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Disruption of an RNA helicase/RNAse III gene in *Arabidopsis* causes unregulated cell division in floral meristems

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SUMMARY

Arabidopsis thaliana **floral meristems are determinate structures that produce a defined number of organs, after which cell division ceases. A new recessive mutant, *carpel factory* (*caf*), converts the floral meristems to an indeterminate state. They produce extra whorls of stamens, and an indefinite number of carpels. Thus, *CAF* appears to suppress cell division in floral meristems. The function of *CAF* is partially redundant with the function of the *CLAVATA* (*CLV*) and *SUPERMAN* (*SUP*) genes, as *caf clv* and *caf sup* double mutants show dramatically enhanced floral meristem over-proliferation. *caf* mutant plants also show other defects, including absence of axillary inflorescence meristems, and abnormally shaped leaves and floral organs. The *CAF* gene was cloned and found to encode a putative protein of 1909 amino acids containing an N-terminal DExH/DEAD-box type RNA helicase domain attached to a C-terminal RNaseIII-like domain. A very similar protein of unknown function is encoded by a fungal and an animal genome. Helicase proteins are involved in a number of processes, including specific mRNA localization and mRNA splicing. RNase III proteins are involved in the processing of rRNA and some mRNA molecules. Thus CAF may act through some type of RNA processing event(s). *CAF* gives rise to two major transcripts of 2.5 and 6.2 kb. In situ hybridization experiments show that *CAF* RNA is expressed throughout all shoot tissues.**

Key words: Flower development, Meristem determinacy, RNaseIII, Helicase, *Arabidopsis thaliana*, *carpel factory* (*caf*)

INTRODUCTION

Shoot apical meristems of angiosperms are the ultimate source of plant aerial structures. These meristems initiate leaves, axillary meristems, and flower meristems, while continually maintaining a pool of undifferentiated cells (Steeves and Sussex, 1989). In *Arabidopsis thaliana* the shoot apical meristem is indeterminate, with floral meristem formation continuing until senescence. Several genes affecting shoot meristem function have been described in *Arabidopsis*. Mutations in the *CLAVATA* (*CLV*) genes (*CLV1*, *CLV2* and *CLV3*) lead to an over-proliferation of meristem cells (Clark et al., 1993, 1995; Alvarez and Smyth, 1994; Kayes and Clark, 1998; Fletcher et al., 1999), while mutations in the *SHOOTMERISTEMLESS* (*STM*), and *WUSCHEL* (*WUS*) loci cause defects in meristem maintenance (Barton and Poethig, 1993; Laux et al., 1996).

The *Arabidopsis* floral meristem differs from the shoot apical meristem both in the type of organs produced (floral meristems produce sepals, petals, stamens and carpels) and in its determinate nature. Several genes have been shown to affect floral meristem identity, and mutations in these genes lead to a partial conversion of the flower meristem into a shoot meristem. In particular, *LEAFY* (*LFY*) and *APETALA1* (*AP1*) mutations produce some loss of floral meristem identity and a partial loss of determinacy, and double mutant combinations of *LFY* and *AP1* lead to a greatly enhanced loss (Huala and Sussex, 1992; Weigel et al., 1992; Bowman et al., 1993). In addition, several mutations affecting apical meristem structure affect floral meristem structure in the same way, with *clv1*, *clv2*, and *clv3* mutations resulting in over-proliferation and partial loss of determinacy in the floral meristem, and mutations in *WUS* and *STM* resulting in a loss of organs, especially in the inner whorls.

Other genes have been shown to affect floral meristem determinacy while not affecting apical meristem development. Mutations in *SUPERMAN* (*SUP*) lead to increased number of stamens and carpels, while *AGAMOUS* (*AG*) mutants show many extra whorls of sepals and petals (Bowman et al., 1991, 1992; Schultz et al., 1991).

Most of these genes have been cloned; some are putative transcription factors while others seem to be involved in cell to cell signaling processes. *AG* and *AP1* encode MADS-Box type transcription factors (Yanofsky et al., 1990; Mandel et al., 1992), *SUP* encodes a zinc-finger protein (Sakai et al., 1995), *STM* and *WUS* encode homeodomain proteins (Long et al.,

1996; Mayer et al., 1998), *LFY* encodes a new type of transcription factor (Parcy et al., 1998), *CLV1* encodes a transmembrane leucine-rich repeat receptor kinase (Clark et al., 1997), and *CLV3* may encode its ligand (Fletcher et al., 1999). Though it seems likely that these genes act through regulation of downstream genes involved in the control of cell division and differentiation functions, none of these target genes nor the specific mechanisms involved are known.

We have identified the *CARPEL FACTORY* (*CAF*) gene, which plays a role in floral meristem determinacy. *caf* mutant flowers show extra stamens and carpels, reminiscent of *sup* mutants, but also show organ morphogenesis defects. The *CAF* gene encodes a protein with similarities to both DExH/DEAD-box type RNA helicases and RNaseIII proteins, suggesting a mechanism for control of floral meristem proliferation. The *Schizosaccharomyces pombe* and *Caenorhabditis elegans* genomes both contain a gene coding for a protein highly similar to that encoded by CAF, indicating that CAF-type RNAse III-helicase proteins may play a role in many eukaryotic organisms.

MATERIALS AND METHODS

Mutant isolation and analysis

The *caf* mutant was isolated in a screen of mutants generated by *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* seeds of ecotype Wassilewskija (Feldmann, 1992). Phenotypic and genetic analysis of *caf* was carried out both in the Wassilewskija background and in a line of *caf* that had been backcrossed to the L*er* ecotype five times. The phenotype was similar in both backgrounds. The *caf* phenotype did not seem to be qualitatively different when grown at 16°C, 23°C, or 29°C. All of the reported analyses of this mutant was carried out at 23°C. Plants were sown in a 1:1:1 mixture of vermiculite:perlite:soil, grown under constant illumination, and watered periodically with a dilute solution of Miracle Grow (20:20:20) plant fertilizer. Scanning electron microscopy (SEM) was performed as described by Bowman et al. (1989). Confocal laser scanning microscopy was performed as described by Running et al. (1995). Negatives and slides were scanned and digitized using Nikon Coolscan slide scanner. Brightness, contrast and color balance were adjusted using Adobe Photoshop 3.0 and figures were printed using a Kodak 8300 digital printer.

CAF gene cloning

Genomic DNA was extracted from plants homozygous for *caf*, partially digested with *Sau*3A1, partially filled with dGTP and dATP, cloned into the partially filled *Xho*I site of lambdaGem-11 (Promega), and packaged with Gigapack II Gold packaging extracts (Stratagene). The cDNA library used was constructed from floral mRNA (Weigel et al., 1992). The longest cDNA (cDNA8) contained 2558 bp of sequence corresponding to the 3′ half of the full cDNA sequence. Three separate rounds of 5′ PCR-RACE were performed using a kit (GIBCO-BRL) as outlined in the manufacturer's instructions.

A *CAF*-containing cosmid clone (CosA) was obtained by screening a Wassilewskija genomic DNA library constructed in a pOCA18 derivative which confers hygromycin resistance to transgenic plants (obtained from the Arabidopsis Biological Resource Center; Olszewski et al., 1988; Schulz et al., 1995). The CosA clone was transformed into the *Agrobacterium tumefaciens* strain ASE, and whole plants were transformed using the vacuum-infiltration method (Bechtold et. al., 1993). Transgenic plants were then crossed to *caf* mutant plants, and F_3 populations of these crosses were selected for lines which were homozygous for kanamycin resistance (and hence were homozygous for the *caf* mutation), and which were segregating for hygromycin resistance (3:1, hygromycin resistant:hygromycin sensitive). Within these populations, all of the hygromycin-resistant plants showed a wild-type phenotype (an example is shown in Fig. 7B) while all of the hygromycin-sensitive plants showed the *caf* mutant phenotype, showing that CosA complements *caf*. Since CosA contained not only the CAF gene, but also an adjacent pyrophosphatase gene whose 3′ end is very close to the T-DNA, there remained a remote possibility that the pyrophosphatase gene was also affected by the T-DNA, and that this could contribute to the *caf* phenotype. To rule out this possibility, we showed by RNA blot analysis that the size and abundance of a 1 kb transcript homologous to the pyrophosphatase gene was unaffected in the *caf* mutant. Furthermore, DNA sequence analysis showed that the pyrophosphatase gene in the *caf* mutant has a sequence which is identical to that of wild-type WS plants. Thus, neither the structure nor expression of the pyrophosphatase gene are affected in *caf*.

For mapping, an *Eco*RI restriction fragment length polymorphism (RFLP) detected with CosA was mapped in a L*er* × Columbia mapping population (Chang et al., 1988). For secondary confirmation, a *Hin*cII RFLP contained within the helicase domain of the *CAF* gene was mapped in a Niederzenz X Columbia mapping population. A similar map position was obtained.

Southern blot analysis showed that the T-DNA insertion at the *CAF* locus is complex, consisting of multiple tandem copies of the T-DNA. The junction between the *CAF* gene and the T-DNA was determined by cloning and sequencing a 6.5 kb *Eco*RI fragment of genomic DNA from a lambda clone isolated from the *caf* genomic library. This clone contained a region of *CAF* corresponding the RNaseIII-like domain, up to amino acid 1836 of CAF (see insertion site shown in Fig. 7A), adjacent to 493 bp of sequence homologous to the right border of the T-DNA followed by 1684 bp of sequence homologous to the vector pBR322, followed by the Trn903, OCS3′, and Trn5-1′-2′ portions of the T-DNA (Feldmann, 1992).

CAF RNA expression

To ensure that our DNA probes were specific for the *CAF* gene, we tested two probes corresponding to the 5′ and 3′ ends of *CAF*, on Southern blots. The N-terminal helicase probe was a 1809 bp PCR product corresponding to positions 475-2283 in the 5815 bp cDNA. The primers used to generate this product were JP237 (5′AATAGGAAACGTACTCGTAATT) and JP236 (5′AATGT-ATGCCAGCACCGTCTT). The C-terminal RNaseIII probe was a 1838 bp PCR product corresponding to positions 3930-5767 in the cDNA. The primers used to generate this were JP121 (5′GTTTCTTCCACCTGAACTA) and JP131 (5′CTACATCTCGT-TGAAGAGAGTA). These probes were hybridized to filters containing genomic DNA cut with the restriction enzymes *Eco*RI, *Xba*I, *Bgl*II, *Hin*dIII, *Hin*cII, or *Eco*RV. Only the fragments predicted from the *CAF* genomic sequence were detected. These Southern blots and the northern blots shown in Fig. 9 were prepared, hybridized, and washed under high stringency conditions as previously described (Chang et al., 1988).

In situ hybridization experiments were carried out using sense or antisense RNA probes synthesized with T7 or T3 polymerase from cDNA8 plasmid DNA that had been linearized with *Eco*RI or *Xho*I. The remainder of the in situ procedure was done according to Drews et al. (1991), with modifications by Sakai et al. (1995). These in situ hybridization experiments were carried out twice independently, with similar results. One of the experiments was carried out both in the presence and absence of 500 μg/ml polyuridylic acid (Pharmacia Biotech, product number 27-4440), to ensure that the poly(A) tail on cDNA8 was not causing an increase in the background hybridization. No difference in signal was observed between the poly(U) plus or minus treatments.

RESULTS

carpel factory floral determinacy defects

During a screen of T-DNA mutagenized lines (Feldmann, 1992) for mutants with abnormal flower development, we found a recessive mutant with indeterminate flowers. The mutant gene was named *CARPEL FACTORY* (*CAF*). *caf* flowers show a lack of floral determinacy that is evident in both the third and fourth whorls. Wild-type *Arabidopsis* flowers produce four organ types, each occupying a separate whorl or concentric ring (Fig. 1A). The first whorl consists of four sepals, the second whorl contains four petals, the third whorl has six stamens, and the fourth or inner whorl is composed of two carpels which later fuse to form the gynoecium. *caf* flowers contain a roughly normal number of sepals, petals, and stamens in the first three whorls (Fig. 1B). However, most *caf* mutant flowers contain one or two extra stamens interior to the third whorl stamens (Fig. 1C). Occasionally, flowers contain multiple whorls of stamens (up to 40 stamens/flower have been observed) (Fig. 1D). These defects are reminiscent of the *sup* mutant phenotype (Schultz et al., 1991; Bowman et al., 1992).

The *caf* gynoecium is severely affected. While wild-type flowers produce a gynoecium consisting of two fused carpels (Fig. 1E), *caf* flowers typically consist of a gynophore (a region of internode elongation between the third and fourth whorl), and several sterile unfused carpels (Fig. 1F). Usually, additional growth can be seen interior to the first whorl of carpels, forming structures which resemble carpels, carpelloid filaments, or filaments with no obvious floral character (Fig. 1G). Occasionally, staminoid structures are also present amongst the carpels (Fig. 1G). These ectopic organs are produced at the flanks of a visible group of undifferentiated cells in the center of the *caf* floral meristem (Fig. 1H-J).

Scanning electron microscopy of floral meristems shows that the region of cells in the fourth whorl is larger in *caf* flowers than in wild type (Fig. 2). Whereas wild-type floral meristems terminate in the development of two carpels (Fig. 2A), *caf* fourth whorl cells continue to proliferate and give rise to additional primordia on the flanks of an indeterminate meristem (Fig. 2B,C).

carpel factory leaf and floral morphogenesis defects

caf affects the shape of most plant organs. As early as 9 days after development, *caf* seedlings can be recognized by the thinner

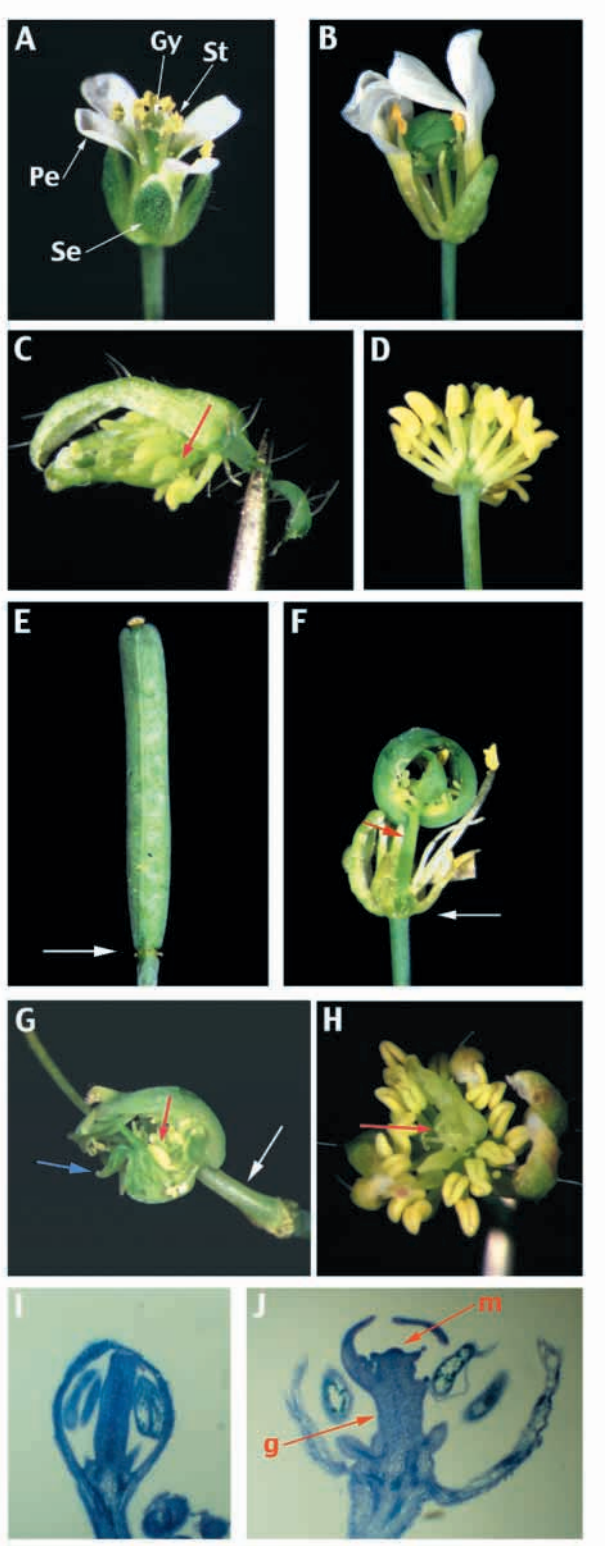


Fig. 1. *carpel factory* flowers produce extra stamens and carpels. (A) A wild-type *Arabidopsis* flower containing four sepals (Se), four petals (Pe), six stamens (St), and a gynoecium (Gy) composed of two fused carpels. (B) A typical *caf* flower containing four sepals, four petals and six stamens in the first three floral whorls. (C) A *caf* flower with two sepals and two petals removed to reveal the stamens. Red arrow points to a stamen interior to the third whorl stamens. (D) A *caf* flower with all sepals and petals removed to reveal four whorls of stamens. This extreme stamen number phenotype is observed in about 1% of the flowers. (E) A wild-type gynoecium consisting to two fused carpels. White arrow shows the abscission zone of the senesced organs of the first three whorls. (F) A *caf* mutant gynoecium showing a gynophore (red arrow) and three sterile unfused carpels. White arrow shows the attachment of the organs of the first three whorls (G) An older *caf* flower in which the organs in the first three whorls have senesced and abscised. The gynoecium consists of a gynophore (white arrow), and many carpels and filamentous organs. Red arrow shows a stamen produced amongst the carpels. Blue arrow shows a filamentous organ. (H) A *caf* flower with an exposed meristem (red arrow). (I) Cross section of a wild-type flower stained with toluidine blue. Two sepals, two anthers, and the central gynoecium are visible. (J) Cross section of a *caf* flower stained with toluidine blue. Two sepals and three anthers are visible as well as a gynophore (g) and a proliferating floral meristem (m) in the center of the flower.

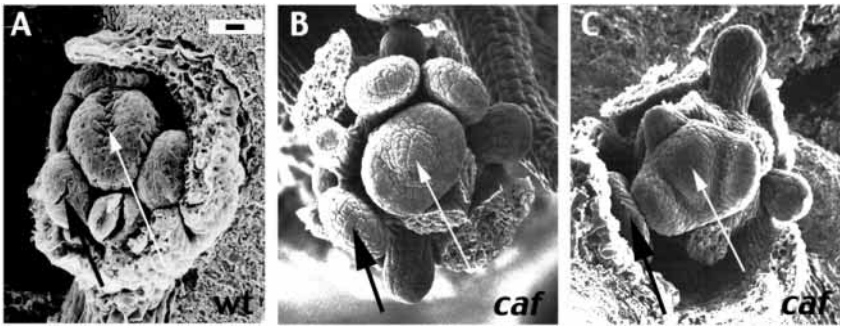


Fig. 2. Wild-type and *caf* fourth whorl development. The three panels show a wild-type (wt) floral meristem at stage 7 (Smyth et al., 1990), which has terminated in the development of two carpel primordia (A), and two *caf* floral meristems (B,C) showing excess cells in the center of the flower after the initiation of carpel primordia. In all three panels, the white arrow indicates the center of the flower, and the black arrow, a stamen. Bar in A (for A-C) is 10 μm.

shape of the cotyledons and rosette leaves and a decrease in the overall size of the seedling (Fig. 3A,B). Mature rosette and cauline leaves are also thinner than wild type (Fig. 3C). Secondary meristems, which normally arise from the axils of rosette leaves and cauline leaves, are almost always absent in *caf* plants (Fig. 3D-G). Decapitating plants does not induce the development of these meristems, suggesting that these meristems are missing or non-functional and not merely inactive. Roughly 10% of the leaves show severe defects. Some leaves are reduced to filamentous structures which resemble the petiole or midrib tissue, but which lack leaf blade character (Fig. 3H). Intermediate leaves are also produced that have patches of blade tissue on the adaxial surface of a filamentous structure resembling the midrib (Fig. 3I). SEM analysis confirms that these filamentous structures have surface features similar to wild-type petioles or midribs (lack of stomata and cells in regular files) (not shown).

The first formed flowers are sometimes highly reduced (Fig. 3J). Some consist of floral pedicels and very few sepal-like organs, while others are reduced to filamentous structures. The reduced flowers are primarily observed within the first 20 flowers.

Some of the *caf* morphological defects can be traced to early defects in the development of meristems. Scanning electron microscope (SEM) analysis of 14-day-old wild-type and *caf* plants revealed that a small percentage of the developing *caf* floral meristems were smaller than wild-type ones (Fig. 4A,B). These smaller primordia probably correspond to the reduced flowers observed on more mature plants. Flowers produced by 30-day-old plants (after elongation of the inflorescence stem) showed fewer defects than those from 14-day-old plants (Fig. 4C,D). We also noted a slight increase in the size of the *caf* apical meristem relative to wild type (Fig. 4A-D). This size difference was confirmed by confocal analysis (Fig. 4E,F).

caf sepals develop in roughly the correct number and position, but are narrower than wild-type sepals (Fig. 1A,B). SEM analysis shows that the sepals are narrower than in wild type at the earliest stages of development (Fig. 4C,D). Though most *caf* petals are similar to wild-type petals (Fig. 1A,B), occasionally they are reduced to filamentous structures (not shown). All stamens contain a reduced number of pollen sacs. Approximately 80% of stamens have an anther consisting of two pollen sacs instead of the wild-type number of four, while the remaining 20% of stamens consist of a filament with no

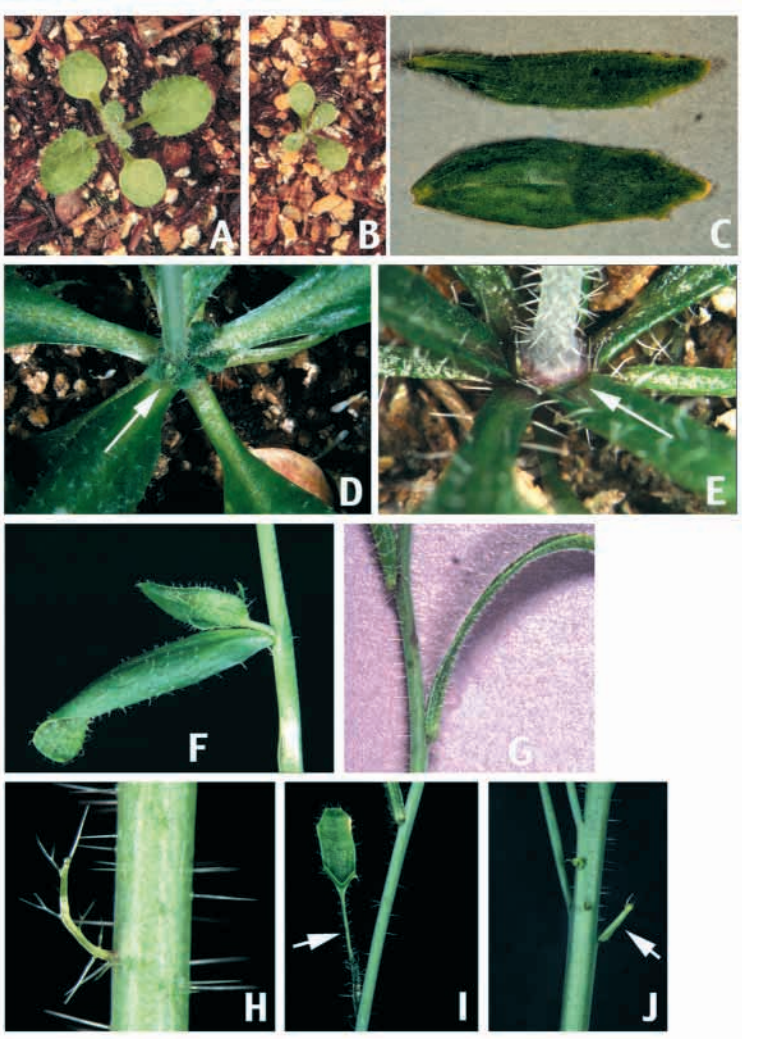


Fig. 3. Morphological defects of *caf* mutants. Nine-day-old wild-type (A) and *caf* (B) seedlings photographed at the same magnification. (C) Mature rosette leaves from wild type (bottom) and *caf* (top). Rosettes of wild type (D) and *caf* (E). White arrows show the axil of a rosette leaf and a stem. An axillary meristem has developed in this position in wild type but not in *caf*. Wild-type (F) and *caf* (G) cauline leaves showing an axillary meristem in wild type but not in *caf*. (H) Filament on a *caf* plant in a position normally occupied by a cauline leaf. (I) *caf* cauline leaf showing two patches of blade tissue on the adaxial surface of a filamentous structure resembling the midrib. The arrow shows the midrib region lacking the leaf blade. (J) Reduced flowers produced during the early development of *caf* inflorescences. White arrow shows a flower consisting of a pedicel and one filamentous organ.

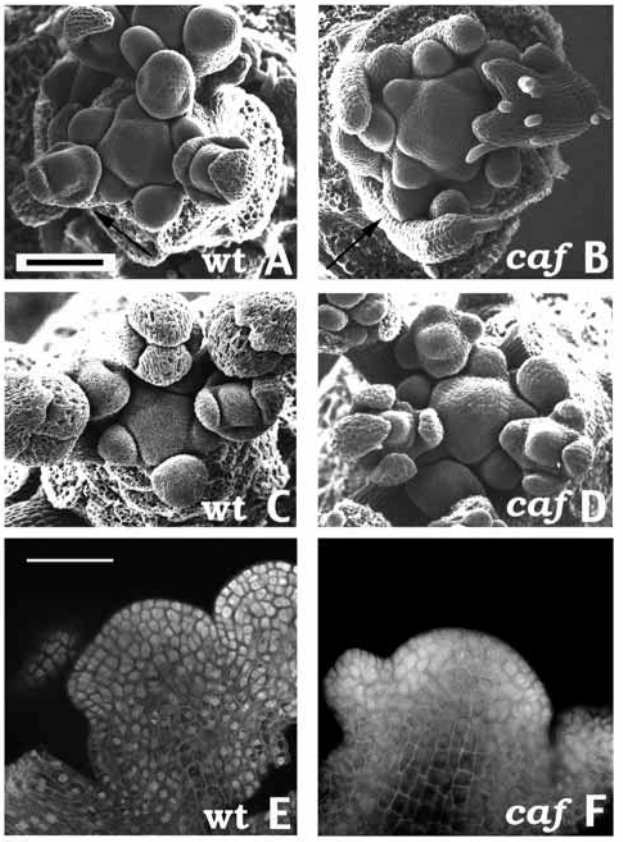


Fig. 4. Apical and floral meristem structure in wild type and *caf*. In 14-day-old plants, wild-type (wt) meristems (A) are producing normal flowers, while some *caf* meristems (B) are producing highly reduced flowers. Arrows indicate an example of a *caf* primordium which has not initiated floral organ primordia, but which occupies a position where in wild type, sepal primordia have already developed. Apical meristems from 30-day-old plants of wild type (C) and *caf* (D). Black bar in A (for A-D) is 100 μm. (E,F) Confocal laser scanning microscopy reveals that in 14-day-old plants, *caf* inflorescence meristems are slightly larger than wild-type meristems. While wild-type meristems were 75±0 μm in diameter, *caf* meristems were 84.6±3.0 μm (numbers are mean ± standard error where n=5). Bar in E (for E and F) is 50 μm.

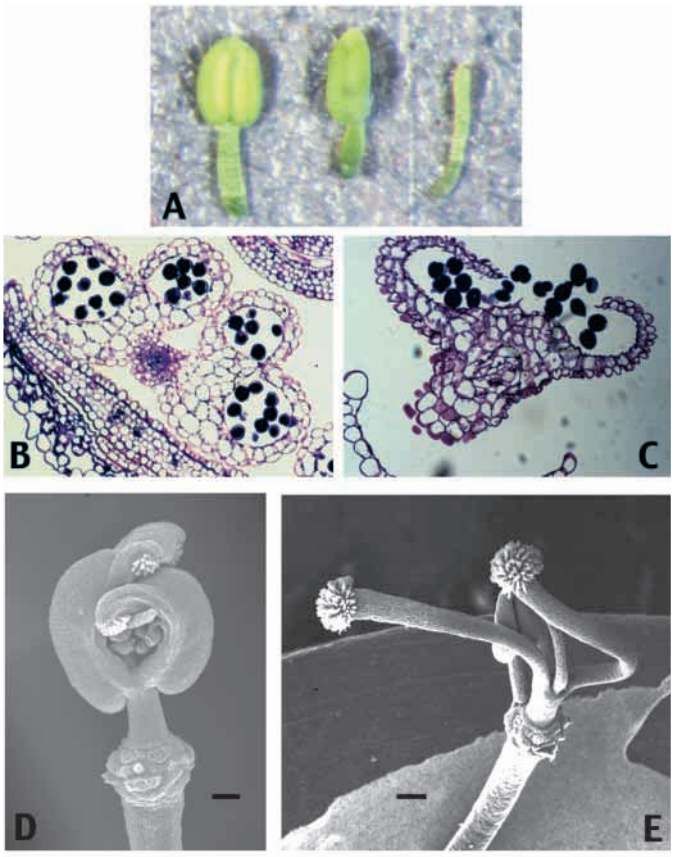


Fig. 5. Floral organ defects of *caf* mutants. (A) A wild-type stamen with four pollen sacs (right), a reduced *caf* stamen with two pollen sacs (middle), and an antherless *caf* stamen (left). (B) A cross section of a wild-type anther and (C) cross section of a *caf* anther containing two pollen sacs, both at the same magnification. (D) A *caf* flower bearing two relatively normal but unfused carpels. (E) A *caf* flower containing filamentous organs tipped with stigmatic tissue. Bars in D and E, 100 μm.

anther at all (Fig. 5A-C). Normal anthers containing 4 pollen sacs have not been observed. Some stamen primordia are much smaller than normal (see Fig. 2B). These smaller primordia may give rise to the antherless stamens observed in mature flowers. The structures produced in the fourth whorl of *caf* flowers are usually carpelloid or filamentous. The carpelloid organs range in phenotype from relatively normal but unfused carpels (Fig. 5D) to filamentous organs tipped with stigmatic tissue (Fig. 5E). While *caf* pollen is functional, *caf* plants are female sterile.

Genetic interactions of *caf* with mutations affecting floral meristem patterning

We constructed double mutants of *caf* with several mutations affecting floral organ identity. *apetala2* (*ap2*) mutations affect the identity of the first and second whorl organs. The *ap2-1* mutation converts the first whorl organs to leaves and the second whorl organs to staminoid petals (Bowman et al., 1989). The *ap2-1 caf* double mutant has a phenotype similar to that of *caf* single mutants, except that it has a floral organ identity phenotype similar to that of *ap2-1* mutants (not shown). *pistillata* (*pi*) mutations affect the identity of the second and third whorl organs, converting the petals and stamens to sepals and carpels (Bowman et al., 1989). The *pi caf* double mutant shows an additive phenotype, exhibiting morphological characteristics and meristem over-proliferation defects of *caf* mutants but the floral organ identity phenotype of *pi* mutants (not shown). *ag* mutations affect the identity of the third and fourth whorl organs and cause floral indeterminacy, such that *ag* flowers produce sepals in the first whorl, petals in the second and third whorls, then a new flower showing the repeating pattern (sepals, petals, petals)$_n$ (Fig. 6A; Bowman et al., 1989). The *ag caf* double mutant has a phenotype similar to *caf* single mutants throughout most of the plant. However, interior to the first whorl sepals *ag-1 caf* double mutant flowers contain only petals (Fig. 6B). This phenotype is reminiscent of *sup ag* double mutant flowers (Bowman et al., 1992; Schultz et al., 1991), which also contain only petals in the inner whorls. In summary, the double mutants of *caf* with the floral homeotic mutants *ap2-1*, *pi-1*, and *ag-1* show a largely additive phenotype; that is, they display the floral meristem over-proliferation phenotype

characteristics of the *caf* mutant, but also show floral organ misspecification in a manner similar to that seen in the floral homeotic single mutants.

We also combined the *caf* mutation with other mutations that affect floral organ number. *sup* mutant flowers display extra cell proliferation in the developing third whorl, resulting in the production of an excess number of stamens (Fig. 6C; Bowman et al., 1992; Schultz et al., 1991). *sup-1 caf* double mutant flowers show greatly enhanced gynophore development and floral indeterminacy (Fig. 6D). After producing the organs in the first three whorls (arrow), double mutant flowers produce many staminoid, carpelloid, and leaf-like organs. Thus, *SUP* and *CAF* appear to be partially redundant in their control of floral determinacy. The *clv3-2* mutant has an enlarged apical meristem and enlarged floral meristems that produce an excess number of organs in all of the floral whorls (Fig. 6E; Clark et al., 1995). Additionally, *clv3-2* plants often produce extra whorls of carpels interior to the fourth whorl (Clark et al., 1995). *clv3-2* and *caf* exhibit an essentially additive interaction with respect to vegetative development. *clv3-2 caf* plants have enlarged apical meristems (similar to *clv3-2* single mutant plants) and produce few secondary meristems (similar to *caf* single mutants) (not shown). However, *clv3-2 caf* flowers exhibit an enhanced floral indeterminacy phenotype, producing a bouquet of stamens, carpels, and filaments (Fig. 6F).

CAF cloning

caf was isolated from a screen of *Agrobacterium* T-DNA insertion mutants (Feldmann, 1992). After 3 back crosses of *caf* to wild-type Landsberg *erecta* (L*er*) plants, a single kanamycin resistance locus cosegregated with the *caf* phenotype, suggesting that *CAF* was tagged with a T-DNA element. To isolate plant sequences flanking the T-DNA, we generated a *caf* genomic lambda library and screened for clones containing homology to the right border of the T-DNA. A clone was isolated that contained homology both to the right border and to plant genomic DNA. Southern blot analysis showed that this plant DNA cosegregated with the *caf* phenotype. Plant DNA flanking the T-DNA insertion was used to probe a cDNA library, and twelve hybridizing clones were found. One of these clones (cDNA8; 2.5 kb in length) was sequenced, and found to contain a long ORF, followed by a 76 base pair 3′ untranslated region, and a short poly(A) tail. PCR analysis showed that of the other 11 cDNA clones, three had 5′ ends which were at a very similar position, while eight were somewhat shorter. To determine the 5′ end of the *CAF* RNA, we performed 5′ RACE and found additional sequences that extended the ORF. Two additional rounds of RACE were performed using primers progressively closer to the 5′ end of the full length RNA. The resulting predicted RNA of 5815 nucleotides (Accession number, AF187317) contains an ORF of 1909 amino acids (Figure 7A). An in-frame stop codon in the genomic sequence nine nucleotides upstream of the predicted RNA sequence suggests that the first methionine shown in Fig. 7 is the start of translation (see Accession number AC007323 for the complete genomic sequence).

A cosmid clone (CosA) with homology to *CAF* was isolated and transformed into wild-type plants, and the resulting transgenic plants were crossed to *caf* mutant plants. This cosmid fully complemented the *caf* mutant floral phenotype (Fig. 7B), as well as all other *caf* phenotypes (not shown). To determine the genomic sequence of *CAF*, three adjacent *Eco*RI fragments were subcloned from CosA and sequenced. These fragments contained 8603 bp of genomic sequence comprising the entire *CAF* coding region and sequences upstream and downstream. The sequence predicts that the *CAF* coding sequence is interrupted by 19 relatively small introns. One of the fragments contained the junction between the putative *CAF* promoter and the cosmid cloning vector. The distance from the vector junction to the 5′ end of the predicted RNA sequence was 675 bp. Since this cosmid complements *caf*, this suggests that all of the relevant 5′ promoter sequences are contained within this 675 bp region. 48 bp downstream from the 3′ end of the *CAF* cDNA sequence

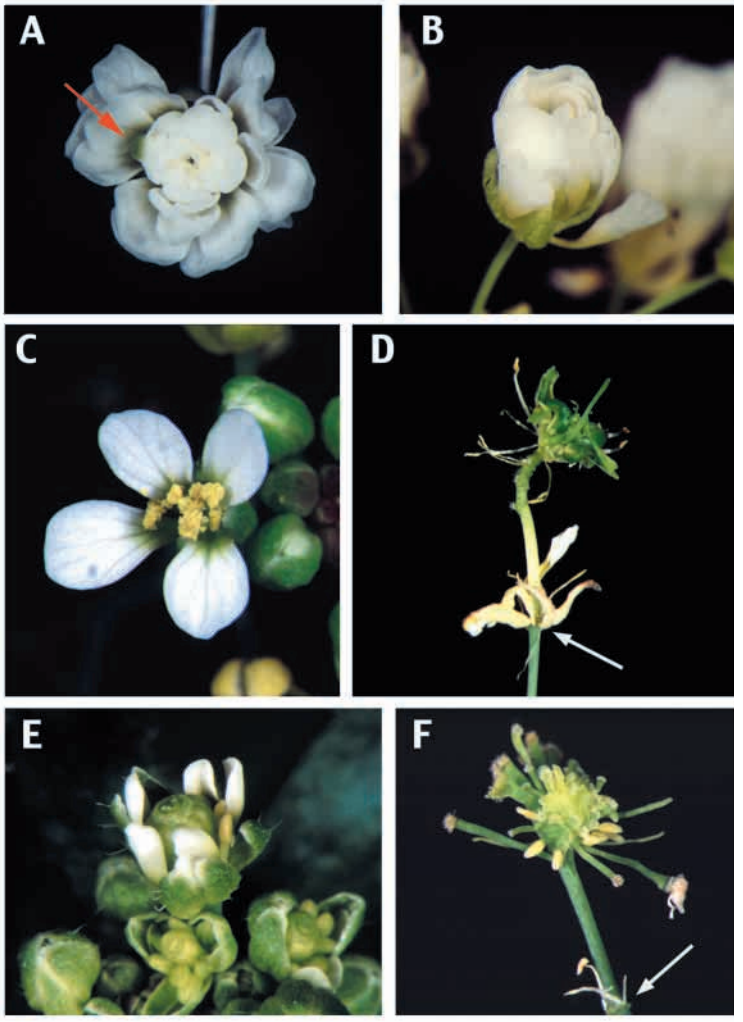


Fig. 6. *agamous-1 caf*, *superman-1 caf*, and *clavata3-2 caf* double mutants. (A) An *ag-1* single mutant flower showing sepals (red arrow) in the inner whorls. (B) An *ag-1 caf* double mutant flower showing only petals in the inner whorls. (C) A *superman-1* mutant flower. (D) A *sup-1 caf* double mutant flower showing greatly enhanced gynophore development and floral indeterminacy. After producing the organs in the first three whorls (arrow), double mutant flowers produce many staminoid, carpelloid, and filamentous organs. (E) *clv3-2* single mutant flowers, showing extra organs in all whorls. (F) *clv3-2 caf* flowers exhibiting enhanced floral indeterminacy. Arrow shows the position of the first three whorls.

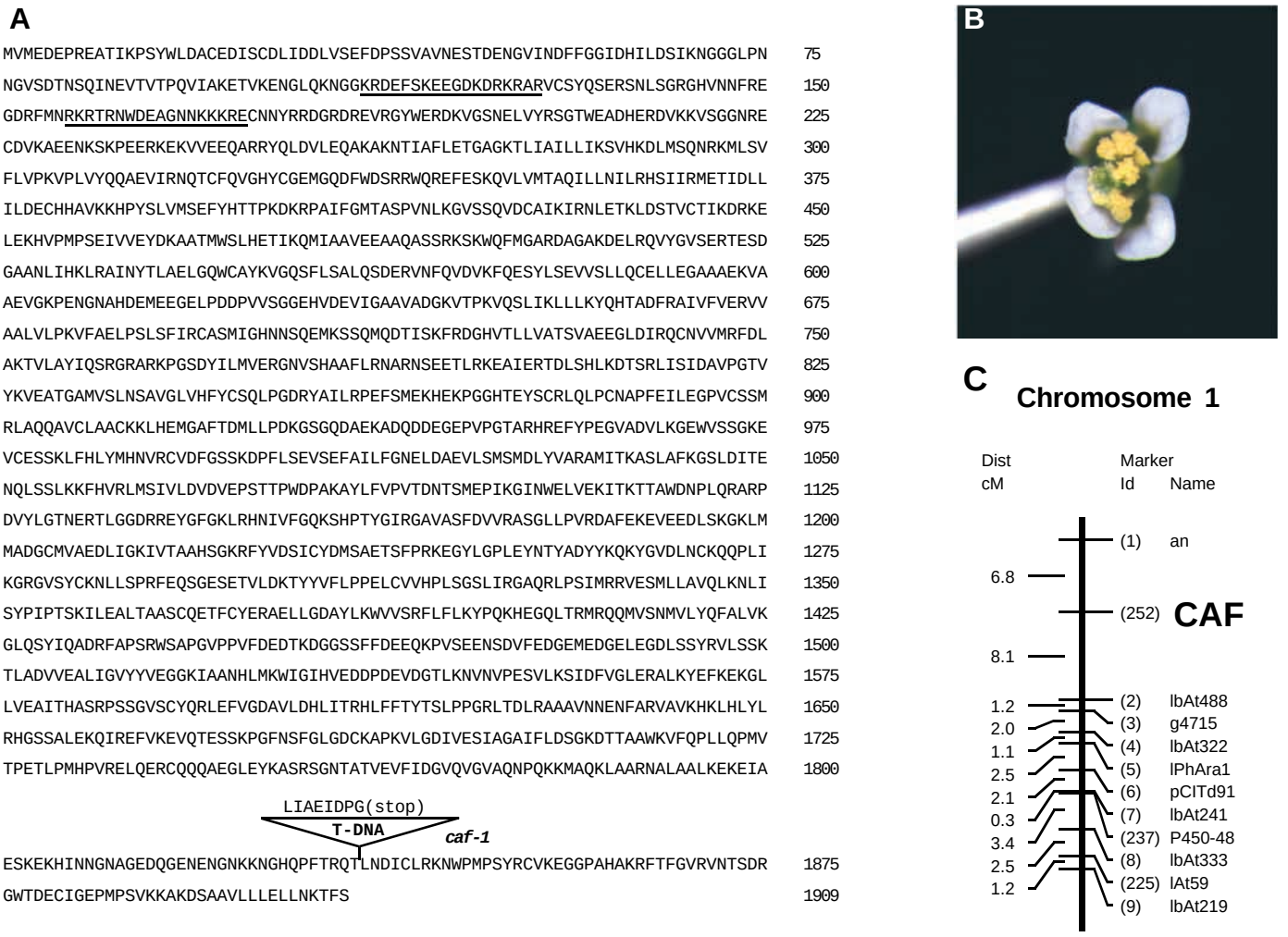


Fig. 7. The *CARPEL FACTORY* gene. (A) Predicted amino acid sequence of the CAF protein. The two underlined regions are putative nuclear localization sequences. Also shown is the position of the T-DNA insertion in the *caf-1* mutant. Above the insertion site is the predicted open reading frame extension conferred by the T-DNA. (B) Flower from a *caf* homozygote which contains a transgenic copy of the wild-type *CAF* gene and exhibits a wild-type phenotype. (C) Map position of the *CAF* gene as deduced by RFLP mapping of an *Eco*RI restriction site present in CosA. *CAF* maps on the top of chromosome 1 distal to lambdaAt488 (see Materials and Methods). Map shows the distance between markers in centimorgans (cM) and the log likelihood value (LOD; Chang et al., 1988). GenBank accession no. AF187317.

was a sequence identical to a 3′ EST clone with homology to pyrophosphatase (EST Z29202), suggesting that there are only 47 bp between the 3′ end of the *CAF* gene and the 3′ end of the adjacent gene. Using restriction fragment length polymorphism mapping, we mapped the *CAF* gene to the top of chromosome one (Fig. 7C).

The T-DNA insertion in the *caf* mutant lies in the 19th exon of the *CAF* gene, and thus separates most of the *CAF* gene from part of the 19th exon, the 19th intron, and the 20th exon. This truncates the predicted CAF protein at amino acid number 1836, replacing the last 73 amino acids of CAF with eight amino acids from the T-DNA sequence (Fig. 7A).

Similarity of CAF with helicase and RNAse III like sequences

Analysis of the predicted CAF sequence with PSORT (Nakai and Kanehisa, 1992) suggests that CAF is a nuclear protein, which contains two putative bipartite nuclear localization signals (underlined in Fig. 7A). The sequence of *CAF* shows extensive similarity along most of its length to a predicted protein of 1374 amino acids in *Schizosaccharomyces pombe* (C8A4.08C) and a predicted protein of 1822 amino acids in *Caenorhabditis elegans* (K12H4.8), both of unknown function. Relative to these proteins, CAF contains an N-terminal extension of 238 amino acids which contains a high content of charged residues (Fig. 8A). In the N-terminal region, these three predicted proteins show similarity to each other (an average of 34% identity) and to a number of DExH and DEAD box type RNA and DNA helicase proteins belonging to helicase superfamily II (Fig. 8B; Gorbalenya et al., 1989; Gorbalenya and Koonin, 1993). The database entries most similar to these helicase domains were three predicted proteins from three different completely sequenced archaebacterial species, one predicted protein from *Saccharomyces cerevisiae*, and two predicted proteins from *C. elegans*, all of unknown function. These sequences represent a distinct family within helicase superfamily II, being much more similar to each other than to other proteins of the DExH and DEAD type [so named

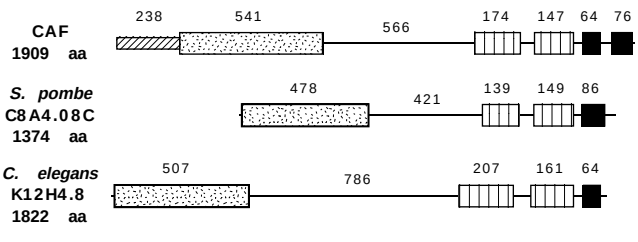
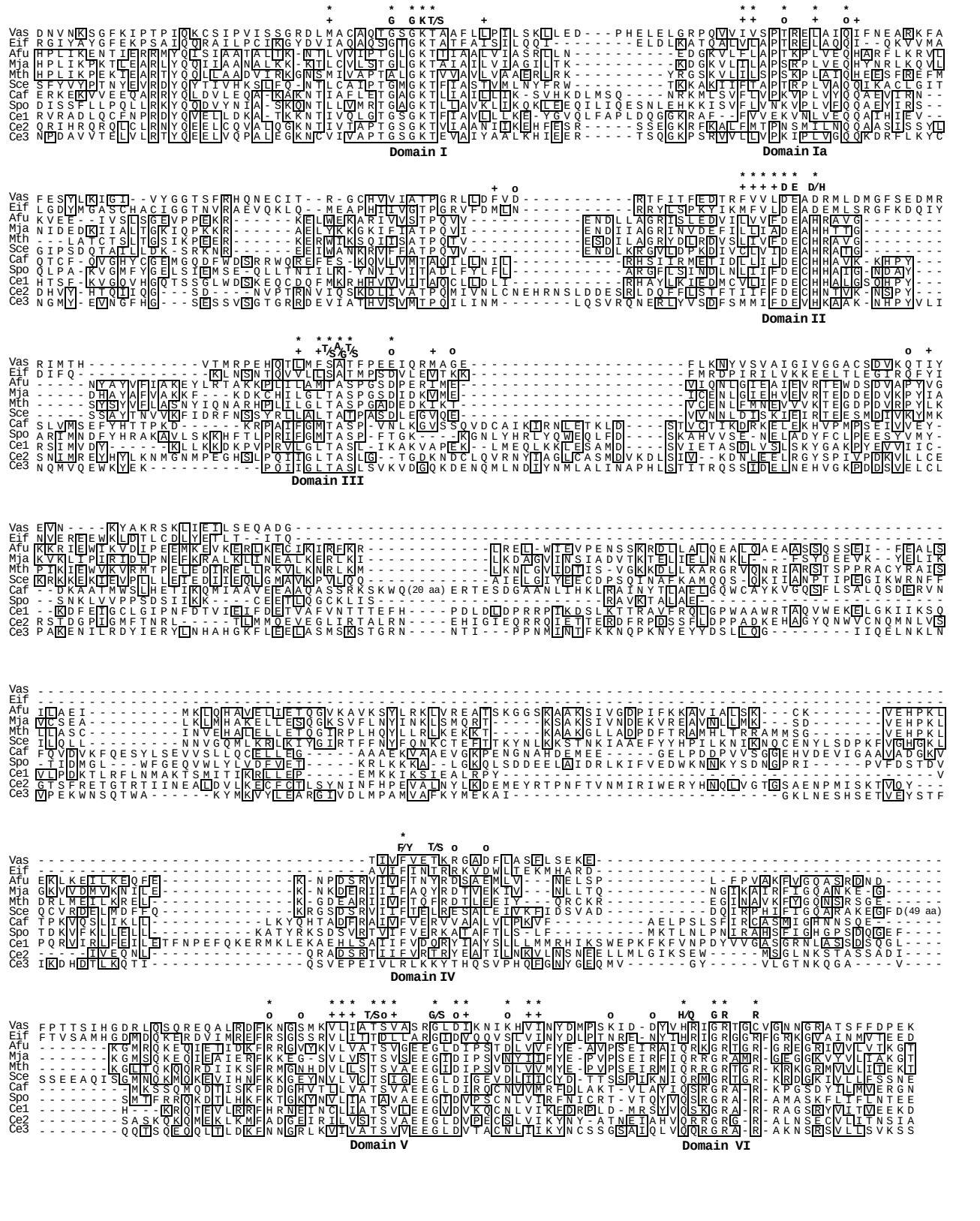
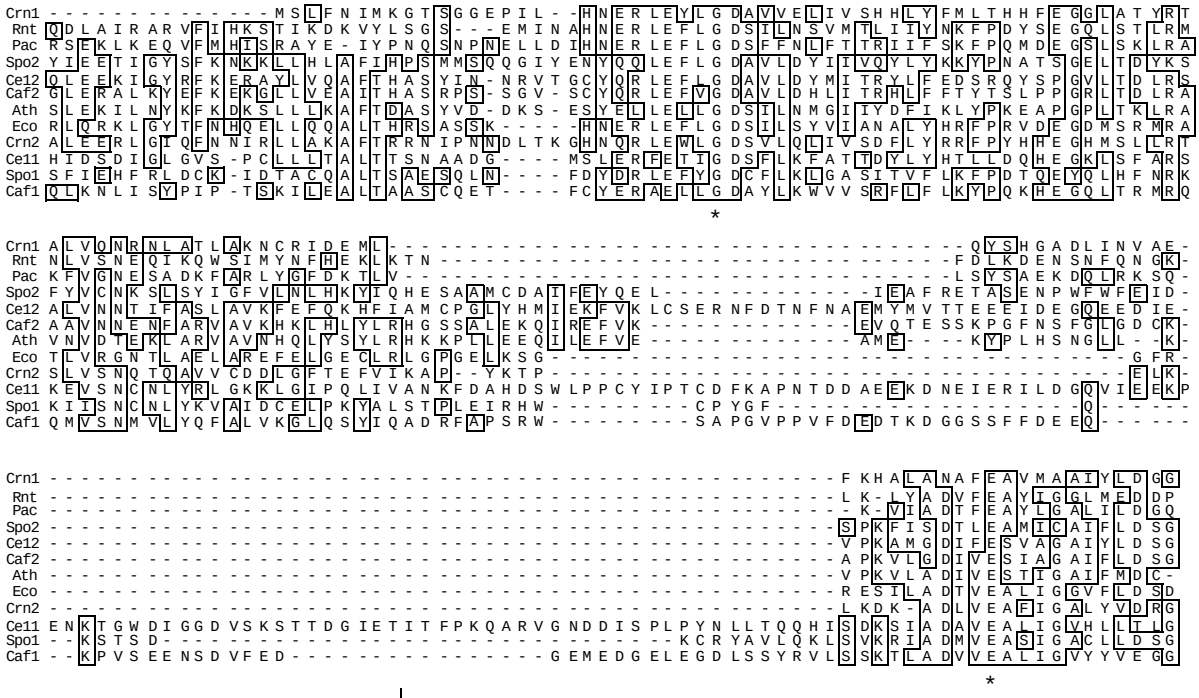
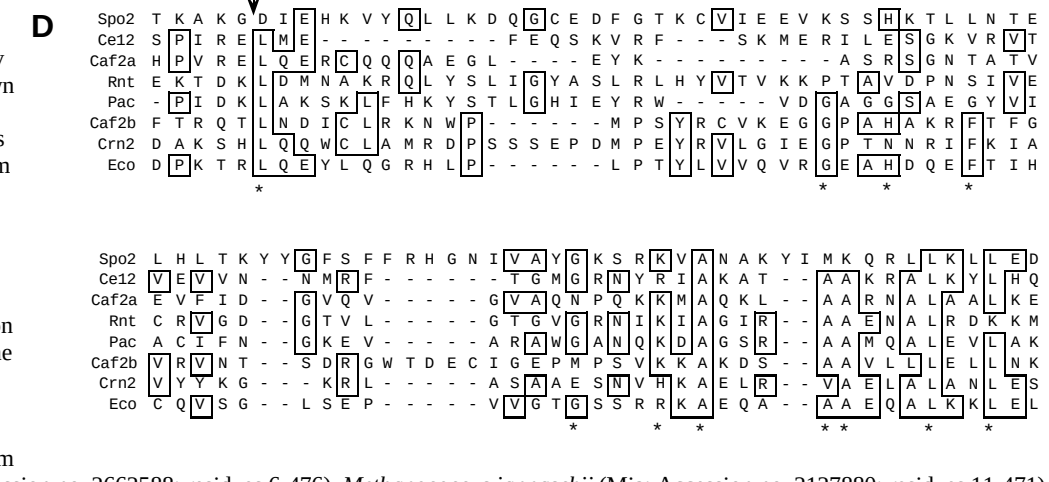


Fig. 8. Similarity of CAF with helicase and RNAse III like sequences. (A) Schematic of the domain structure of CAF and two similar predicted sequences from *S. pombe* (C8A4.08C; Accession no. Q09884) and *C. elegans* (K12H4.8; Accession no. P34529). Hatched box denotes an N-terminal CAF sequence with a high content of charged residues that is not found in the other two proteins. Stippled boxes denote the ATPase/Helicase domains. Vertically hatched boxes denote the RNaseIII-like domains, which are duplicated in all three predicted proteins. Black boxes denote the double stranded RNA binding domains, which are







duplicated in CAF but not in C8A4.08C or K12H4.8. Lines denote regions that show homology to each other, but not to other known sequences. The number of amino acids (aa) present in each domain is noted above each segment. Diagram is drawn to scale. (B) The N-terminal helicase-like domain of CAF (Caf; residues 239-779) was aligned with similar domains present in the predicted C8A4.08C protein of *S. pombe* (Spo; Accession no. Q09884, residues 2-479) and the predicted K12H4.8 protein of *C. elegans* (Ce1; Accession no. P34529; residues 3-509), and other predicted helicase-like proteins from *Archaeoglobus fulgidus* (Afu; Accession no. 2662588; residues 6-476), *Methanococcus jannaschii* (Mja; Accession no. 2127880; residues 11-471), *Methanobacterium thermoautotrophicum* (Mth; Accession no. 2622527; residues 7-478), *S. cerevisiae* (Sce; Accession no. P40562; residues 77-624), and *C. elegans* (Ce2; Accession no. 2662588; residues 283-822 and Ce3; Accession no. 1667297; residues 361-854). Also included in the alignment are the *Drosophila melanogaster* protein VASA (Vas; Accession no. P09052; residues 258-597) and the mouse protein eiF-4A1 (Eif; Accession no. FIMS4A; residues 29-364). A consensus sequence of seven domains (I-VI) present in these proteins is shown above the alignment and numbered as in Gorbelenya et al. (1989). + denotes the hydrophobic residues (I,L,V,M,F,Y,W); o denotes the charged or polar residues (S,T,D,E,N,Q,K,R). Asterisks mark positions where at least 10 of the 11 sequences matched the consensus. Identical residues are boxed in columns where at least 3 of the residues are identical. The (20 aa) and (49 aa) notations denote either 20 or 49 amino acids, which were present in only one protein and were removed from the alignment. (C) Similarity of CAF to the conserved regions of the catalytic domains of RNaseIII-like proteins. The duplicate RNaseIII-like domains of CAF [(Caf1; residues 1345-1518)(Caf2; residues 1561-1707)], C8A4.08C [(Spo1; residues 900-1038)(Spo2; residues 1085-1233)], K12H4.8 [(Ce11; residues 1295-1501)(Ce12; residues 1557-1717)], and RNC_CAEEL (Accession no. 001326) [(Crn1; residues 1-110)(Crn2; residues 164-271)] were aligned with similar domains present in *E. coli* RNaseIII (Eco; Accession no. P05797; residues 8-128), *S. cerevisiae* RNaseIII (Rnt; Accession no. Q02555; residues 206-331), the *Pac1* gene of *S. pombe* (Pac; Accession no. P22192; residues 138-262), and the predicted ATFCA3 protein from *A. thaliana* (Ath; Accession no. Z97338; residues 56-195). Identical residues are boxed in columns where at least 3 of the residues are identical. Asterisks mark conserved positions that are sites of inactivating point mutations in *E. coli* RNaseIII (Court, 1993). (D) Double stranded RNA binding domains (dsRNAbds) of CAF. Two putative dsRNAbds of CAF [(caf2a; residues 1733-1796)(caf2b; residues 1831-1906)] were aligned with similar domains found in C8A4.08C (Spo2; residues 1256-1341), K12H4.8 (Ce12; residues 1745-1808), RNC_CAEEL (Crn2; residues 298-373), *E. coli* RNaseIII (Eco; residues 155-225), *S. cerevisiae* RNaseIII (Rnt; residues 361-437), and the *Pac1* gene of *S. pombe* (Pac; residues 286-356). Identical residues are boxed in columns where at least 3 of the residues are identical. Asterisks denote positions where the residues are highly conserved in a variety of additional dsRNAbds (Kharrat et al., 1995). Arrow indicates the position of the T-DNA insertion in the second dsRNAbd of CAF (caf2B). All alignments were performed using PILEUP and PRETTYPLOT [Genetics Computer Group, (1991), Madison, Wisconsin], using a gap creation penalty of 3.0 and a gap extension penalty of 0.05.

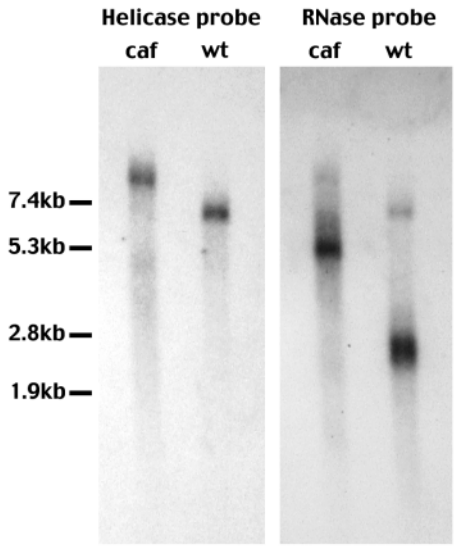


Fig. 9. *CAF* RNA species in wild-type and *caf* mutant plants. A Northern blot containing 2 μg of poly(A) RNA from *caf* mutant plants (caf) or wild-type plants (wt) was hybridized with a probe from the 5′ end of the *CAF* RNA (helicase probe; see Materials and Methods) and exposed for 11 days. The same blot was stripped, exposed for 13 days to ensure that no signal remained, and then hybridized with a probe from the 3′ end of the *CAF* RNA (RNaseIII probe; see Materials and Methods), and exposed for 8 days. Size markers shown are from an RNA ladder which was run on the same gel (Boehringer Mannheim RNA Molecular marker I).

for the conserved residues DExH and DEAD, with x representing any amino acid (Gorbalenya, et al., 1989)]. Fig. 8B shows an alignment of CAF and the related sequences along with eIF-4A and VASA, two RNA helicase proteins of the DEAD box type. CAF contains sequence similarity within all seven of the most conserved domains present in these DEAD/DExH proteins (Fig. 8B; Gorbelenya et al., 1989). These include domains I and II which are present in a wide variety of ATPases, and domains III through VI which are specific to helicases. Mutation analysis has shown these domains to be important for ATPase and/or helicase activity (reviewed by Gorbalenya and Koonin, 1993).

The middle portion of the CAF protein (amino acids 780-1344) shares dispersed sequence identity with the above mentioned *S. pombe* (C8A4.08C; 21% identity) and *C. elegans* (K12H4.8; 18% identity) sequences, but is not similar to other known proteins. However, CAF and these two related proteins contain a C-terminal domain with sequence similarity to each other (average of 31% identity), to several bacterial and yeast RNAse III proteins (Nashimoto and Uchida, 1985; Elela et al., 1996; Iino et al., 1991; Xu et al., 1990) and to predicted proteins from a number of species, including *A. thaliana*, *S. pombe* and *C. elegans* (Fig. 8C). CAF, C8A4.08C, K12H4.8 and another predicted protein from *C. elegans* (RNC_CAEEL) each contain adjacent duplicated domains with similarity to the RNaseIII catalytic domain (Fig. 8A, C). These sequences contain two absolutely conserved residues which are the sites of inactivating point mutations in *E. coli* RNase III (Court, 1993). At the extreme C terminus, these proteins contain domains similar to double stranded RNA binding domains (dsRNAbds) found in RNaseIII and in a variety of additional RNA binding proteins (Fig. 8D; Kharrat et al., 1995). CAF contains a duplication of this dsRNAbd, which is not found in any of the other known RNaseIII-like proteins (Fig. 8A,D). The most C-terminal of these putative dsRNAbds is disrupted by the insertion of the T-DNA in the *caf* mutant, indicating that this sequence is necessary for wild-type CAF function.

CAF RNA expression

To test whether *CAF* is a single copy gene in *Arabidopsis*, or is a member of a family of closely related genes, we performed DNA blot analysis with probes from both the helicase and RNaseIII domains. Under high stringency conditions, both

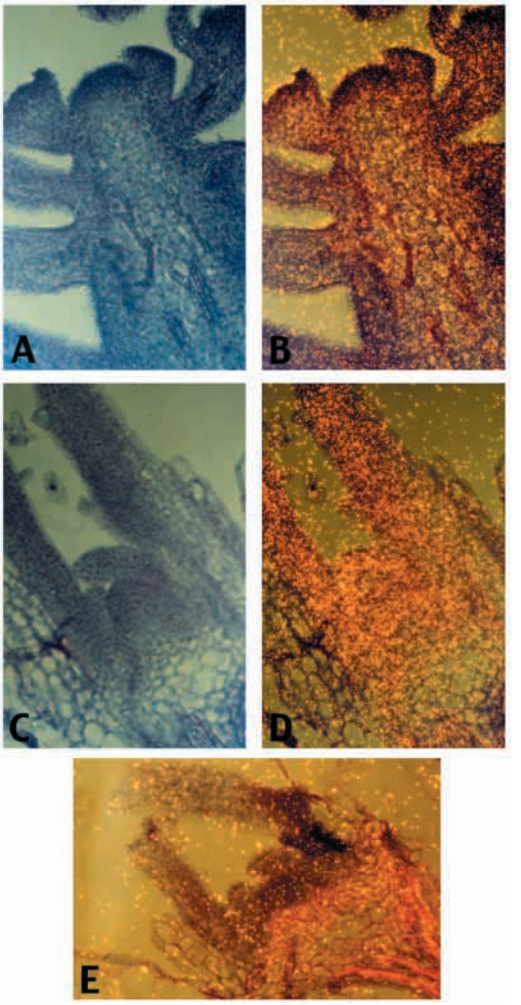


Fig. 10. *CAF* RNA expression pattern. Sections of inflorescences or seedlings were hybridized with either a *CAF* antisense probe (A-D) or a control *CAF* sense probe (E), and exposed for 30 days. (A) Bright-field photograph of a longitudinal section of an inflorescence meristem bearing flowers on its flanks. (B) Dark-field/bright-field double exposure of the same section showing the expression pattern of *CAF*. Yellow spots represent silver grains exposed by the ^{35}S-labeled *CAF* probe. (C) Bright-field photograph of a longitudinal section of a 7.5-day-old seedling showing the apical meristem and developing leaves on its flanks. (D) Dark-field/bright-field double exposure of the same section. (E) Dark-field/bright-field double exposure of a 7.5-day-old seedling showing the background level of signal from the sense probe. All photographs were taken at the same magnification.

probes detected only the restriction fragments predicted by the *CAF* genomic sequence (see Materials and Methods). These results show that *CAF* is single copy in the *Arabidopsis* genome, and that under these conditions, the probes used are specific for the *CAF* gene.

To analyze the expression pattern of *CAF*, RNA blot analysis was performed with probes to both the helicase and RNaseIII domains. The helicase probe detected an RNA of approximately 6.2 kb, consistent with predictions from the cDNA and genomic sequences (Fig. 9A). However, the RNaseIII domain detected two RNA species, a 6.2 kb RNA and an additional 2.5 kb RNA (Fig. 9B). Thus *CAF* produces two predominant RNA species, one apparently encoding the full 1909 amino acid protein containing both the helicase and RNaseIII like domains, and one containing only the RNaseIII domain. Neither the 6.2 kb nor the 2.5 kb RNA species were detected in RNA extracted from the *caf* mutant. Instead, two larger transcripts of approximately 4.8 kb and 8.7 kb were present (Fig. 9A,B), presumably because transcription of the mutant *caf* gene extends into the T-DNA and adds approximately 2.4 kb to the size of each of the two major RNA species. The abundance of these aberrant *caf* mutant RNAs is similar to that of the wild-type *CAF* RNAs.

Results from RNA blot and RT-PCR experiments indicate that these two transcripts are present in vegetative tissue (leaves and stems) from 2-week old plants, and present in inflorescence tips from 4-week old plants (data not shown), suggesting that *CAF* RNA may be expressed ubiquitously throughout the shoot. To confirm these results and to test whether *CAF* could be expressed in a cell layer-specific manner, in situ hybridization experiments were performed (Fig. 10). A low level of *CAF* RNA was found in all cells of the apical and floral meristems, and in the flowers, cauline leaves and stems. Thus *CAF* RNA is expressed evenly throughout most shoot tissues. This expression pattern is consistent with the *caf* mutant phenotype, in that most shoot tissues show some defect in the mutant.

DISCUSSION

The *caf* mutant was initially chosen for study because of its dramatic defect in floral meristem determinacy. *caf* mutants exhibit over-proliferation of the floral meristem, such that flowers contain excess numbers of stamens and carpels. The *caf* mutant phenotype is distinct from that of the *ag* mutants, which also show reduced floral determinacy. Whereas *ag* mutations cause a reiteration of the floral program so that flowers show a repeating pattern of sepals and petals, *caf* flowers show unregulated cell division in the center of the flower, but show normal floral organ identity. *caf* mutants are also defective in other aspects of plant development. *caf* mutant plants lack nearly all of the axillary inflorescence meristems normally found in the wild type, and possess abnormally shaped cotyledons, leaves, sepals, stamens and carpels. Thus the wild-type *CAF* gene plays a role in specifying the determinate growth of the floral meristem, but also functions in the specification of axillary meristems and in the morphogenesis of organs.

It seems possible that the morphogenesis defects seen in the *caf* mutant could be explained as a secondary consequence of the defect in *caf* apical and floral meristem structure, in which case, one might expect that *CAF* activity could be localized to the meristems. However, we find that *CAF* RNA is evenly expressed throughout most shoot tissues, including apical and floral meristems, floral organs, stems and leaves. *CAF* is expressed in actively dividing cells at roughly the same level as in differentiated cells. Thus it seems more likely that *CAF* may act in flowers to pattern meristem determinacy, and act separately in other tissues to promote the proper development of axillary meristems, leaves and floral organs. However, it is still possible that the different floral and vegetative abnormalities found in *caf* are mechanistically related. For instance, one could interpret many of the vegetative and floral phenotypes as being losses in cell division control, with too much cell division giving rise to the extra carpels and too little cell division contributing to a lack axillary meristems and the reduced size of leaves, flowers and anthers.

The mutant allele of *caf* that we have studied, which is the only mutant allele known, seems unlikely to be a complete loss-of-function mutation. First, northern blot experiments show that the *CAF* RNA is expressed at a normal level in the *caf* mutant. Second, the T-DNA insertion in *caf* is predicted to prematurely truncate the *CAF* protein at amino acid number 1836, replacing the last 73 amino acids of CAF with eight amino acids from the T-DNA. This would leave 96% of the CAF protein intact in the mutant. Third, in several mutant screens carried out in our laboratory for mutations affecting floral structure, we have failed to find additional *caf* alleles. Thus, it is possible that stronger alleles of *caf* might have a lethal phenotype, or have a phenotype that would not have been detected in our screens. Given that *CAF* homologues exist in fungi and animals, it seems likely that *CAF* may carry out a conserved cell biological function (since plants and animals arrived independently at multicellularity), and therefore that *CAF* could be an essential gene, strong mutations in which might lead to a lethal phenotype. Thus the *caf* mutation described here may be a weak allele which uncovers *CAF*′s role in the regulation of cell division in floral meristems.

The genetic interactions of *caf* with other floral mutations support a role for *CAF* in controlling floral meristem proliferation. The double mutant phenotypes of *caf* combined with the floral homeotic mutants *ap2-1*, *pi-1*, and *ag-1* are roughly additive. However, *caf* shows a synergistic interaction in combinations with two other mutations, *sup-1* and *clv3-2*, which increase cell proliferation in floral meristems, but which do not seem to directly control organ identity. Epistasis was not found in the double mutant combinations of *caf* with *sup* or *clv3* mutations, suggesting that *CAF* may, at least in part, control meristem activity through a mechanism somewhat different than that of *SUP* or *CLV3*.

The *caf* mutant phenotype resembles that of the *sup* mutants, in two ways. First both types of mutations cause an increase in the number of stamens and carpels (Gaiser et al., 1995; Jacobsen and Meyerowitz, 1997). Second, in an *ag* mutant background, both mutations cause a conversion of the inner whorl sepals to petals. The *caf* and *sup* mutations differ greatly however in their effects outside of the flower. Whereas the *caf* mutation affects the morphogenesis of most of the organs of the shoot, the *sup* mutations have no detectable effect on the development of non-floral tissues.

The N terminus of the predicted CAF protein is similar to DEAD/DExH type helicase/ATPase proteins from a number of organisms. CAF and these related proteins (all of unknown

function) form a new family of related sequences within the helicase superfamily II (Gorbalenya et al., 1989; Gorbalenya and Koonin, 1993). eiF-4A is the founding member of the DEAD box RNA helicase family and is thought to function in unwinding mRNA during translational initiation (Nielsen et al., 1985; Rozen et al., 1990). VASA is a *Drosophila* DEAD protein which is required for the formation of posterior pole plasm (Lasko and Ashburner, 1988; Hay et al., 1988). It acts as a positive regulator of *oskar* RNA translation (Webster et al., 1997 and references therein), and is required for proper localization and translation of *nanos* RNA (Gavis et al., 1996). Both eif-4A (in combination with another factor, eif-4B) and VASA have been shown to possess ATP-dependent RNA helicase activity in vitro (Rozen et al., 1990; Liang, et al., 1994). Other helicase-related proteins are known to be involved in mRNA splicing (reviewed by Kramer, 1996). For instance, PRP2, a DEAH type protein, is thought to activate the precatalytic spliceosome (Kim and Lin, 1996). RAD3 is a DEAH family helicase-like protein, which acts in DNA repair and has DNA-dependent ATPase and DNA helicase activity (Harosh and Deschavanne, 1991). Another DEAD protein appears to regulate entry into mitosis in *Schizosaccharomyces pombe* (Warbrick and Glover, 1994). Other functions ascribed to helicase proteins include rRNA processing (Kressler et al., 1997), mRNA stability (Iost and Dreyfus, 1994), and mRNA degradation (Anderson and Parker, 1996). Given the diverse functions of helicase proteins, it is difficult to predict a specific function for the helicase domain of CAF, however, it is likely that this domain participates in nucleic acid dependent ATPase activity and may act to unwind RNA or DNA.

The C-terminal portion of CAF and the related *S. pombe* (C8A4.08C) and *C. elegans* (K12H4.8) proteins contain homology to RNaseIII proteins. Relative to the RNaseIII proteins from bacteria and yeast, these proteins have a duplication of the putative RNase catalytic domain, followed by either one or two double stranded RNA binding domains (dsRNAbds). RNaseIII proteins cleave specific regions of RNA that form base-paired stem-loop structures (reviewed by Court, 1993). Though RNaseIII cleaves only particular RNAs, the sequence requirements for this specific cleavage are unknown. In *E. coli*, RNase III is involved in the processing of ribosomal precursor RNAs and certain mRNA molecules (Court, 1993). In *S. cerevisiae*, RNaseIII proteins are known to process rRNAs and small nuclear RNAs (Elela et al., 1996; Chanfreau et al., 1997; Rotondo et al., 1995). Unlike other known RNaseIII proteins, including the homologous *C. elegans* and *S. pombe* proteins, CAF contains a second putative dsRNAbd. This putative dsRNAbd is essential for wild-type CAF function since disruption of this sequence by the insertion of the T-DNA results in the *caf* mutant phenotype. The altered structure of CAF relative to RNaseIII, and the fact that the other RNaseIII homologues known to be present in *A. thaliana* do not contain helicase domains, suggest that CAF may have a different function than the bacterial and yeast RNaseIII proteins.

Given its homology to both RNA helicase proteins and RNaseIII proteins, it seems likely that CAF acts as an RNA processing enzyme. Extrapolating from the known functions of RNA helicase proteins and RNaseIII proteins, and considering that CAF is predicted to reside in the nucleus, CAF could act in processes such as mRNA stability, mRNA splicing, rRNA 3′ end maturation, or snRNA processing. The helicase and RNaseIII domains may act together to process RNAs, with the helicase possibly acting to unwind or facilitate the recognition of RNAs which are then cleaved by RNaseIII. Indeed, precedent for the association of helicase and RNAse proteins already exists; the DEAD box protein Rh1B exists in a complex (the degradosome) with the endonuclease RNase E and the 3′ to 5′ exonuclease polynucleotide phosphorylase (reviewed by Anderson and Parker, 1996). Since CAF-like proteins are found in the genomes of *C. elegans* and *S. pombe*, this suggests that CAF may carry out a similar function in all three kingdoms.

It is not clear how the 2.5 kb and 6 kb transcripts of *CAF* are derived. One possibility is alternative splicing. However, in our PCR-RACE experiments we did not detect chimeric transcripts containing upstream exons, as might be expected if exon skipping were occurring. A second possibility is that the full length RNA is cleaved, and that only the 3′ cleavage product stably accumulates. Indeed it is tempting to think that the CAF protein itself could participate in such a cleavage reaction. However, analysis of the RNA structure in the *caf* mutant suggests that the *CAF* RNA processing still occurs, since both a large and a small transcript accumulate (Fig. 9). Thus, if CAF does process its own transcript, the second dsRNAbd probably does not play a role in this process, as this is absent in the *caf* mutant. A third alternative is that the *CAF* gene contains two promoters, one that initiates at the beginning of the gene and another that initiates in the middle. This would be consistent with the observation that smaller transcripts with homology to the 5′ helicase domain are not detected.

Why does mutation of the putative CAF RNA processing enzyme result in the observed loss of control of cell division seen in *caf* mutant floral meristems? One possibility is that CAF is an mRNA processing enzyme, and that the *caf* mutant phenotype results from the misregulation of one or more specific mRNAs, at least one of which plays a major role in the control of floral meristem determinacy. CAF would likely affect the processing of additional RNAs as well, which are important in other processes throughout the plant, such as those needed for the proper specification of the normal number of pollen sacs in an anther, for the specification of the axillary inflorescence meristems, and for the proper shape of most organs of the shoot.

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REFERENCES

Alvarez, J. and Smyth, D. R. (1994). Flower development in *clavata3*, a mutation that produces enlarged floral meristems. In: *Arabidopsis: An Atlas of Morphology and Development.* (ed. J. Bowman), pp. 254-257. New York: Springer-Verlag.

Anderson, J. S., Parker, R. (1996) RNA turnover: The helicase story unwinds. *Curr. Biol.* **6**, 780-782.

Barton, M. K. and Poethig, R. S. (1993). Formation of the shoot apical meristem in *Arabidopsis thaliana*: an analysis of development in the wild-type and in the *SHOOT MERISTEMLESS* mutant. *Development* **119**, 823-831.

Bechtold, N., Ellis, J. and Pelletier, G. (1993) In planta Agrobacterium-mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. C. R. Acad. Sci. Paris, *Sciences de la vie -Life Sciences* **316**, 1194-1199.

Bowman, J. L., Alvarez, J., Weigel, D., Meyerowitz, E. M. and Smyth, D.

R. (1993). Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. *Development* **119**, 721-743.

Bowman, J. L., Sakai, H., Jack, T., Weigel, D., Mayer, U. and Meyerowitz, E. M. (1992). *SUPERMAN*, a regulator of floral homeotic genes in *Arabidopsis*. *Development* **114**, 599-615.

Bowman, J. L., Smyth, D. R. and Meyerowitz, E. M. (1989). Genes directing flower development in Arabidopsis. *Plant Cell* **1**, 37-52.

Bowman, J. L., Smyth, D. R. and Meyerowitz, E. M. (1991). Genetic interactions among floral homeotic genes of *Arabidopsis*. *Development* **112**, 1-20.

Chanfreau, G., Elela, S. A., Ares, M. and Guthrie, C. (1997). Alternative 3′-end processing of U5 snRNA by RNase III. *Genes Dev.* **11**, 2741-2751.

Chang, C., Bowman, J. L., DeJohn, A. W., Lander, E. and Meyerowitz, E. M. (1988). Restriction fragment length polymorphism linkage map for *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **85**, 6856.

Clark, S. E., Running, M. P. and Meyerowitz, E. M. (1993). *CLAVATA1*, a regulator of meristem and flower development in *Arabidopsis*. *Development* **119**, 397-418.

Clark, S. E., Running, M. P. and Meyerowitz, E. M. (1995). *CLAVATA3* is a specific regulator of shoot and floral meristem development affecting the same processes as *CLAVATA1*. *Development* **121**, 2057-2067.

Clark, S. E., Williams, R. W. and Meyerowitz, E. M. (1997). the *CLAVATA1* gene encodes a putative receptor kinase that controls shoot and floral meristem size in Arabidopsis. *Cell* **89**, 575-585.

Court, D. (1993). RNA processing and degradation by RNaseIII. In *Control of messenger RNA Stability*. (ed. J. Belasco), pp. 71-116. Academic Press, Inc.

Drews, G. N., Bowman, J. L. and Meyerowitz, E. M. (1991). Negative regulation of the Arabidopsis homeotic gene *AGAMOUS* by the *APETALA2* product. *Cell* **65**, 991-1002.

Elela, S. A., Igel, H. and Ares, M. (1996). RNaseIII cleaves eukaryotic preribosomal RNBA at a U3 snoRNP-dependent site. *Cell* **85**, 115-124.

Feldmann, K. A. (1992). T-DNA insertion mutagenesis in Arabidopsis: Seed infection/transformation. In: *Methods in Arabidopsis Research* (ed. C. Koncz, N.-H. Chua, and J. Schell), pp. 274-289. Singapore: World Scientific.

Fletcher, J. C., Brand, U., Running, M. P., Simon, R. and Meyerowitz, E. M. (1999). Signaling of cell fate decisions by CLAVATA3 in Arabidopsis shoot meristems. *Science* **283**, 1911-1914.

Gaiser J., C., Robinson-Beers K. and Gasser C. S. (1995). The Arabidopsis *SUPERMAN* gene mediates asymmetric growth of the outer integument of ovules. *Plant Cell* **7**, 333-345.

Gavis, E. R., Lunsford, L., Bergsten, S. E. and Lehmann, R. (1996). A conserved 90 nucleotide element mediates translational repression of *nanos* RNA. *Development* **122**, 2791-2800.

Gorbalenya, A. E. and Koonin, E. V. (1993). Helicases: amino acid sequence comparisons and structure-function relationships. *Curr. Opin. Struct. Biol.* **3**, 419-429.

Gorbalenya, A. E., Koonin, E. V., Donchenko, A. P. and Blinov, V. M. (1989). Two related superfamilies of putative helicses involved in replication, recombination, repair and expression of DNA and RNA genomes. *Nucl. Acids Res*. **17**, 4713-4730.

Harosh, I. and Deschavanne, P. (1991). The RAD3 gene is a member of the DEAH family RNA helicase-like protein. *Nucl. Acids Res.* **19**, 6331.

Hay, B., Jan, L. Y. and Jan, Y. N. (1988). A Protein Component of *Drosophila* polar granules is encoded by *vasa* and has extensive sequence similarity to ATP-dependent helicases. *Cell* **55**, 577-587.

Huala, E. and Sussex, I. M. (1992). *LEAFY* interacts with floral homeotic genes to regulate *Arabidopsis* floral development. *Plant Cell* **4**, 901-913.

Iino, Y., Sugimoto, A. and Yamamoto, M. (1991). S. pombe pac1+, whose overexpression inhibits sexual development, encodes a ribonucease III-like RNase. *EMBO J.* **10**, 221-226.

Iost, I., Dreyfus, M. (1994). mRNAs can be stabilized by DEAD-box proteins. *Nature* **372,** 193-196.

Jacobsen, S. E. and Meyerowitz, E. M. (1997). Hypermethylated *SUPERMAN* epigenetic alleles in Arabidopsis. *Science* **277**, 1100-1103.

Kayes, J. M. and Clark, S. E. (1998). *CLAVATA2*, a regulator of meristem and organ development in *Arabidopsis*. *Development* **125**, 3843-3851.

Kharrat, A., Macias, M. J., Gibson, T. J., Nilges, M. and Pastore, A. (1995). Structure of the dsRNA binding domain of *E. coli* RNase III. *EMBO J*. **14**, 3572-3584.

Kim, S. H. and Lin, R. J. (1996). Spliceosome activation by PRP2 ATPase prior to the first transesterification reaction of Pre-mRNA Splicing. *Mol. Cell Biol.* **16**, 6810-6819.

Kramer, A. (1996) The Structure and function of proteins involved in mammalian pre-mRNA splicing. *Annu. Rev. Biochem.* **65**, 367-409.

Kressler, D., De La Cruz, J., Rojo, M. and Linder, P. (1997) Fal1p is an essential DEAD-Box protein involved in 40S-ribosomal-subunit biogenesis in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **17**, 7283-7294.

Lasko, P. F., Ashburner, M. (1988) The product of the *Drosophila* gene *vasa* is very similar to eukaryotic initiation factor-4A. *Nature* **335**, 611-617.

Laux, T., Meyer, K. F. X., Berger, K. and Jurgens, G. (1996). The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. *Development* **122**, 87-96.

Liang, L., Diehl-Jones, W. and Lasko, P. (1994) Localization of vasa protein to the *Drosophila* pole plasm is independent of its RNA-binding and helicase activities. *Development* **120**, 1201-1211.

Long, J. A., Moan, E. I., Medford, J. I. and Barton, M. K. (1996). A member of the *KNOTTED* class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis*. *Nature* **379**, 66-69.

Mandel, A. M., Gustafson-Brown, C., Savidge, B. and Yanofsky, M. F. (1992). Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. *Nature* **360**, 273-277.

Mayer, K. F. X., Schoof, H., Haecker, A., Lenhard, M., Jurgens, G. and Laux, T. (1998). Role of WUSCHEL in regulating stem cell fate in the Arabidopsis shoot meristem. *Cell* **95**, 805-815.

Nakai, K. and Kanehisa, M. (1992). A knowledge base for predicting protein localization sites in eukaryotic cells. *Genomics* **14**, 897-911.

Nashimoto, H. and Uchida, H. (1985). DNA sequencing of the *Escherichia coli* ribonuclease III gene and its mutations. *Mol. Gen. Genet*. **201**, 25-29.

Nielsen, P. J., McMaster, G. K. and Trachsel, H. (1985). Cloning of eukaryotic protein synthesis initiation factor genes: isolation and characterization of cDNA clones encoding factor eIF-4A. *Nucl. Acids Res*. **13**, 6867-6880.

Olszewski, N. E., Martin, F. B. and Ausubel, F. M. (1988). Specialized binary vector for plant transformation-expression of the *Arabidopsis thaliana* AHAS gene in *Nicotiana tabacum*. *Nucl. Acids Res.* **16**, 10756-10782.

Parcy, F., Nilsson, O., Busch, M. A., Lee, I. and Weigel, D. (1998). A genetic framework for floral patterning. *Nature* **395**, 561-566.

Rotondo, G., Gillespie, M. and Frendewey, D. (1995). Rescue of the fission yeast snRNA synthesis mutant *snm1* by overexpression of the double-strand-specific Pac1 ribonuclease. *Mol. Gen. Genet.* **247**, 698-708.

Rozen, F., Edery, I., Meerovitch, K., Dever, T. E., Merrick, W. C. and Sonenberg, N. (1990). Bidirectional RNA helicase activity of eucaryotic translation initiation factors 4A and 4F. *Mol. Cell Biol.* **10**, 1134-1144.

Running, M. P., Clark, S. E. and Meyerowitz E. M. (1995). Confocal microscopy of the shoot apex. In *Methods in Cell Biology: Plant Cell Biology*, Vol. 49, (ed. D. W. Galbraith, D. P. Burque and H. J. Bohnert), pp. 215-227. Academic Press: San Diego.

Sakai, H., Medrano, L. J. and Meyerowitz, E. M. (1995). Role of *SUPERMAN* in maintaining *Arabidopsis* floral whorl boundaries. *Nature* **378**, 199-203.

Schultz, E. A., Pickett, F. B. and Haughn, G. W. (1991). The FLO10 gene product regulates the expression domain of homeotic genes AP3 and PI in *Arabidopsis flowers*. *Plant Cell* **3**, 1221-1237.

Schulz, B., Bennett, M. J., Dilkes, B. P. and Feldmann, K. A. (1995). T-DNA tagging in Arabidopsis thaliana: Cloning by gene disruption. In *Plant. Mol. Biol. Manual K3*, pp. 1-17. Kluwer Academic Publishers, Dordrecht, Netherlands.

Smyth, D. R., Bowman, J. L. and Meyerowitz, E. M. (1990). Early flower development in *Arabidopsis*. *Plant Cell* **2**, 755-767.

Steeves, T. A. and Sussex, I. M. (1991). *Patterns in Plant Development.* Cambridge, U.K.: Cambridge University Press.

Warbrick, E. and Glover, D. (1994). A *Drosophila* gene encoding a DEAD box RNA helicase can suppress loss of *wee1/mik1* function in *Schizosaccharomyces pombe. Mol Gen. Genet.* **245**, 654-657.

Webster, P. J., Liang, L., Berg, C. A., Lasko, P. and Macdonald, P. M. (1997). Translational repressor *bruno* plays multiple roles in development and is widely conserved. *Genes Dev.* **11**, 2510-2521.

Weigel, D., Alvarez, J., Smyth, D. R., Yanofsky, M. F. and Meyerowitz, E. M. (1992). *LEAFY* controls floral meristem identity in Arabidopsis. *Cell* **69**, 843-859.

Xu, H. P., Riggs, M., Rodgers, L. and Wigler, M. (1990). A gene from *S. pombe* with homology to E. coli RNase III blocks conjugation and sporulation when overexpressed in wild-type cells. *Nucl. Acids Res.* **18**, 5304.

Yanofsky, M. F., Ma., H., Bowman, J. L., Drews, G. N., Feldmann, K. A. and Meyerowitz, E. M. (1990). The protein encoded by the *Arabidopsis* homeotic gene *AGAMOUS* resembles transcription factors. *Nature* **346**, 35-39.