 50°C for 30 seconds. Specimens were then photographed under bright-field illumination.

Organs used for SEM were obtained from plants grown under identical conditions to those described above. Samples were fixed in FAA solution overnight and dehydrated in a graded ethanol series at room temperature. Isoamyl acetate was then gradually substituted for the ethanol. Tissue samples were critical-point dried in liquid carbon dioxide. After mounting of individual samples on stubs for SEM, the tissue was sputter-coated with gold and palladium. A Hitachi HCP-2 type SEM was used for examination of specimens, and samples were photographed on Kodak TMAX 100 film.

Grafting experiments

For grafting experiments, a cleft-graft technique was used. The basal part of a scion (i.e., donor) was cut into a wedge with a stainless-steel razor blade. The prepared scion was inserted into the slit of a stock (i.e., acceptor) that had been slit with a razor blade, and cut surfaces were carefully realigned. The stock and scion were then bound with a strip of several-mm-wide 'Band-Aid' tape (Johnson and Johnson, USA). Culture conditions were the same as in the other experiments.

Detachment of flower buds

For simplicity, the first flower bud that differentiated was named the first flower bud, the second one was named the second flower bud, etc. The relationship between the developmental stage of the first or second flower bud (Smyth et al., 1990) and the length of the inflorescence was examined. Decapitation was carried out by cutting off the apical region that included the third flower bud of the inflorescence at various stages. Detachment of the first flower bud from the inflorescence was carried out by cutting off the peduncle. 2 days after the decapitation or detachment of a flower bud, the developmental stage of oldest flower bud and the length of the inflorescence were recorded.

Analysis of double mutant

The following developmental mutant lines were used for construction of double mutants with *acl1-1*: *flo5* (*clv1-2*) (supplied by Dr G. Haughn of the University of Saskatchewan, Saskatoon, Canada), *ap1-1* (supplied by Dr M. Koornneef of the Agricultural University, Wageningen, the Netherlands), *terminal flower* (isolated from the M2 generation as *acaulis* mutants and then used for this study), and *leafy-6* (supplied by Dr D. Weigle of the California Institute of Technology, Pasadena, CA, USA).

erecta (er) and *hy2* mutants were also used. An *er* mutation was obtained after the crossing of *co er* strain (Rédei's) with Columbia wild type and elimination of the *co* mutation. This allele was tentatively named *er-101*. An *hy2* mutation was obtained by S. Naito and named *hy2-314* allele.

RESULTS

Mutant isolation and genetic analysis of mutants

In an attempt to isolate mutations that affect the elongation of the flower stalk we screened 5000 EMS-treated M2 families with a Columbia wild-type background (in Tokyo). The common dwarf mutants were not included in this study and are distinguished by short internodes and various reproductive anomalies (Fig. 1). Many of the dwarf mutants respond favorably to treatments with gibberellic acid (see, for review, Rédei and Koncz, 1992).

Three mutants with stalks of greatly reduced length were isolated (Fig. 2). A similar mutation had been obtained earlier (in Columbia) after X-ray irradiation (Rédei, 1990; Koncz and Rédei, unpublished data). These mutants, unlike the dwarf mutants, have fully fertile flowers, fruits of normal size and completely normal seed sets. This type of mutation was named *acaulis* (*acl* in the three-letter nomenclature), for stalkless (Fig. 1). The wild-type gene is, thus, named *ACAULIS* (*ACL*). These mutants were selfed twice and then backcrossed to a Columbia wild type. All the mutations except for ATYK2033 were found to be recessive. ATYK2033 was found to be semi-dominant. The resulting *acaulis* plants were detected in F_2 generations and were selfed more than four times before further study. Reciprocal crossing of four mutants was performed in order to determine the genetic basis of the Acaulis$^-$ phenotypes. The results defined two complementation groups that generate the Acaulis$^-$ phenotype. The first locus, represented by more than a single mutant allele, was named *acl1* and the alleles were designated *acl1-1* (in strain 294-321 isolated by one of us, GPR), *acl1-2* (in strain ATYK2032), and *acl1-3* (in strain ATYK2034). The other locus was named *acl2* and the allele was designated *acl2-1* (in strain ATYK2033). Fig. 2 shows the Acaulis$^-$ phenotype of each mutant in a comparison with Columbia wild type. For detailed studies we chose to examine the *acl1* locus with three alleles that are associated with different degrees of expression (Fig. 2).

The chromosomal location of the *acl1* locus was determined by use of testers that represented the five linkage groups in *Arabidopsis*. In the 124 progeny of F_2 plants obtained from crossing the *acl1-1* mutant and the MSU15 tester, four *acl1 bp* double homozygotes and no *acl1 ap2* homozygote were obtained. From this result the *acl1* locus was calculated to lie 0±9.0 cM from the *ap2* locus and 45.2 cM from the *bp* locus in linkage group 4 (at map position 4-63; Koornneef and Stam, 1992). From 133 progeny of F_2 plants obtained from the *acl1-1* × *ag* cross, no *acl1 ag* homozygote was obtained. The other tested markers localized on the other chromosomes, such as *er*, *flo5*, *gi-2*, *gl1*, *hy2*, *as*, *an*, *tt4*, and *th1*, segregated independently of the *acl1* locus.

Gross morphology of *acl1* mutants

Fig. 3 shows the morphological characteristics of the *acl1-1* mutant and the wild type. We should note that the control of the elongation of flower stalks by the *acl1* mutation was not absolute and the length of flower stalks varied among plants grown side by side, for unidentified reasons. The variations among mutant plants are shown in Fig. 3B,C, as two groups: the most severely affected plants (indicated by open squares on the curve marked 's') and the least severely affected plants (open squares). Fig. 3A illustrates the changes in length of flower stalks during the life cycle of normal and mutant plants. The mutant (*acl1-1*) had a defect in the elongation of flower stalks that resulted in an approximately 30-fold reduction in length at maturity. The number of flowers also decreased (Fig. 3B). However, the number of leaves (including cauline leaves and rosette leaves) did not change very much (Fig. 3C). Thus, the defect in the elongation of flower stalks due to the *acl1-1* mutation was not caused by a general reduction in plant growth. The length of a flower stalk varied from almost 0 mm to several cm. The stalks were thin and not straight, and they had small and twisted cauline leaves (schematic illustration in Figs 1, 2).

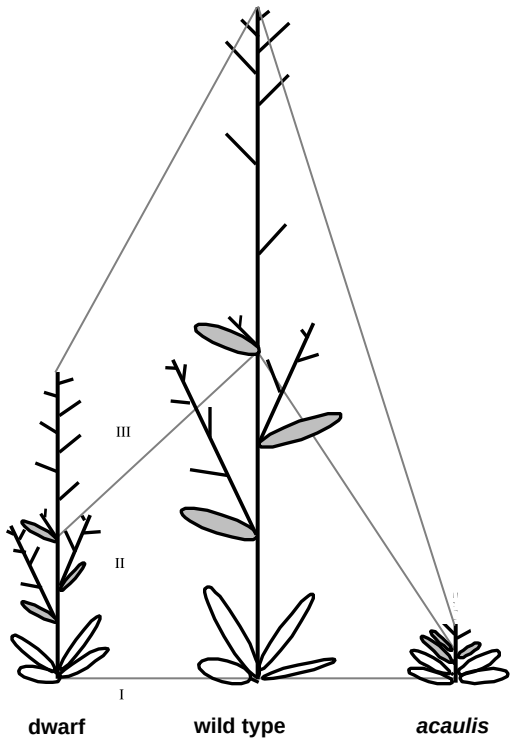

Fig. 1. Differences between the *acaulis* phenotype and a dwarf mutation. Cauline leaves are shaded. The dwarf mutant does not lack any of the constituent phytomers of the plant body. The *acaulis* mutants contain a reduced number of flower bearing phytomers. I, II, and III indicate the regions of type 1, type 2, and type 3 metamers, respectively (Schultz and Haughn, 1991).

Table 1. Comparison of number of nodes of each metamer between Columbia wild and *acl1* mutants

	Type 1 metamer	Type 2 metamer	Type 3 metamer
Wild type	9.6±1.9 (*n*=82)	2.9±0.8 (*n*=93)	20.7± 4.4 (*n*=19)
acl1-1	8.0±1.2 (*n*=25)	2.6±0.6 (*n*=25)	2.4±0.6 (*n*=28)
acl1-3	9.0±1.5 (*n*=16)	2.6±0.5 (*n*=16)	7.1±1.6 (*n*=16)

n, number of plants examined.
±, standard error.

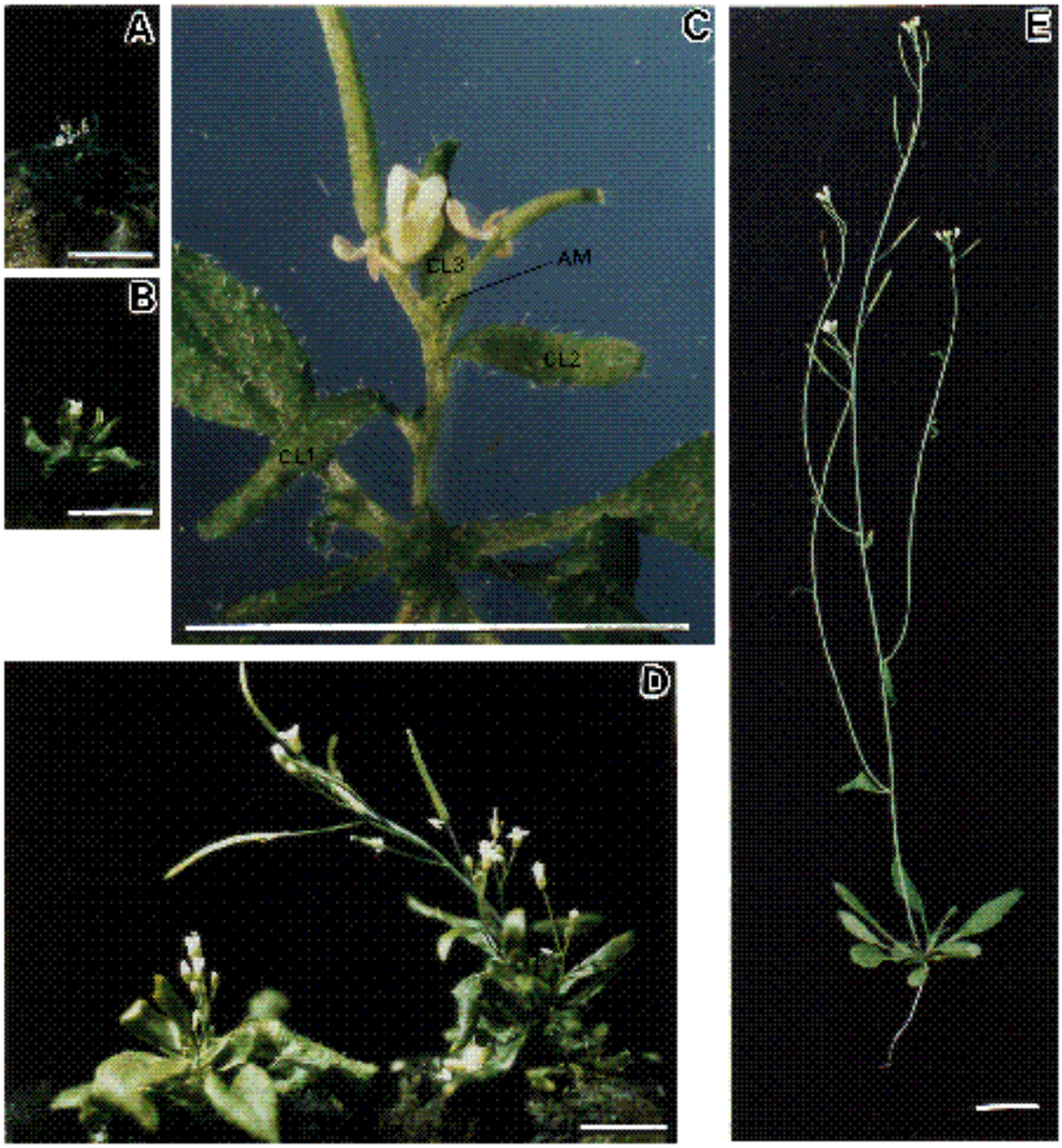

Fig. 2. Comparison of *acaulis* mutants with the wild type. All the *acl* mutants and wild-type plants were cultivated for 25 days at 22ºC under continuous light. Bars, 1 cm. (A) Mutant strain 294-321. (B) Mutant strain ATYK2032. (C) Magnified view of mutant strain 294-321. Some rosette leaves were detached in order to reveal the main stem. CL1, CL2, and CL3 indicate cauline leaves 1, 2, and 3. AM, apical meristem. (D) Mutant strain ATYK2033 (left) and mutant strain ATYK2034 (right). (E) Columbia wild-type plant.

We employed metamer terminology (Schultz and Haughn, 1991) in order to describe our *acl1* mutants. As shown in Table 1 and Fig. 2, wild-type plants had about three phytomers in the type 2 metamer, which was characterized by the presence of a cauline leaf and an elongated internode. *acl1-1* and *acl1-3* plants also had about 3 phytomers on the type 2 metamer. In type 2 metamers, the length of each internode of *acl1-3* mutants was 22%, 18%, and 14% (from bottom to top) of that of wild-type plants (mean values from 16 *acl1-3* plants and 20 wild-type plants). Thus, the number of components of type 2 metamer in *acl1* mutants is normal and only elongation is defective.

In type 3 metamers, a lack of components was found. As shown in Table 1, the number of phytomers of type 3 metamers, was 2.4 in the *acl1-1* mutant, 7.1 in the *acl1-3* mutant, and 20.7 in the wild type. A defect in the elongation of internodes was also recognized in type 3 metamers (the length of the botom internode in *aca1-3* mutants was 15% that of the wild type). We examined secondary lateral shoots from both *acl1* and wild-type inflorescences to determine

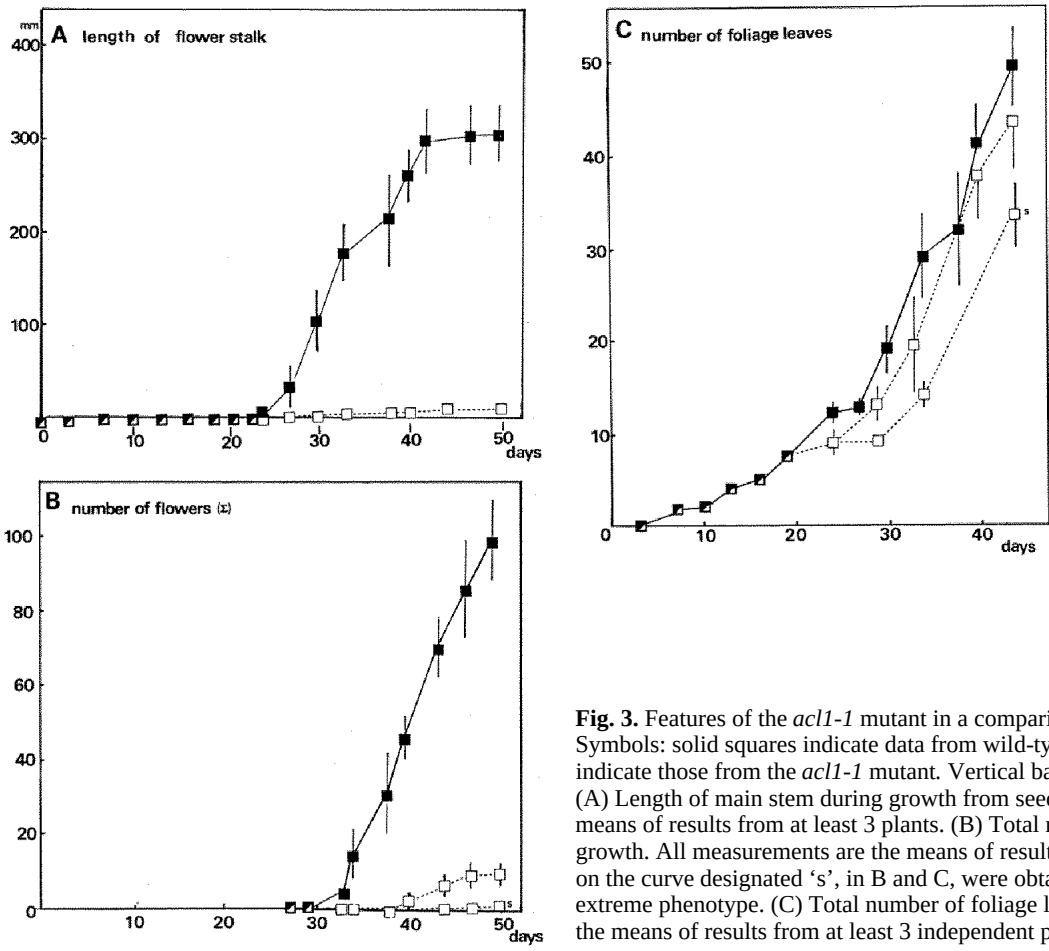

Fig. 3. Features of the *acl1-1* mutant in a comparison with the wild type. Symbols: solid squares indicate data from wild-type plants and open squares indicate those from the *acl1-1* mutant. Vertical bars indicate standard errors. (A) Length of main stem during growth from seeds. All measurements are the means of results from at least 3 plants. (B) Total number of flowers during growth. All measurements are the means of results from at least 3 plants. Data on the curve designated 's', in B and C, were obtained from plants with a more extreme phenotype. (C) Total number of foliage leaves. All measurements are the means of results from at least 3 independent plants.

Table 2. Measurements of leaves of Columbia wild type and *acl1-1***

		Columbia wild	*acl1-1*
Leaf blade (mm)	Length	14.5±1.8	3.8±0.4
	Width	6.9±0.6	2.0±0.0
	Thickness	0.14	0.14
Parenchymatous cell (μm)†	Width	64.4±15.0	15.2±2.0
	Thickness	44.1±7.8	17.4±3.6
Number of cell layers		2.0±0.8	6.6±0.6
Intercellular space (%)		19.9	2.8
Stomatal index‡	Upper side	0.26±0.02	0.27
	Lower side	0.27±0.02	0.24
Area of epidermal cell (mm^2)	Upper side	0.25	0.08
	Lower side	0.19	0.07

*Cells in 7th and 8th foliage leaves of 26-day-old plantlets at 22°C.
†Cells in the layer just beneath epidermal cells.
‡Number of stomata/total number of epidermal cells.
±, standard error.

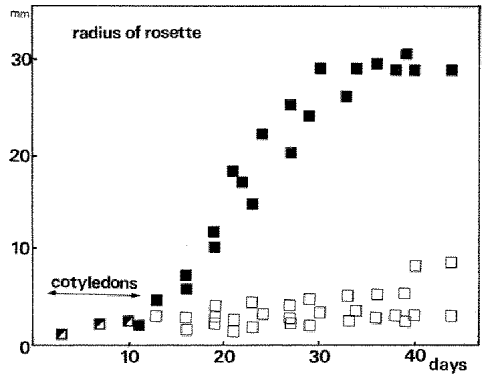

Fig. 4. The increase in the length of the leaves of a rosette. Data are plotted for individual plants. Plants up to 10 days old (the period indicated by an arrow) had only cotyledons, and the values given for that period represent the lengths of cotyledons.

whether the development of *acl1* secondary lateral shoots was similar to that of shoots on main stems. Secondary lateral shoots of *acl1* mutants had the same gross morphology as those on main stems.

We noted an additional defect in the morphology of leaves of *acl1* mutants (Fig. 2). Rosette leaves of mature *acl1* plants were small, irregularly buckled and twisted and they tended to curl downwards. In all mutant lines, this altered leaf morphology cosegregated with the above-mentioned Acaulis⁻ phenotype. Initially, seedlings with the first pair of foliage leaves were similar to wild-type seedlings. The altered leaf morphology usually appeared after the development of third foliage leaves or sometimes after that of fourth foliage leaves, depending on growth conditions. This observation agreed with that of Medford et al. (1992). However, we also observed that first foliage leaves

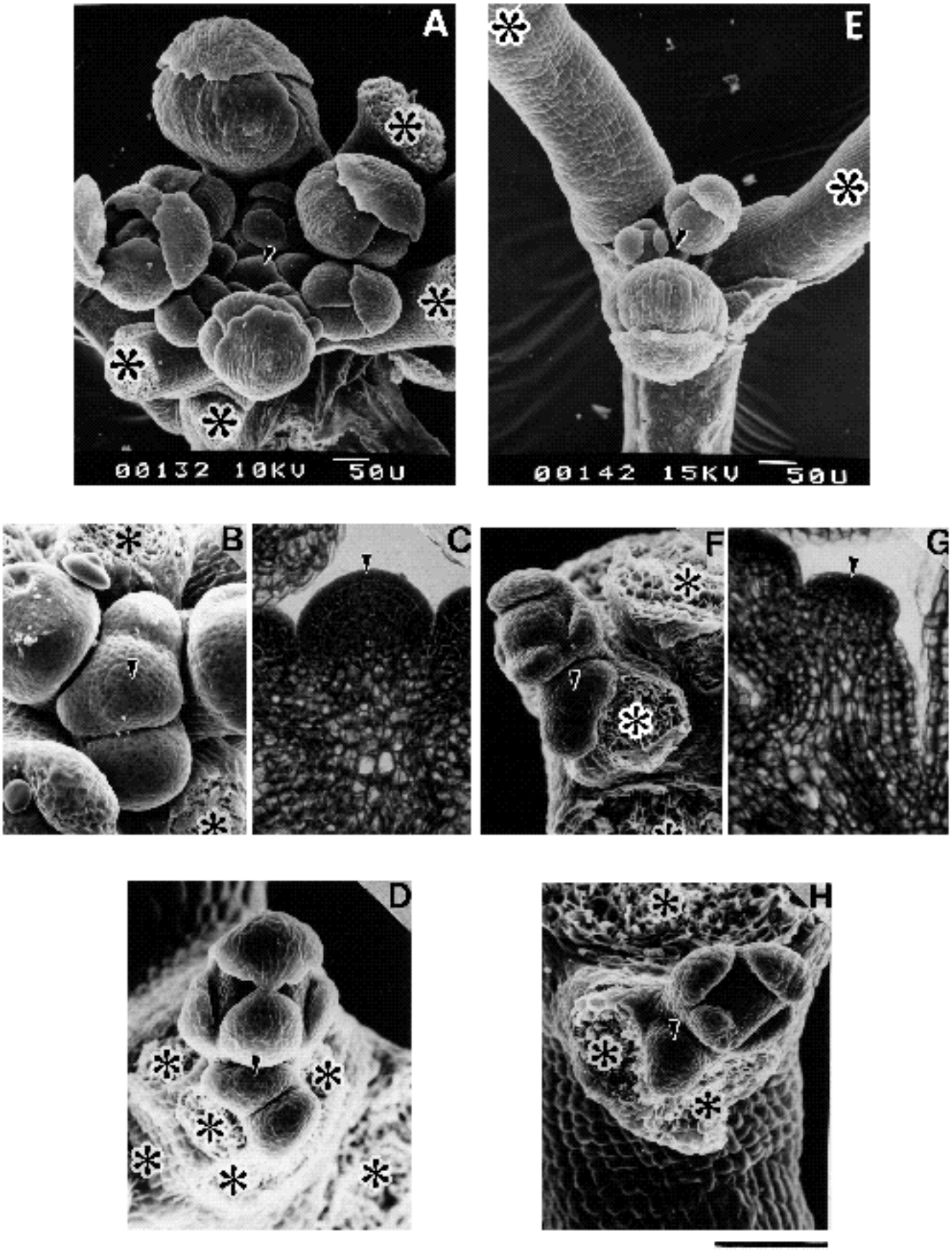

Fig. 5

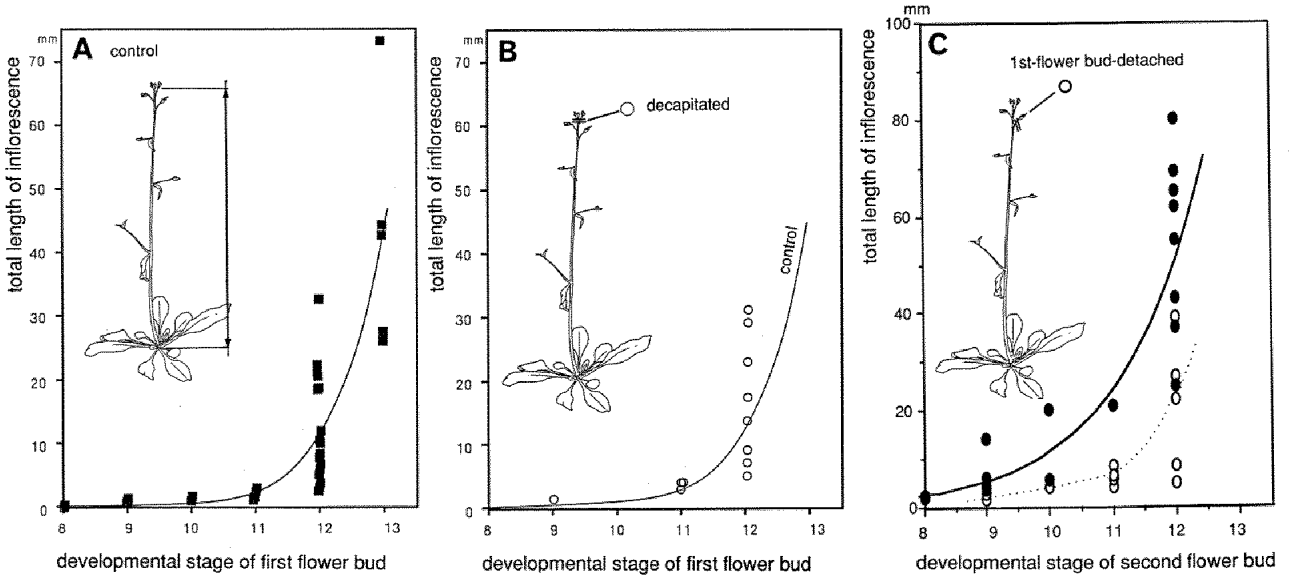

Fig. 6. Relationship between the developmental stage of the flower bud and the length of the inflorescence. Each point indicates the result from one plant. (A) Relationship between the developmental stage of the first flower bud and the length of the inflorescence of a wild-type plant under normal conditions. (B) Relationship between the developmental stage of the first flower bud and the length of the inflorescence of a wild-type plant, 2 days after detachment of the apical region of the inflorescence. Curve labeled 'control' shows data obtained under normal conditions as in A. (C) Relationship between the developmental stage of the second flower bud and the length of the inflorescence of a wild-type plant, 2 days after detachment of the first flower bud (open circles) and in normal plants (closed circles).

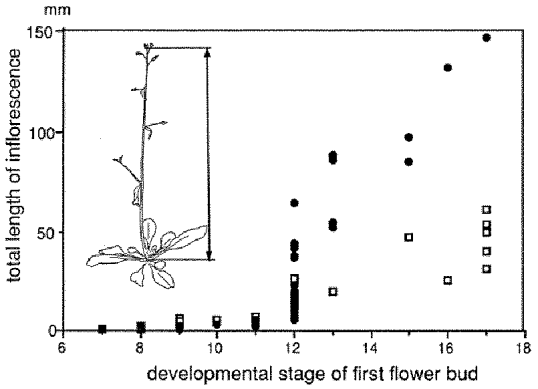

Fig. 7. Elongation pattern of the total inflorescence. Closed circles indicate the lengths of type 2 metamers of wild-type plants. Open squares indicate those of the *acl1-3* mutant. Each point indicates the result from a single plant.

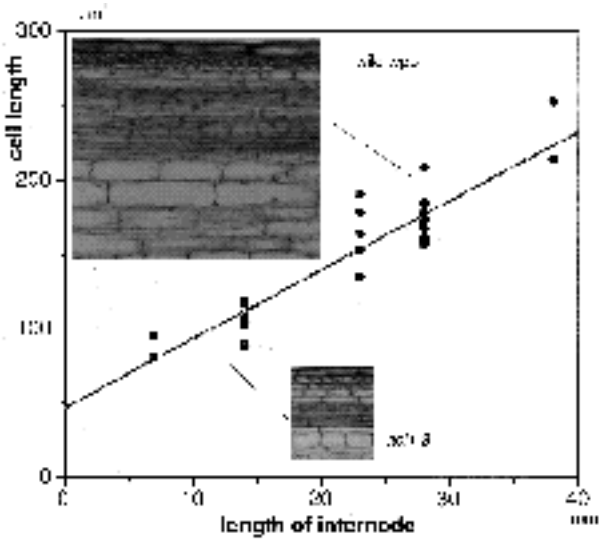

Fig. 8. Comparison of the lengths of entire internodes and lengths of internodal cells. The mean value of the length of 30 cells on one section (A) was plotted against the length of the internode (B). Closed circles indicate the data from the wild type. Closed squares indicate data from the *acl1-3* mutant. Each point indicates the result from a single section.

Fig. 5. SEM images and sections of apices of the inflorescences and apical meristems of *acl1-1* and wild-type plants. An arrowhead indicates the apical meristem; an asterisk indicates a cut-off peduncle that carried a flower. Bar, 50 μm. (A) Apical region of an inflorescence from a wild-type plant, 29 days after sowing. (B) Magnified view of an apical region from a wild-type plant, 29 days after sowing. (C) Magnified view of section of an apical region from a wild-type plant, 29 days after sowing. (D) Magnified view of an apical region from a wild-type plant, 42 days after sowing. (E) Apical region of an inflorescence from an *acl1-1* plant, 29 days after sowing. (F) Magnified view of an apical region from an *acl1-1* plant, 29 days after sowing. (G) Magnified view of section of an apical region from an *acl1-1* plant, 29 days after sowing. (H) Magnified view of an apical region from an *acl1-1* plant, 42 days after sowing.

were irregular in shape in the severest cases. The seventh and eighth (i.e., mature) leaves were about 3.5 times shorter than wild-type leaves and the same was true of their width (Table 2). Fig. 4 shows measurements of the radii of rosettes. The mutant rosettes had a smaller radius after development of foliage leaves, and radii of rosettes were 3- to 10-fold smaller than those of the wild type at maturity, reflecting the differences in the dimensions of leaves. The

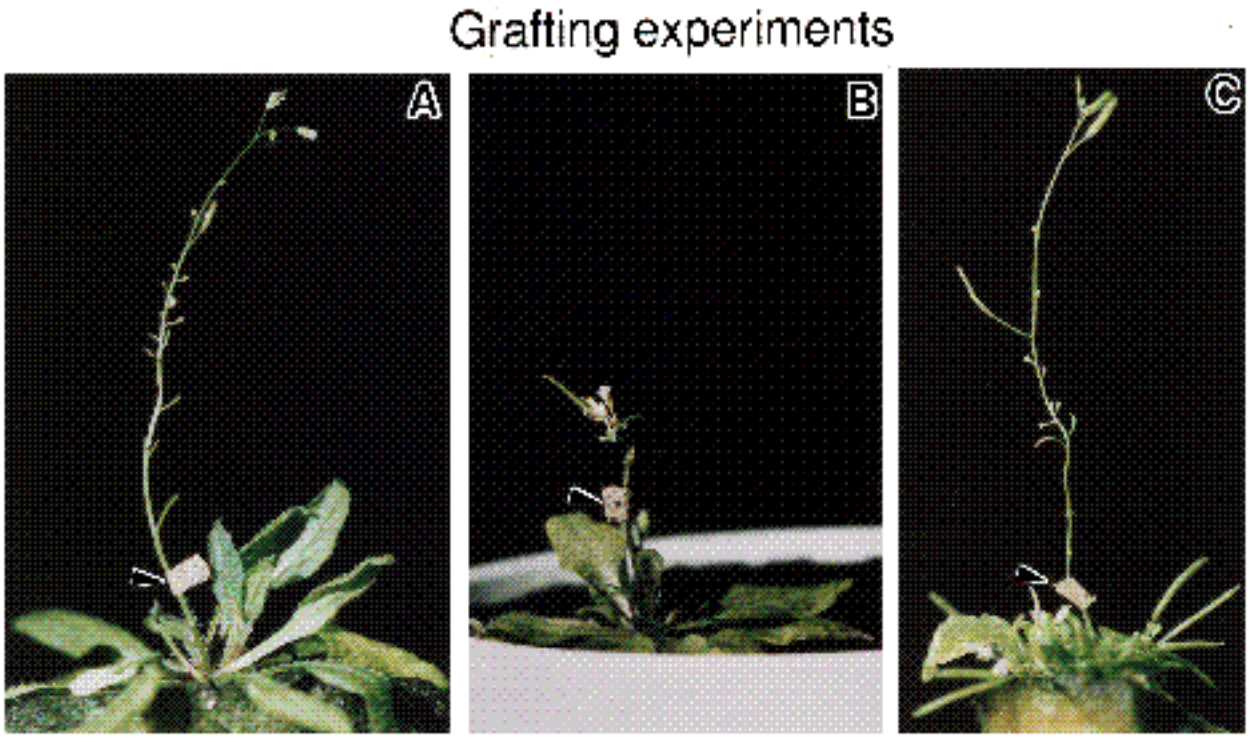

Fig. 9. Grafting experiments. 30-day-old plants were used for both scion and stock. See Materials and methods for details. Photographs were taken two weeks after grafting. Arrowheads indicate grafted joints. (A) A rosette-distal portion of a wild-type inflorescence grafted onto a rosette-proximal portion of a wild-type inflorescence. (B) A rosette-distal portion of an inflorescence from an *acl1-1* mutant grafted onto a rosette-proximal portion from a Columbia wild type. All secondary inflorescences of the wild-type plant were removed. (C) A rosette-distal portion of a wild-type inflorescence grafted onto a rosette-proximal portion of an *acl1-1* mutant inflorescence.

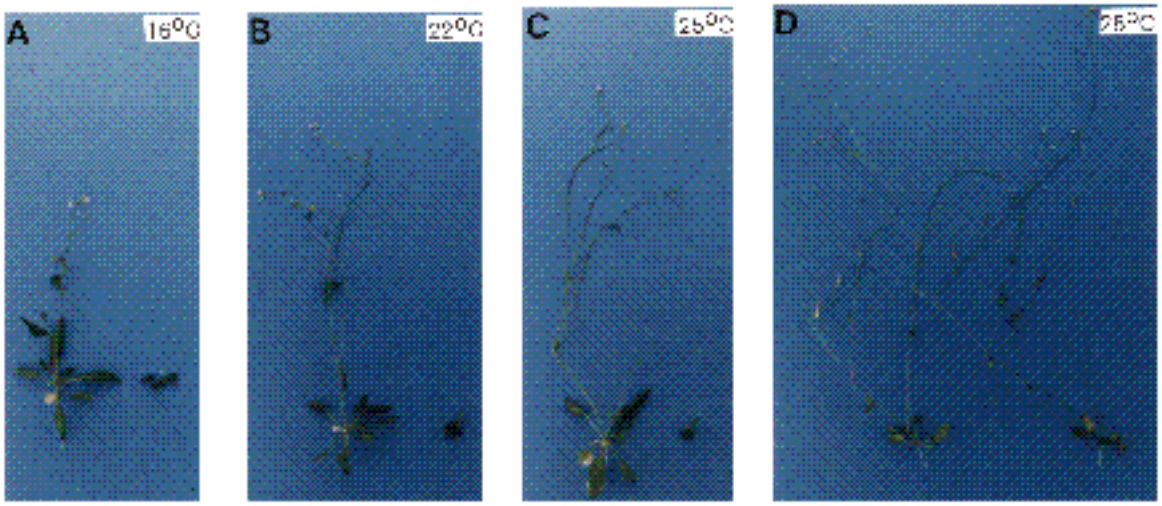

Fig. 10. Cold-sensitive phenotype of the *acl1* mutation. All plants shown were grown at 22°C for 1 week after sowing, and they were then transferred to and grown at the temperature indicated for an additional 3 weeks. (A-D) Columbia wild-type plants (left) and *acl1-1* mutants (right).

defect in leaves was further studied by light-microscopic examinations of sectioned leaves. The measurements of leaf cells obtained in this way are also summarized in Table 2. Comparing the sizes of parenchymatous cells and epidermal cells between leaves, we found that all the cells in a given leaf were smaller in the presence of the *acl1-1* mutation. The intercellular spaces were also smaller in the mutants. The number of cell layers was increased about 3-fold to compensate for the decrease in cell volume caused by the mutation (Table 2). The stomatal indices of wild-type plants and *acl1-1* were the same.

Ultrastructure of apices of inflorescences

In order to determine how the *acl1-1* mutation interrupts the

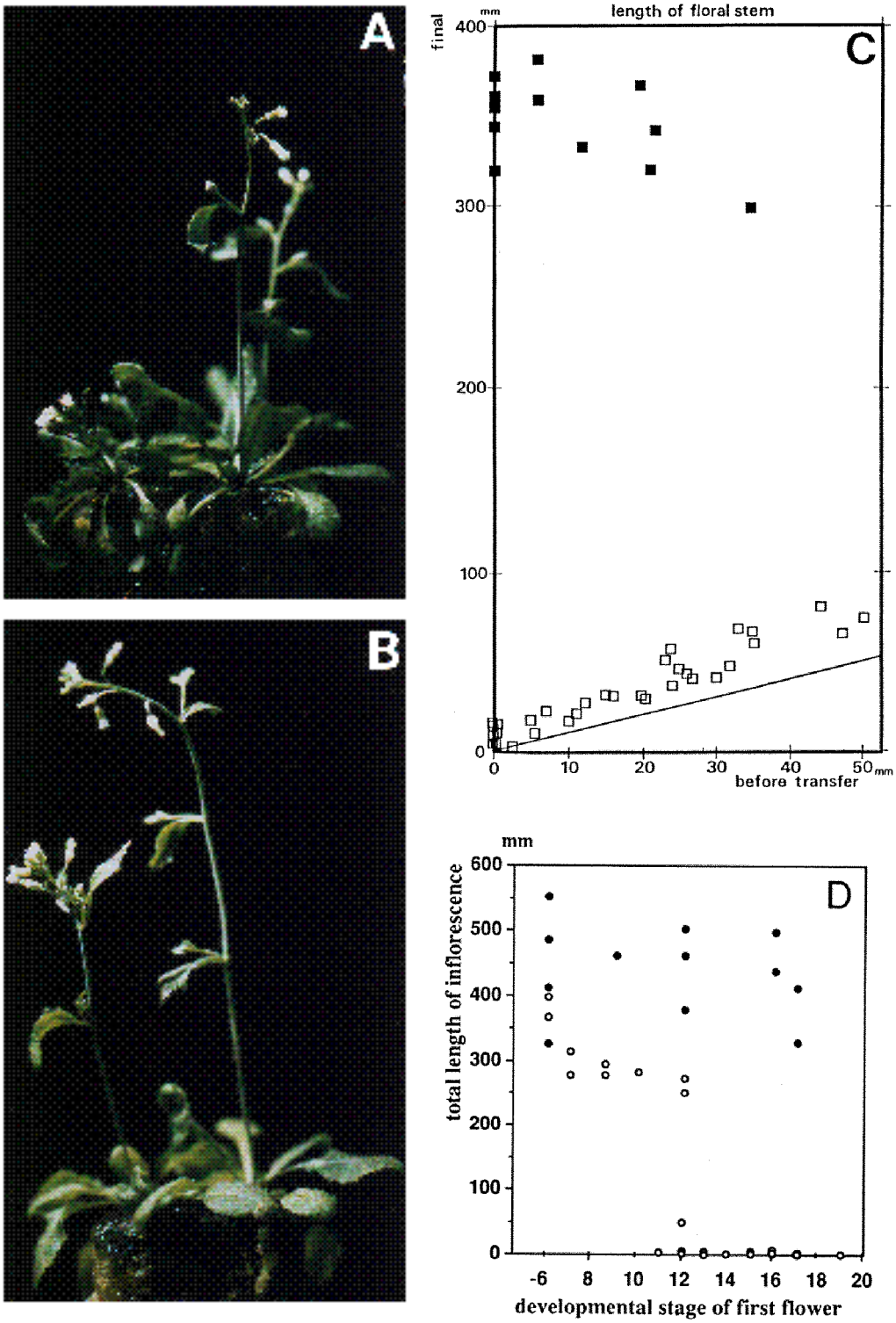

Fig. 11. Temperature-shift experiment. (A) Plants were cultivated at 28°C for 22 days after sowing and then transferred to 16°C. The photograph was taken 9 days after the transfer to 16°C. Columbia wild-type (right) and *acl1-1* mutant plants (left) are shown. (B) Plants were cultivated at 28°C for 26 days (when inflorescences began to elongate) and then transferred to 16°C. The photograph was taken 5 days after the transfer to 16°C. Columbia wild-type (right) and *acl1-1* mutant plants (left) are shown. (C) Elongation of main stems after a shift from 28°C to 16°C. The lengths of main stems of the plants before and after the transfer were plotted. Lengths of main stems after transfer were measured after cessation of the growth of the main stem (about 20 days after transfer in the case of wild-type plants). Each point indicates a result from a single plant. Filled squares, wild-type; open squares, *acl1-1* mutant. (D) Elongation of main stems after a shift from 22°C to 28°C. The lengths of main stems of the plants before and after the transfer were plotted. Lengths of main stems after transfer were measured after cessation of the growth of the main stem (about 20 days after transfer in the case of wild-type plants). Each point indicates the result from a single plant.

development of inflorescences, the apical regions of inflorescences were observed by scanning electron microscopy (SEM). In the case of wild-type plants, the apex of 42-day-old plants was similar to that of 29-day-old plants in terms of the retention of apical domes (Fig. 5A,B,D). The apical meristem continued to differentiate flower buds for more than two weeks and flower buds at various stages of development were apparent in inflorescence apices as a result. In contrast, the apex of inflorescences of *acl1-1* plants differentiated only a few flower buds (Fig. 5E), and then ceased further development with more aborted flower buds (Fig. 5F,H). This pattern of development resulted in the Acaulis$^-$ phenotype. The cessation of production of flower buds in the *acl1-1* mutant was not caused by the disappearance of the inflorescence meristem itself, because the apical meristem appeared to be morphologically normal and dome-shaped (Fig. 5F-H). The terminal morphology of the 29-day-old inflorescence of *acl1-1* plants was similar to that of old (42-day-old) wild-type plants with respect to the presence of some aborted flower buds adjacent to the apical dome (Fig. 5D,F). The diameter of 29-day-old wild-type apical meristems was 45.6±6.5 μm (mean value ± s.e. for 5 independent apices). The diameter of 29-day-old apical meristem of *acl1-1* mutant plants was 22.9±2.7 μm and that of old (with aborted flower buds, in the final stage of development) wild-type apical meristems was 24.6±2.1 μm. The small size of the apical dome was confirmed by observations of sections of shoot apices (Fig. 5C,G). The difference in size of the apical region seemed to occur only after floral induction and no abnormality was recognized in the vegetative stage from both SEM observations and sectional views (data not shown). The size of cells in apical domes was observed to be the same in the wild type and *acl1-1* mutants (Fig. 5C,G).

Lateral shoots in the *acl1-1* mutant had the same characteristics as shoots on main stems in terms of ultrastructural morphology as seen by SEM. The observations by SEM did not reveal any significant differences between the *acl1-1* mutant and its parent in other organs, namely, flowers and seeds (data not shown).

Elongation of flower stalks in wild-type and *acl1* plants

To characterize the defects associated with the *acl1* mutations, we first examined wild-type plants of ecotype Columbia for the elongation of flower stalks. Consistent with previous descriptions by Smyth et al. (1990) of ecotype Landsberg *erecta*, the main inflorescence began to elongate rapidly around 18 days after sowing at the developmental stage 9 (after the definition of Smyth et al., 1990), as the first flower bud differentiated (Fig. 6A). This correlation was unchanged in the *acl1-3* mutant (Fig. 7). In order to determine whether a flower bud or an apical meristem is responsible for the elongation of a flower stalk, we performed decapitation and flower bud-detachment experiments. When apical regions above the third flower bud were detached from plants at the bolting stage, the elongation pattern of the inflorescence was not affected (Fig. 6B). However, when young first flower buds were detached from inflorescences, elongation of such inflorescences was delayed (Fig. 6C). Thus, the bolting process in wild-type plants is apparently regulated not by the apical meristem but by the developmental stage of the first flower bud itself (Fig. 6B,C).

Anatomical study of internodes

To clarify whether the defect in elongation of internodes is caused by a decrease in cell number or inadequate cell size, an anatomical study was performed of sections of second internodes of type 2 metamers from the wild type and a leaky mutant, *acl1-3*. As shown in Fig. 8, the length of the internode was proportional to the length of the internodal cell in both the wild type and the *acl1-3* mutant (Fig. 8). This result suggests that cell numbers were unchanged by the *acl1* mutation.

Grafting experiments

In order to determine the effects of leaves on the development of inflorescences in mutants, grafting experiments were carried out. Distal parts of inflorescences of *acl1-1* plants were grafted onto proximal parts of inflorescences of wild-type plants as stocks (i.e., acceptors). As a control experiment, grafting between wild-type stock and wild-type scions (i.e., donors) was performed. In 4 out of 7 grafting experiments, successful connection (knitting) and normal growth were observed (Fig. 9A). Successful connection between the wild-type stock and the *acl1-1* scion was achieved in 3 of 9 graftings. As shown in Fig. 9B, the grafted *acl1-1* inflorescences were not affected by growth on the wild-type plants. And, as shown in Fig. 9C, the grafted wild-type inflorescences were not affected by growth on the *acl1-1* plants in 3 successfully connected plants of 5 grafting experiments.

Cold sensitivity of the phenotype caused by *acl1* mutations

To find clues that might facilitate the analysis of the mutation, we examined the effects of various changes in temperature, illumination, and nutrients on the severity of the phenotype. Of the conditions examined, only temperature reproducibly affected the severity of the phenotype. We found that growth at 28°C resulted in the absence of the defective phenotype, and leaves and inflorescences of plants with any of the three *acl1* mutations were normal (Fig. 10D). When plants were germinated and cultured at 22°C for 7-10 days and then transferred to 25°C, 28°C and 16°C, wild-type plants showed evidence only of retardation of growth after incubation at lower temperatures (Fig. 10A). By contrast, the *acl1-1* plants grown at 28°C showed discrete differences in morphology to those grown at 16°C (Fig. 10A), 22°C (Fig. 10B) or 25°C (Fig. 10C). The same results were obtained with other *acl1* mutations (*acl1-2* and *acl1-3*).

In order to examine the effects of temperature in greater detail, we performed a set of temperature-shift experiments. When *acl1-1* mutant plants were cultured at a permissive temperature, 28°C, for about 3 weeks after sowing, the plants produced normal (wild-type) leaves in the form of a rosette. These plants were then transferred to a restrictive temperature, 16°C. They developed very short flower stalks on the top of the normal rosette after the transfer (Fig. 11A). At the permissive temperature (28°C), bolting began 22 days after sowing. When *acl1-1* mutant plants with normal inflo-

rescences, which had been cultivated at 28°C for 26 days, were transferred to 16°C, further elongation of the internodes stopped (Fig. 11B). In wild-type plants, the elongation of the internodes did not cease and the final lengths of inflorescences were not affected by a shift from 28°C to 16°C (Fig. 11). Whenever bolting *acl1-1* mutant plants grown at 28°C were transferred to 16°C, elongation continued for about another 10 mm and then ceased (Fig. 11C). When mutant plants grown at 28°C were transferred to 16°C before bolting, the resultant inflorescences varied in length from almost 0 mm to about 10 mm (Fig. 11C).

Shift experiments in which plants were transferred from a restrictive temperature (22°C) to a permissive one (28°C), showed a dramatic effect on flower stalk development (Fig. 11). When we shifted mutant plants with their first flower buds at stages less than stage 12, from 22°C to 28°C, the flower stalks started to elongate and normal sized inflorescences were formed. However, the flower stalk failed to elongate in the case of plants with first flower buds that were beyond stage 12 (Fig. 11D).

Analysis of double mutants

In order to determine the role of the *ACL1* gene within the developmental network, some developmental mutations that affect the morphology of inflorescences and/or flowers were introduced into the *acl1-1* mutant and double mutants were generated.

For the double mutant analysis we employed the following mutations: *lfy* and *tfl1*, which affect the initiation of floral primordia and the morphology of flower stalks; *ap1*, which affects the formation of flowers; and *clv1* (*flo5*) which affects the development of shoot apices. Fig 12 shows some of the phenotypes of double mutants. The *acl1-1 tfl* double mutant made only a few flowers, as did *acl1*, but it differentiated a terminal flower (Fig. 12E). *acl1-1 lfy-6* double mutants made only a few flower-like structures that were composed of carpelloid organs (Fig. 13), which is a characteristic of the *lfy* mutation (Schultz and Haughn, 1991). The increase in the number of cauline leaves, one of the phenotypes known to be caused by the *lfy* mutation, was also observed in the *acl1-1 lfy* double mutant (Fig. 12F). *acl1-1 ap1-1* (Fig. 12G) and *acl1-1 flo5* (*clv1-2*) (Fig. 12H) double mutants differentiated flowers with the phenotypes expected of *flo5* or *ap1* mutants on the short flower stalks caused by the *acl1-1* mutation. Therefore, *tfl*, *lfy*, *flo5* (*clv1*) and *ap1* mutations are additive with respect to the *acl1-1* mutation.

In order to gain insight into the control of flower stalk growth, we also examined the effects of the mutations *erecta* (*er*, Rédei, 1962) and *hy2*, which appear to influence the length of internodes. The internodes of *acl1-1 er-101* or *acl1-1 hy2-314* double mutants were longer than those of *acl1-1*. Table 3 summarizes the measurement of the number of flowers on the main axis. The double mutants had twice the number of flowers as the *acl1-1* single mutant. The morphology of leaves did not change after the introduction of the *er-101* or *hy2-314* mutation.

DISCUSSION

In this report we have described a set of *acl1* mutants with flower stalks that are almost absent or are much reduced in length and with very small numbers of flowers. The *acl1* mutations are associated with single recessive traits and the *acl1* locus was mapped to linkage group 4. In the wild-type main inflorescence (i.e., flower stalk with flowers), an apex continues to grow and produce flower buds indefinitely, in principle. The apex of the inflorescence of the *acl1-1* mutant cannot grow beyond a certain point. Apparently, cessation of the differentiation of each floral meristem occurs and then the flower buds near the apical region cease development or *vice versa*. Analysis of apical meristems using cross sections and SEM revealed no morphological abnormalities in apical regions of the *acl1-1* mutant. Although a decrease in diameter of the apical dome was observed at an earlier stage in the *acl1-1* mutant than in wild-type plants, this decrease should be a reflection of cessation of the development of the apical dome at an earlier stage. We conclude that the *acl1-1* mutant ceases to elongate its flower stalk, not because of loss of the inflorescence meristem but, apparently, because of the arrest of further development of meristems, for the following two reasons. The *acl1-1 tfl1* double mutant still retains the capacity to differentiate terminal flowers and the *acl1* mutants can recover a normal phenotype by the temperature shift after a certain period of time.

Foliage leaves of the *acl1* mutants are very small and twisted. Both the small size of leaf cells and the smaller intercellular spaces imply that the process of elongation of the leaf cells ceases soon after their differentiation. As in other plant species, leaf cells derived from one leaf primordium divide repeatedly and differentiate according to each cell's fate (Pyke et al., 1991). After their differentiation, leaf cells of *A. thaliana* expand to the adult size by increasing the volume of each vacuole and the surface area of the cell wall. The change in leaf morphology in the mutant is caused by a defect in the last process.

acl1 mutants have a defect in elongation of inflorescences

Development of inflorescences in *Arabidopsis thaliana* involves two phases. First, the primary inflorescence meristem produces cauline leaves associated with secondary lateral inflorescences. Then, the inflorescence meristem switches to the formation of flowers. This situation is well documented by reference to the metamer concept of Schultz and Haughn (1991). Wild-type plants of *A. thaliana* produce three types of metamer: type 1, rosette; type 2, coflorescence-bearing with cauline leaves; and type 3, flower-bearing without bract (Fig. 1).

Using the metamer concept, we can describe the *acl1* mutation with relative ease. Compared with wild-type plants, the mutants are defective in the elongation of type 2 and type 3 metamers but not in the start of elongation. The *acl1* mutants have all of the three types of metamers, but they have very short type 2 metamers and precociously terminate type 3-like metamers after formation of a few flowers. This explanation of the defect is also applicable to the lateral shoots, i.e., the coflorescences (or branches).

The *acl1* mutants that we have isolated to date ceased both the elongation of main stems and further development of floral meristems. In order to analyze this linkage between the length of internodes and the number of flowers, we intro-

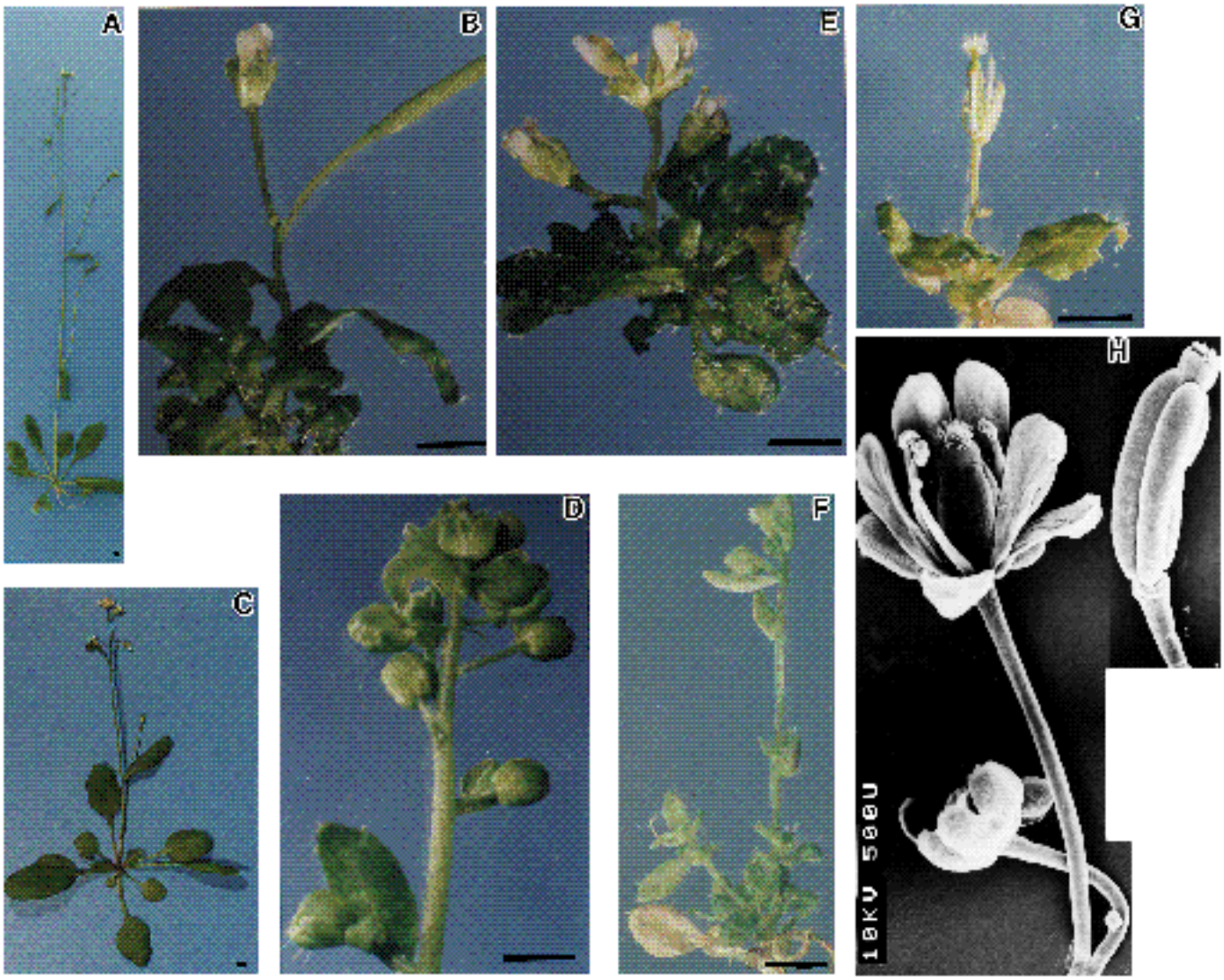

Fig. 12. (A-G) Analysis of double mutant grown at 22°C. Bar, 2 mm. (A) Wild type. (B) *acl1-1* mutant. (C) *terminal flower* mutant. (D) *leafy6* mutant. (E) *acl1-1 terminal flower* double mutant. (F) *acl1-1 leafy* double mutant. (G) *acl1-1 ap1* double mutant. (H) SEM view of inflorescence of *acl1-1 flo5* double mutant. Bar, 50 μm.

duced *hy2* and *er* mutations into *acl1-1* mutants. The *hy2* mutant has long internodes (Koonneef et al., 1980) and the *er* mutant was believed to be short (Rédei, 1962). We found that *er-101* mutants are generally short but the first internode in the type 2-metamer elongates (our unpublished observations). When we could increase the length of the first internodes of type-2 metamer by a *hy2-314* or *er-101* mutation, the double mutant produced more flowers.

Thus, there is tight linkage between the length of flower stalks and floral development, although bolting is regulated independently from the development of the first few flowers (Smyth et al., 1990). In order to determine what organs are responsible for the synchronization of the two independent processes, we performed decapitation and flower bud-detachment experiments. Decapitation did not influence the tight linkage between the developmental stage of the first flower bud and bolting, but detachment of the first flower bud delayed bolting. Thus, the start of the bolting process is dependent on the start of a certain program(s) that is involved in the development of some floral organ(s) in the first flower bud and not on the shoot apex. Considering that *lfy* and *pin-formed* (Goto et al., 1991) mutants without normal flowers can exhibit bolting, this effect of the first flower bud would be secondary.

ACL1 belongs to a new category of inflorescence gene

Some genes have been identified within the genetic network that control the development of inflorescences and flowers. *LFY* and *TFL1* are responsible for the initiation of development of floral primordia (Schultz et al., 1991, Shannon et al., 1991). Floral homeotic genes, such as *AG*, *AP1*, *AP3*, and *PI* (Bowman et al., 1991), are categorized as genes responsible for the formation of flowers after the onset of switches by *LFY* and *TFL1*. The *CLV1* gene appears to control the development of shoot apices (Lyser and Furner, 1992). From the analyses of double mutants, the function of the *ACL1* gene within the network of inflorescence-devel-

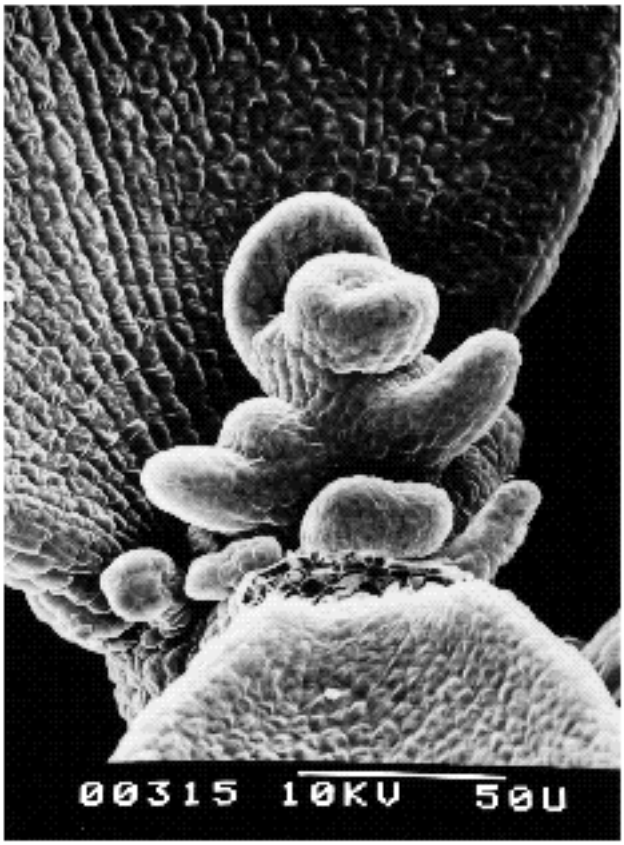

Fig. 13. SEM view of the terminal flower produced on the tip of the *acl1-1 lfy-6* double mutant grown at 22°C.

Table 3. Effect of *acl1-1*, *er*, and *hy2* mutations on the number of flowers on main stem

	Strain	Number of flowers*	Relative number of flowers (*acl1-1*=1)	No. of plants examined
Experiment 1	Wild type	21.8±4.9	9.1	36
	acl1-1	2.4±0.6	1	28
Experiment 2	*acl1-1*	2.4±0.6	1	15
	acl1-1/er	5.6±2.1	2.3	18
	er	23.8±4.1	9.9	8
Experiment 3	*acl1-1*	1.8±0.6	1	14
	acl1-1/er	4.1±0.8	2.3	8
	acl1-1/hy2	3.2±1.3	1.8	29

*Standard error.

opment is different from that of other known genes such as *AP1*, *CLV1*, *LFY* and *TFL*. The effect of another homeotic mutation *agamous* is very interesting because *agamous* mutants retain the capacity to develop further flowers at floral apices (Bowman et al., 1989). However, no *ag acl1-1* double mutant appeared among about 300 F_2 progeny examined because the two mutations are closely linked to one another.

Possible function of the *ACL1* gene

The mutational phenomena in leaves and internodes of *acl1-1* plants can be explained by the arrest of cell expansion. Such a change is categorized as neoteny (lack of completion of development), a kind of heterochronia (Guerrant, 1982), wherein the mature organ has juvenile features. Thus, the *ACL1* gene must have an important role in cell maturation (elongation) in both leaf cells and internodal cells. Another important role is continuous production of flower-bearing phytomers in flower stalks.

The temperature-shift experiments revealed two aspects of the function of the *ACL1* gene. The $Acl1^+$ product is required constantly. If the $Acl1^+$ product is missing or not functioning at a critical point or for a certain period of time, the *acl1* mutants cannot recover normal development.

Since *acl1* mutants expressed pleiotropic defects, the *acl1* genetic defect may affect general and indirect function. The defects may be the consequence of some phytohormone imbalance. However, the addition of several growth regulators, including phytohormones and their analogs, failed to have any clear effect on the phenotype under our experimental conditions. The grafting experiments showed that the *acl1* mutation does not affect a diffusible substance. An auxin-insensitive mutant, *axr1*, has a defect in internode elongation. This is caused by a decrease in cell number in the internodes but not by a decrease in cell length (Lincoln et al., 1990). This phenotype is different from the dwarfism of *acl1* mutants which is caused by a decrease in cell length. Another auxin-insensitive mutant, *axr2*, has a defect in inflorescences that is caused by a decrease in cell length but not by a decrease in cell number (Timpte et al., 1992). This is the same as the *acl1* mutants in this study, but *axr2* mutants differ from *acl1* mutants, which do not have a defect in the hypocotyl.

There is also another possible function of the *ACL1* gene. It may be responsible for a specific cell-wall or cytoskeletal component that is required for both the expansion of cells in leaves/internodes and the division of meristem cells for the elongation of inflorescences. However, it is difficult to imagine a component that is necessary for the division of cells in inflorescence meristems but not for the division of cells in leaves and flower stalks.

A third possible explanation of *ACL1* function is that the primary defect in the *acl1* mutant is in the maturation (elongation) of cells, with the cessation of development of inflorescence meristems being a secondary effect. Since we suggested a linkage between the development of internodes and of flowers, negative feedback regulation of floral development from internodal development might be postulated. If there is such negative feedback, the cessation of development of further flowers in *acl1* mutants would be the result of feedback regulation from internodes that cannot elongate. Results of double mutants of *acl1-1 hy2* and *acl1-1 er* favor our negative-regulatory hypothesis. Although some compensatory mechanisms in reproductive organs must also be hypothesized, given that cells in the reproductive organs are not affected, as are vegetative cells, by *acl1* mutations, the negative-feedback hypothesis appears to explain the phenotypes observed in *acl1* mutants rather simply. Proof of the negative-feedback hypothesis awaits future study. Since there is a large gap in our understanding of the relationship between gross multicellular morphology and subcellular structures in plant development, many other possibilities can be postulated. Further physiological, morphological, and

molecular investigations of the gross morphology of *Arabidopsis* must be performed.

A very similar mutation, known as the *det* (*determinate*) mutation, was identified in *Pisum sativum* L. (Singer et al., 1990). The *det* mutant of pea also exhibits arrested development of inflorescence apices at early stages and produces a limited number of flowers, with the apical dome remaining intact. From these apparent similarities, it is possible that the *acl1* mutation of *Arabidopsis* and the *det* mutation of pea may have a common mechanism.

Further clarification of the mechanism of action of the normal and mutant genes can be expected from molecular analysis. The *acl1-1* mutation was obtained by X-ray irradiation, which is known to cause deletions and other chromosomal defects. Preliminary genetic analysis has shown a reduction of 30% or more in the transmission of the *acl1-1* allele than would logically be expected, and such reductions are frequently an indicator of the involvement of a deletion at the locus of interest and/or in its vicinity. Therefore, an attempt will be made to isolate the *ACL1* gene by the 'genomic subtraction' procedure of Sun et al. (1992).

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