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Chromatin assembly factor CAF-1 is required for cellular differentiation during plant development

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Chromatin assembly factor CAF-1 facilitates the formation of nucleosomes on newly replicated DNA in vitro. However, the role of CAF-1 in development is poorly understood because mutants are not available in most multicellular model organisms. Biochemical evidence suggests that FASCIATA1, FASCIATA2 and MSI1 form CAF-1 in *Arabidopsis thaliana*. Because *fasciata* mutants are viable, CAF-1 is not essential for cell division in plants. *Arabidopsis* CAF-1 mutants have defects in shoot apical meristems; in addition, CAF-1 is required to establish seedling architecture, leaf size and trichome differentiation. CAF-1 is needed to restrict branching of trichomes on rosette leaves. Increased trichome branching in CAF-1 mutants is not strictly correlated with increased nuclear DNA content. In addition, *fas2 glabra3* double mutants show an additive genetic interaction, demonstrating that CAF-1 acts genetically parallel to the *GLABRA3*-containing, endoreduplication-coupled trichome branching pathway. However, CAF-1 is often needed to restrict endoreduplication, because seedlings of most CAF-1 mutants have increased ploidy. Notably, in the Landsberg *erecta* background, loss of CAF-1 does not affect ploidy, demonstrating that loss of CAF-1 can be compensated in some *Arabidopsis* accessions. These results reveal that the functions of FAS1, FAS2 and MSI1 are not restricted to meristems, but are also needed to control genome replication at multiple steps of development.

KEY WORDS: *Arabidopsis*, CAF-1, Chromatin, Endoreduplication, MSI1, Shoot apical meristem, Trichome

INTRODUCTION

During the cell cycle, nuclear DNA must be fully replicated and equally distributed to the daughter cells. The genetic information encoded in the DNA does not only consist of the primary nucleotide sequence along the chromosomes, but also has an important epigenetic component that is established by the chromatin state(s) across the regulated loci. Biochemical and genetic studies have identified many proteins that are involved in the establishment or maintenance of chromatin. One of the well-studied protein complexes involved in chromatin metabolism is chromatin assembly factor CAF-1 (for a review, see Krude and Keller, 2001; Mello and Almouzni, 2001; Loyola and Almouzni, 2004). This heterotrimeric complex is found in yeast, animals and plants, and facilitates nucleosome assembly on newly replicated DNA (Smith and Stillman, 1989; Bulger et al., 1995; Verreault et al., 1996; Kaufman et al., 1997; Kaya et al., 2001). Human CAF1 binds newly synthesized H3-H4 histone dimers and, because its largest subunit (p150) physically interacts with the proliferating cell nuclear antigen PCNA, these histones are targeted to regions of DNA synthesis during replication or repair (Gaillard et al., 1996; Verreault et al., 1996; Shibahara and Stillman, 1999; Moggs et al., 2000; Zhang et al., 2000; Krawitz et al., 2002). Importantly, all three CAF-1 subunits physically interact with other proteins as well: the smallest subunit, p48, is found in multiple complexes affecting chromatin dynamics (for a review, see Hennig et al., 2005); the second largest subunit, p60, interacts with the histone chaperone anti-silencing factor 1 (Mello et al., 2002); and p150 interacts with heterochromatin protein 1, methyl-binding protein 1 and the Bloom's syndrome DNA-helicase BLM (Murzina et al., 1999; Reese et al., 2003; Jiao et al., 2004; Sarraf and Stancheva, 2004). However, it is unclear whether CAF-1 formation and the other interactions described above occur simultaneously, or whether they are exclusive, indicating CAF-1-independent functions of the subunits.

Yeast mutants deficient in CAF-1 subunits are viable but show enhanced sensitivity to UV light (Kaufman et al., 1997; Game et al., 1999). In addition, maintenance of silencing at mating type loci and near the telomeres is impaired (Enomoto et al., 1997; Kaufman et al., 1997; Monson et al., 1997; Enomoto and Berman, 1998; Smith et al., 1999; Taddei et al., 1999). Unlike in yeast, much less is known about the role of CAF-1 in the life cycle of multicellular organisms, mainly because mutants are not readily available. Transgenic approaches were therefore used to analyze CAF-1 function during the development of model organisms. For example, expression of a truncated version of human p150 interfered with the maintenance of transcriptional gene silencing in mammalian cells (Tchenio et al., 2001), and expression of a truncated version of p150 in *Xenopus* caused severe defects in embryo development, suggesting that CAF-1 is essential in vertebrates (Quivy et al., 2001). This hypothesis was strongly supported by RNAi knock-down experiments of p150 in human cell lines. Such cells showed delayed DNA replication and accumulated in S-phase (Hoek and Stillman, 2003). Similarly, silencing of p60 expression by RNAi caused apoptosis in dividing, but not in quiescent, human cells (Nabatiyan and Krude, 2004).

The model plant *Arabidopsis thaliana* is currently the only multicellular organism for which mutants in all three CAF-1 subunits are available. Reconstitution experiments showed that FASCIATA 1 (FAS1, a homolog of p150), FAS2 (a homolog of p60) and *Arabidopsis* MSI1 (a homolog of p48) form a functional CAF-1 complex in vitro (Kaya et al., 2001). Mutants deficient in FAS1 and FAS2 were originally identified because of their fasciated phenotype (Reinholz, 1966; Leyser and Furner, 1992). Fasciation describes a set of developmental abnormalities

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caused by defects of the shoot apical meristem (SAM) that includes altered phyllotaxis, broadening and bifurcation of the stem, and alterations in the number of floral organs (Worsdell, 1905). The SAM contains slowly dividing stem cells that give rise to all cells in above-ground, post-embryonic plant organs (Scheres et al., 2004). Proper organization of the SAM depends on several proteins, including the key regulator WUSCHEL (Laux et al., 1996). The fasciated phenotype of *fas1* and *fas2* is caused at least in part by loss of the restricted spatial expression pattern of *WUSCHEL* (Kaya et al., 2001). In addition to SAM defects, *fas1* and *fas2* have defects in the root apical meristem (RAM), which produces the cells needed for post-embryonic root growth (Scheres et al., 2004). This is because the restricted spatial expression patterns of the RAM key regulator *SCARECROW* are partially lost in the *fasciata* mutants (Kaya et al., 2001). Surprisingly, plants lacking both FAS1 and FAS2 subunits of CAF-1 are viable and fertile, but express some silenced transposons or transgenes (Takeda et al., 2004; Ono et al., 2006). By contrast, *msi1-1* null mutants are not viable and seeds abort early during embryo development (Köhler et al., 2003; Guitton et al., 2004). MSI1 co-suppression (*msi1-cs*) plants with strongly reduced protein levels (>90% reduction) are viable but show severe developmental defects, including homeotic transformation of floral organs and sterility (Hennig et al., 2003). Because such defects were not observed in the *fasciata* mutants, it was concluded that they are caused by the reduced function of other MSI1-containing complexes, such as the MEDEA (MEA) or CURLY LEAF Polycomb group complex (Hennig et al., 2003; Köhler et al., 2003; Hennig et al., 2005; Schönrock et al., 2006a).

Thus, CAF-1 is required for the execution of normal developmental programs in the apical meristems, but roles for CAF-1 in other tissues are much less understood. Here, we show that expression of *FAS1* and *FAS2* is high in actively dividing cells, and that several aspects of development, including seedling growth and leaf hair differentiation, are impaired in *Arabidopsis* mutants lacking CAF-1. Therefore, CAF-1 plays a more general role in plant development than has been previously appreciated.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of Columbia (Col), Landsberg *erecta* (L*er*) and Enkheim (En) *Arabidopsis thaliana* wild-type accessions, and of *fas1-1* (Reinholz, 1966; Kaya et al., 2001), *fas2-1* (Leyser and Furner, 1992; Kaya et al., 2001) and *gl3-1* (Koornneef et al., 1982; Payne et al., 2000) mutants were obtained from the Nottingham Arabidopsis Stock Centre. New *fas1* and *fas2* alleles were identified in T-DNA insertion mutant collections (Sessions et al., 2002; Alonso et al., 2003), and named *fas1-4* and *fas2-4* (see Figs S1, S2 in the supplementary material). Plants were kept in Conviron growth chambers with mixed cold fluorescent and incandescent light (110 to 140 μmol/m^{2}s, 21±2°C) under long-day (LD, 16 hour light) photoperiods, unless indicated otherwise. For characterization of seedling development, seeds were plated on four layers of water-soaked filter paper, which were placed into clear plastic boxes. A 48-hour dark treatment at 4°C was followed by induction of germination by white light for 10 hours and further incubation of seedlings in the dark at 23°C or under LD photoperiods.

Generation of transgenic plants with reduced MSI1 protein levels

In contrast to the viable *fas1-1* and *fas2-1* mutants, which have premature stop codons and are most likely null alleles (Kaya et al., 2001), loss of MSI1 is lethal (Köhler et al., 2003; Guitton et al., 2004). Therefore, we constructed transgenic lines in which the level of MSI1 was only moderately decreased by a *MSI1* antisense construct. A 532-bp *Cla*I/*Xba*I fragment of the *MSI1* coding sequence was fused in antisense orientation to the cauliflower mosaic virus (CaMV) 35S promoter in the binary vector pSLK7292 and transformed into Col wild-type plants. We selected the line 1ASb7 (*msi1-as*), which segregated a single transgene in a 1:3 ratio and had 30-50% of wild-type MSI1 protein levels (see Fig. S3A in the supplementary material), for further characterization, but the phenotypes described below were observed for several independent lines. Similar to *fas1-1* and *fas2-1*, but in contrast to *msi1-cs* and *msi1-1*, *msi1-as* plants were fertile and showed no seed abortion. Development of *msi1-as* plants was delayed, and plants had a reduced rosette size, a delayed flowering time and reduced growth of primary shoots, although more severe symptoms of fasciation, such as stem bifurcation, were not observed (see Fig. S3C in the supplementary material; data not shown). MSI1 shares only 25-52% identity with the other MSI1-like proteins in *Arabidopsis*, suggesting that expression of the *MSI1* antisense-RNA construct would not affect expression of *MSI2-5*; and semi-quantitative RT-PCR did not reveal any changes in *MSI2-5* transcript levels in *msi1-as* plants (see Fig. S3B in the supplementary material). In addition, we could not detect elevated transcript levels for *AGAMOUS* (*AG*) or *APETALA2* (*AP2*) in *msi1-as* leaves (see Fig. S3D in the supplementary material). *AG* and *AP2* are floral homeotic genes that are ectopically expressed in non-floral tissues of *msi1-cs* plants, but not in *fas1-1* or *fas2-1* mutants (Hennig et al., 2003).

Protein gel blot analysis

Protein extracts and protein gel blots using a specific anti-MSI1 antiserum were performed as described previously (Ach et al., 1997; Hennig et al., 2003).

RNA isolation and RT-PCR

RNA was extracted as previously described (Hennig et al., 2003). For RT-PCR analysis, 0.4-1.0 μg total RNA was treated with DNase I. The DNA-free RNA (0.2-1.0 μg) was reverse-transcribed using an oligodT primer and MMLV reverse transcriptase (Clonetech, Palo Alto, CA). Aliquots of the generated cDNA were used as template for PCR with gene-specific primers (Table 1).

Analysis of hypocotyl development

Dark-grown seedlings were mounted in water and observed with differential interference contrast (DIC) optics. Pictures were recorded with an AxioCam HRc CCD camera (Zeiss, Jena, Germany) and analyzed using ImageJ software, which is freely available at http://rsb.info.nih.gov/ij/index.html.

Ploidy analysis

Tissue was cut into small pieces in 400 μl nuclear extraction buffer (Partec, Münster, Germany), incubated for 30 minutes on ice, filtered through a 30 μm mesh, mixed with 1 ml nuclear staining buffer (Partec) and, after further

Table 1. Sequences of gene-specific primers used for RT-PCR

Gene	Forward primer	Reverse primer
MSI2	ATTGGCCACAGCTTCCTCAGA	TTGGAAGACCTCTCCCTCATGG
MSI3	TGCGCCATTACATGTCCTGA	TCCCCAACCCTGTTGATATCC
MSI4	ACGAAGGTTGAAAAAGCGCA	CGATCAAACAACCGGACAGTG
MSI5	GGACAAAGTGGTCGACTTCCA	CCGCCATATCTGCAATGTACC
AG	TCGGACAATTCTAACACCGGA	CCCATCAATTGCCTGTTGG
AP2	ATTTGGGTTTGTTCGACACC	ATGACTCGGCATTGAGTTCC
GAPDHα	ATGGCTTCGGTTACTTTCTCTGTC	TTCTTGGCACCAGCTTCAAT
FAS2	GCTCTAGAATTCATGAAGGGAGGTACGATACAG	GAGAGGCTACATACTTTGTTAAG
FAS1	CTTCCCATTCTTCATCACTATCAACTTC	TGTTCAGGCAATTGACAACGC

incubation on ice for 10 minutes, analyzed with a Partec Ploidy Analyzer. For quantification, the results of three to six independent preparations were averaged.

Ploidy of trichome nuclei was determined as described (Walker et al., 2000). Briefly, young rosette leaves were fixed in FAA (50% ethanol, 5% glacial acetic, 10% formaldehyde). Staining was performed for 1.5 hours with 130 μg/ml DAPI in McIlvaines buffer (60 mM citric acid, 80 mM sodium phosphate, pH 4.1). Samples were washed once for 15 minutes and once for 1 hour with McIlvaines buffer, and mounted in McIlvaines buffer with 50% glycerol. Fluorescence was recorded with a MagnaFire CCD camera (Optronics, Goleta, CA), or with an Apogee Alta U32 CCD camera (Apogee Instruments, Roseville, CA). Images were quantified using ImageJ. Total fluorescence of at least 30 representative nuclei per experiment was determined and calibrated using guard cell nuclei ($n \geqslant 30$), which are considered to be strictly diploid (Walker et al., 2000).

Analysis of leaf histology

Plants were grown until bolting before leaves were harvested. Leaf area was determined by scanning the leaves and measuring their size with ImageJ. Leaves were then fixed in ethanol:acetic acid (9:1) at room temperature for 2.5 hours, dehydrated in 90% then 70% ethanol for 1 hour each, and cleared in clearing solution [66.7% (w/v) chloral hydrate, 8.3% (w/v) glycerol in water] at 4°C overnight. Samples were mounted in clearing solution and investigated with DIC optics. Images were recorded with an AxioCam HRc CCD camera and analyzed with ImageJ.

RESULTS

Lack of CAF-1 causes defects in seedling development

The current model proposes that CAF-1 facilitates nucleosome assembly during S-phase of the cell cycle (Loyola and Almouzni, 2004). Accordingly, the expression of the two large CAF-1 subunits increases in proliferating *Arabidopsis* cells, and is cell cycle regulated in *Arabidopsis* and yeast (Kaya et al., 2001) (see Fig. S4 in the supplementary material). By contrast, expression of the small subunit MSI1 does not strongly correlate with cell proliferation or cell cycle. These expression patterns reflect the more widespread role of MSI1 in other chromatin complexes (Hennig et al., 2005). The expression data suggest that CAF-1 is required in dividing cells. However, to date, only defects in meristem function have been studied in detail for *fasciata* mutants (Reinholz, 1966; Leyser and Furner, 1992; Kaya et al., 2001). To test whether CAF-1 function is required in other organs as well, we characterized meristem-independent cell proliferation and differentiation in CAF-1 mutants. *fas1* and *fas2* alleles have been previously characterized in Landsberg *erecta*, Enkheim or Nossen (Reinholz, 1966; Leyser et al., 1992; Kaya et al., 2001). To test whether any defects observed are genuine traits of CAF-1 loss or are caused by the genetic background of the different accessions, we identified novel *fas1* and *fas2* alleles in Columbia – *fas1-4* and *fas2-4* (see Figs S1, S2 in the supplementary material). The *fas1-4* and *fas2-4* phenotypes are similar to the phenotypes of other *fasciata* mutants: the plants are smaller than wild type and develop fasciation.

Seedling organs such as hypocotyl and cotyledons are not formed by the apical meristems, but are formed during embryogenesis. Therefore, we analyzed seedlings of CAF-1 mutants in more detail. General seedling morphology was not severely affected in *fas1*, *fas2* or *msi1-as* seedlings grown in long-day photoperiods (Fig. 1A, see also Figs S1, S2 in the supplementary material), but *msi1-as* seedlings consistently showed cotyledon epinasty, i.e. a downward curling of the cotyledons. As this phenotype was not observed in *fas1* or *fas2* seedlings, but was sometimes apparent in *msi1-cs* lines (P. Taranto, PhD thesis, University of California, Berkeley, 1998), we conclude that it is not related to CAF-1 but rather to a different MSI1-function.

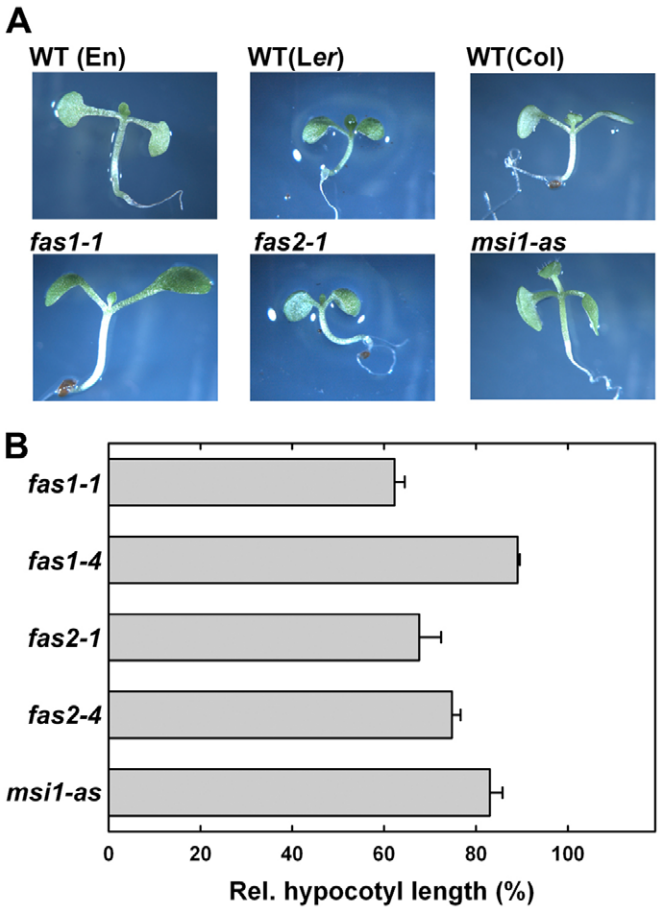

Fig. 1. Development of CAF-1 mutant seedlings. (**A**) Representative 9-day-old light-grown seedlings of CAF-1 mutants and their corresponding wild types. (**B**) After induction of germination by a 10-hour exposure to white light, seeds were kept in the dark at 23°C. Hypocotyl lengths of at least 20 seedlings were determined after 4 days and expressed relative to their corresponding wild types. Means±s.e.m. of at least three independent experiments are shown.

Dark-grown seedlings of all three CAF-1 mutants had shorter hypocotyls than did wild type (Fig. 1B); this phenotype was most pronounced for *fas1-1*, and weakest for *fas1-4* and *msi1-as*. Note that some *FAS1* transcript is made in the *fas1-4* insertion mutant (see Fig. S1 in the supplementary material), demonstrating that *fas1-1* but not *fas1-4* is a null allele. By contrast, both *fas2* alleles appear to be null (see Fig. S2 in the supplementary material). When seedlings were grown under continuous white light, all plants responded strongly with inhibition of hypocotyl elongation (data not shown). The short hypocotyls formed in the light precluded the detection of length differences between CAF-1 mutants and wild type.

Post-germination hypocotyl growth in the dark results mostly from cell elongation and only a few cell divisions occur (Gendreau et al., 1997). To determine whether the shorter hypocotyls in dark-grown CAF-1 mutant seedlings are caused by defects in cell division or elongation, we counted hypocotyl epidermal cell number and measured cell size (Fig. 2A,B). The results show that hypocotyl epidermal cells of both *fas2* alleles were significantly shorter than in the wild-type, whereas cell size was not, or only weakly, affected in *fas1-1*, *fas1-4* and *msi1-as*. These results suggest that the reduced size of hypocotyl epidermal cells is a genuine trait of *fas2* mutants and is not caused by the genetic background. Moreover, loss of

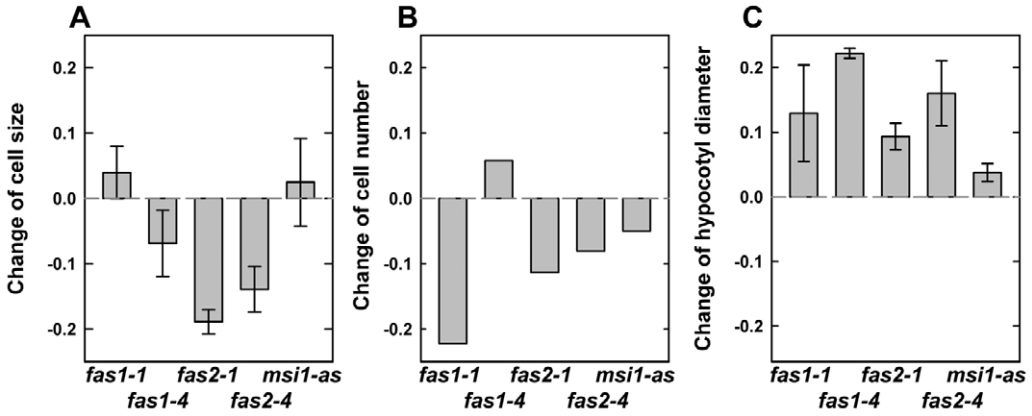

Fig. 2. Hypocotyl cell size, number and hypocotyl diameter in CAF-1 mutant seedlings. Seedlings of CAF-1 mutants and their corresponding wild types were grown as described for Fig. 1B, except that the seedlings were grown for 9 days. (**A**) The size of epidermal cells of the hypocotyl was measured for 10 seedlings of the mutants and wild types (about 300 cells per genotype). (**B**) The total number of epidermal cells along the hypocotyl axis was estimated from the hypocotyl length and the average cell size. (**C**) The diameters of hypocotyls of CAF-1 mutants and wild-type seedlings were determined. For A and C, the means±s.e.m. of the relative differences between mutant and wild type from three to five independent experiments are shown.

CAF-1 does not necessarily cause size reductions for hypocotyl epidermal cells. By contrast, seedlings lacking any of the three CAF-1 subunits had reduced cell numbers in their hypocotyls. This reduction was strongest in *fas1-1* and weakest in *msi1-as*. Only the weak *fas1-4* allele had slightly increased cell numbers. Interestingly, hypocotyls of *fas1*, *fas2* and *msi1-as* seedlings were significantly thicker than hypocotyls of wild-type plants (Fig. 2C). Notably, the increase in hypocotyl diameter was strongest in the weak *fas1-4* allele, whereas changes in cell size and cell number were smallest in this allele. In summary, CAF-1 subunits are therefore not only required to control cell proliferation in the SAM and RAM, but also for normal cell proliferation in hypocotyls.

Hypocotyl cells of *fas1-1* undergo increased DNA endoreduplication

Elongating hypocotyl cells endoreduplicate their DNA more in dark-grown than in light-grown seedlings (Gendreau et al., 1997), and cell elongation and endoreduplication are often tightly coupled. Because cell elongation is impaired in *fas2* hypocotyls, we measured DNA endoreduplication in dark- and light-grown CAF-1 mutant seedlings. However, rather than a decrease in endoreduplication, seedlings grown in long-day photoperiods or in the dark showed increased DNA endoreduplication (Fig. 3A,B): the fraction of 2C nuclei decreased from 30-40% to 20-30%, whereas the fraction of 8C and 16C nuclei increased. This shift in ploidy was strongest for *fas1-1*, *fas1-4* and *fas2-4*, but was less pronounced for *msi1-as*. We also measured ploidy directly in nuclei of *fas1-1* and wild-type hypocotyl cells, and found that the increased DNA content was caused, at least partially, by higher ploidy in the hypocotyl (data not shown). Interestingly, ploidy patterns in *fas2-1* did not differ from those of wild-type Landsberg *erecta*. Thus, CAF-1 deficiency does not generally affect DNA endoreduplication, and the reduced cell size in dark-grown *fas2-1* and *fas2-4* hypocotyls does not correlate with reduced endoreduplication.

Size of leaf epidermal cells is altered in CAF-1 mutants

The SAM produces leaf primordia, but the final leaf morphology is mostly independent of SAM function (Byrne, 2005; Fleming, 2005). The smaller rosette diameter and the serration of rosette leaves in *fasciata* mutants in the Col and En accessions (Reinholz, 1966; Leyser and Furner, 1992; Serrano-Cartagena et al., 1999; Kaya et al., 2001) suggested that cell proliferation and/or expansion was affected during leaf development. To test this hypothesis, the histology of rosette leaves was investigated. In addition to the *fas1-1* allele in En, we included the novel *fas1-4* allele in the Col background. The phenotype of *fas1-4* plants was similar to that of other *fasciata* mutants, including alterations in phyllotaxis and reduced shoot height (see Fig. S1 in the supplementary material). Furthermore, *fas1-4* rosette leaves were serrated, similar to the rosette leaves of *fas1-1* and *fas2-4* plants. Growth of rosette leaves occurs in two phases: outgrowth through cell division and enlargement of the blade through cell expansion (Beemster et al., 2005). Cross sections through the first or second rosette leaves of plants at bolting did not reveal any obvious differences in internal leaf histology between wild type and mutants (data not shown). By contrast, epidermal cells of these leaves were significantly larger in the CAF-1 mutants than in the wild type (Fig. 4A,B). This effect was much stronger in the *fas1-4* and *fas2-4* mutants (1.5 to 2-fold increase) than in the *msi1-as* plants (1.2-fold increase).

Next, we determined the number of leaf epidermal cells. Consistent with the observation that CAF-1 mutations interfere with regulation of cell division rates during hypocotyl development, the number of epidermal cells was reduced in rosette leaves of both *fas1-4* and *fas2-4* (Fig. 4C). By contrast, the number of epidermal cells was not significantly altered in *msi1-as* plants. Similar results were obtained when the third and fourth rosette leaves were analyzed (Fig. 4D,E). These results show that CAF-1 is required not only to control cell proliferation in the SAM, RAM and hypocotyls, but also for normal cell proliferation in expanding rosette leaves. In addition, these results suggest that the phenotype of *fasciata* mutants does not become more severe during plant development.

Trichome differentiation is altered in CAF-1 mutants

Trichomes are leaf hairs that originate from the leaf or stem epidermis. In *Arabidopsis*, each trichome consists of a single cell and therefore provides an ideal system with which to study cell differentiation. Lateral inhibition maintains a regular spacing of trichomes in wild-type leaves. After trichome fate commitment, cells

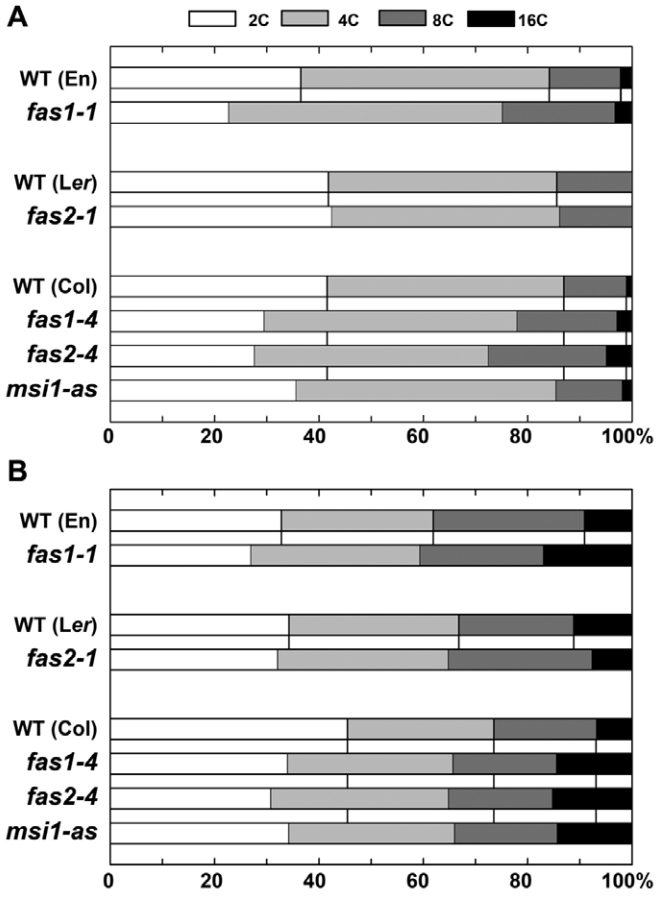

Fig. 3. Ploidy in CAF-1 mutant seedlings. Seedlings were grown as described for Fig. 2 or exposed to LD conditions for 9 days. (**A**) Ploidy was measured for seedlings grown under LD photoperiods. (**B**) Ploidy was measured for etiolated CAF-1 seedlings. For three to four independent preparations, areas of 2C (white), 4C (light gray), 8C (dark gray) and 16C peaks (black bars) were averaged. For all measurements, the s.d. was smaller than 4%.

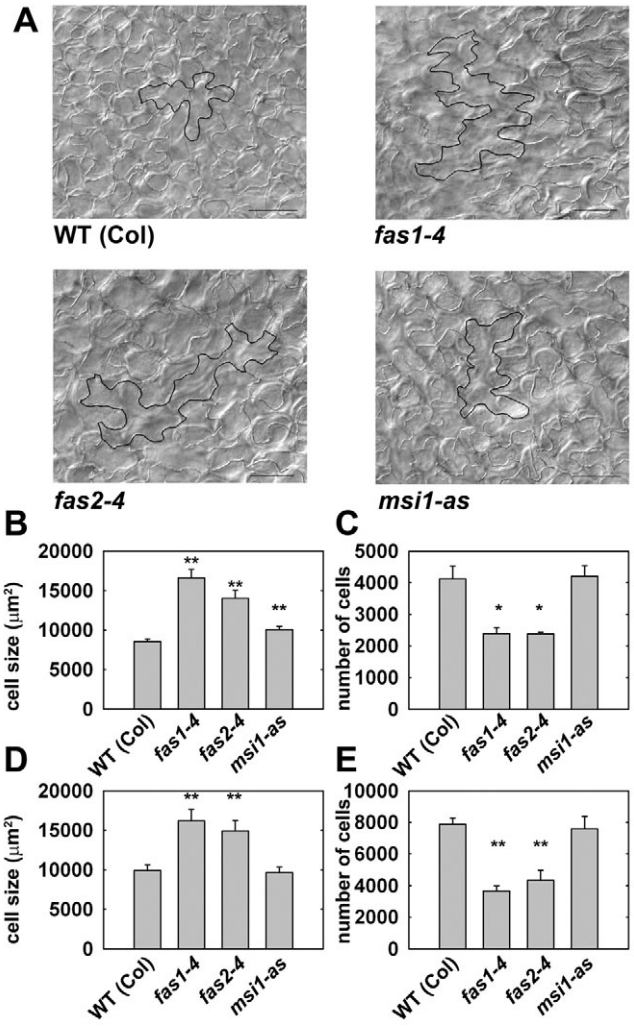

Fig. 4. Leaf development in CAF-1 mutants. (**A**) Jigsaw-like shape of epidermal cells in CAF-1 mutants. Scale bar: 100 μm. (**B**,**D**) Size of epidermal cells on the first and second (B), or third and fourth (D) rosette leaves of CAF-1 mutants. Shown are the means±s.e.m. for one representative experiment ($n \geq 63$). (**C**,**E**) Number of epidermal cells on rosette leaves. (C) Epidermal cell number of the first and second rosette leaf. Shown is the average (means±s.e.m.) of three (*msi1-as*: two) independent experiments. (E) Epidermal cell number of the third and fourth leaf. Shown are the means±s.e.m. of one experiment ($n \geq 7$). Asterisks indicate *P*-values (*t*-test) <0.05; double asterisks indicate *P*-values <0.005.

stop dividing but continue to synthesize DNA. The differentiating trichome cell extends out of the leaf surface, undergoes two branching events and finally elongates extensively (Schnittger and Hülskamp, 2002; Larkin et al., 2003). The majority of trichomes on *Arabidopsis* leaves have three branches (Fig. 5A). By contrast, CAF-1 mutants contain many trichomes that develop more than three branches (Fig. 5B,G). In general, only about 80% of trichomes in CAF-1 mutants have three branches, but up to 30% have four to six branches (Fig. 5G). Similar results were obtained when the third and fourth leaves were analyzed (data not shown), suggesting that this trichome phenotype is stable throughout development. The leaves of CAF-1 mutants did not contain clustered trichomes suggesting that regular trichome initiation does not require CAF-1.

In wild-type *Arabidopsis* plants, trichomes are single cells with only one nucleus (Larkin et al., 2003). Microscopic investigation of DAPI-stained trichomes revealed that trichomes of CAF-1 mutants are also single cells with one nucleus (Fig. 5C-F). During trichome differentiation, four rounds of DNA endoreduplication usually occur, which establish a relative DNA content of 32C instead of the usual 2C of diploid cells. Because increased trichome branching is often correlated with extra DNA endoreduplication, we measured the DNA content in trichomes of CAF-1 mutants (Fig. 5H). Only trichome nuclei of *fas1-1* and *fas1-4* consistently contained more DNA in all experiments, suggesting that many trichome cells undergo at least one extra round of DNA endoreduplication. Although *fas2-4* had a non-significant tendency for increased ploidy, this was not evident in all experiments, despite consistently increased trichome branching. Because *fas2-1*, *fas2-4* and *msi1-as* trichomes develop extra branches without significantly increased ploidy levels, we reasoned that CAF-1 controls trichome branching via a pathway that is independent of DNA endoreduplication.

FAS1, FAS2 and MSI1 act together in trichome development

Although the similar phenotypes of *fas1*, *fas2* and *msi1-as* plants strongly suggest that FAS1, FAS2 and MSI1 function in CAF-1 during trichome development, the different effects on ploidy make

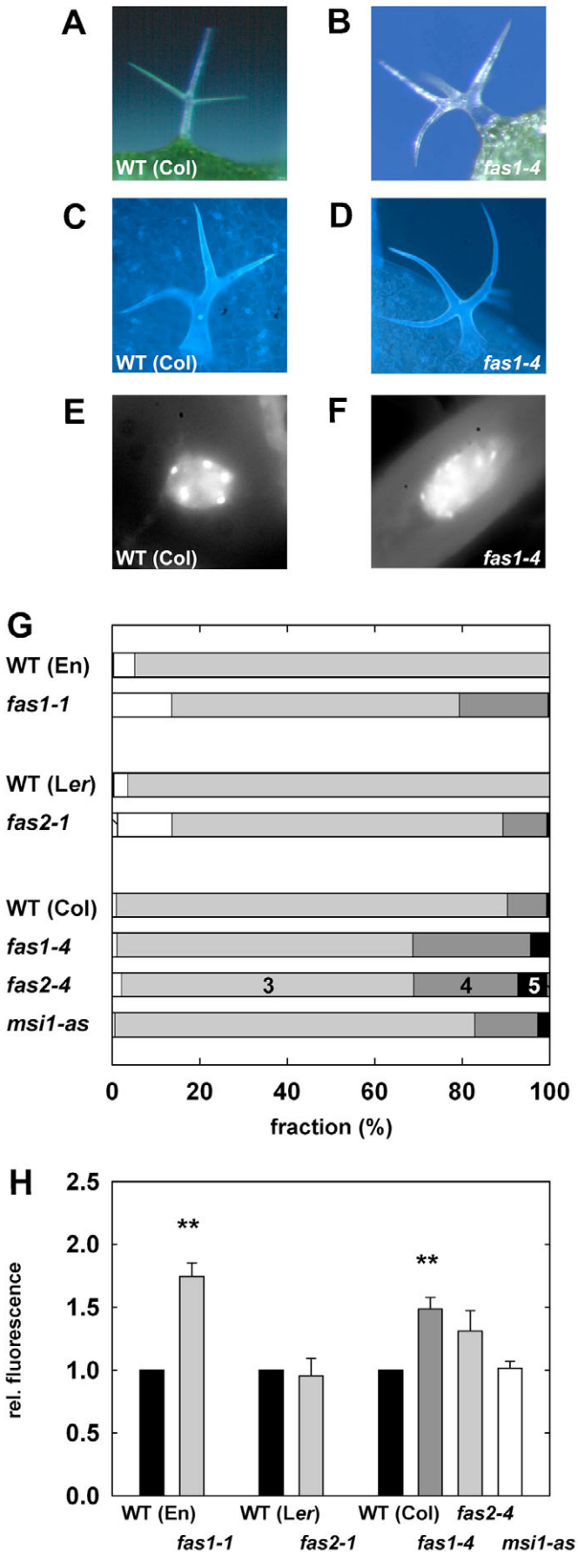

Fig. 5. Trichome differentiation in CAF-1 mutants. (**A**,**B**) Trichomes from leaves of wild-type (Col) plants usually have three branches (A), whereas trichomes on *fas1-4* leaves develop up to six branches (B). (**C-F**) Trichomes of wild-type (Col) and *fas1-4* plants are single cells with one nucleus. (C,D) DAPI-stained trichomes of wild type (Col) and *fas1-4*, respectively. (E,F) Close-up view of nuclei in DAPI-stained trichomes of wild type (Col) and *fas1-4*, respectively. (**G**) Trichome branching for the first two rosette leaves of at least six plants per experiment. Shown are the average values of several independent experiments. Segments represent fractions of trichomes with 1 (white hatched), 2 (white), 3 (light gray), 4 (dark gray), 5 (black) and 6 (dark gray, hatched) branches. (**H**) Ploidy of trichome nuclei from the first two rosette leaves. DNA in trichome nuclei was stained with DAPI and the fluorescence intensity values were normalized to the fluorescence of guard cell nuclei from the same specimen; these are displayed relative to wild-type intensities. Shown are the average values of several independent experiments (means±s.e.m.). Double asterisks indicate *P*-values (*t*-test) <0.005.

it possible that they affect trichome development independently of CAF-1. To test this possibility genetically, we generated *fas1-4 fas2-4*, *fas1-4 msi1-as* and fas2-*4 msi1-as* double mutants. All of these double mutants were viable, similar to the previously described *fas1-1 fas2-1* double mutant (Hennig et al., 2003), and no obvious synergistic effects were observed during development. In particular, quantification of trichome branching showed no additive or synergistic effects between *fas1-4*, *fas2-4* and *msi1-as* (Fig. 6A). Similarly, *fas1-1 fas2-1*, *fas1-1 msi1-as* and *fas2-1 msi1-as* double mutants generated by inter-accession crossings did not show increased trichome branching (data not shown). These results demonstrate that *FAS1*, *FAS2* and *MSI1* function in the same genetic pathway to control trichome development, possibly by acting together in the CAF-1 complex.

Loss of CAF-1 affects trichome branching independently of endoreduplication

GLABRA3 (*GL3*) is a positive regulator of trichome cell-fate determination. *GL3* controls trichome branching via the control of endoreduplication (Hülskamp et al., 1994), and *gl3-1* mutants produce trichomes with less than three branches (Payne et al., 2000). To genetically test the hypothesis that CAF-1 controls trichome development in a pathway parallel to the endoreduplication-dependent pathway, in which *GL3* acts, we generated *fas2-1 gl3-1* double mutants. Whereas *fas2-1* had almost no single-ended but many three-ended trichomes, *gl3-1* had 24% single-ended and only 2% three-ended trichomes. By contrast, the trichome branching phenotype of the *fas2-1 gl3-1* double mutant was intermediate to the two single mutants, with only 4% single-ended trichomes and 10% three-ended trichomes (Fig. 6B). Because *FAS2* and *GL3* had an additive rather than epistatic interaction, CAF-1 acts in a pathway genetically parallel to the *GL3*-containing endoreduplication pathway.

DISCUSSION

CAF-1 is required for control of hypocotyl growth

Previously, CAF-1 function in plants has been described mainly for the SAM and RAM (Reinholz, 1966; Leyser and Furner, 1992; Kaya et al., 2001). In particular, defects in the organization of the SAM were suggested to cause abnormal phyllotaxis and a thickening or even bifurcation of the stem. These defects, together with the reduced growth rate of the primary shoot, are most likely the consequence of disruptions in normal SAM function. However, the *fasciata* mutants

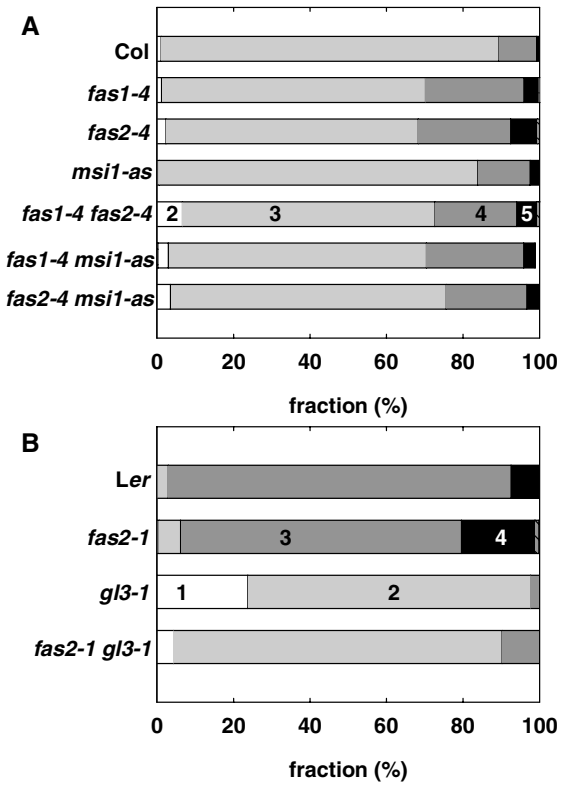

Fig. 6. Epistasis analysis of CAF-1 function for trichome branching. (**A**) Trichome branching in *fas1-4*, *fas2-4*, *msi1-as* and double mutants. Branching was quantified for all trichomes on the first two rosette leaves of at least six plants per experiment. Segments represent fractions of trichomes with 2 (white), 3 (light gray), 4 (dark gray), 5 (black) and 6 (dark gray, hatched) branches. (**B**) Trichome branching in *fas2-1*, *gl3-1* and double mutants. Branching was quantified for all trichomes on the first two rosette leaves of at least six plants per experiment. Segments represent fractions of trichomes with 1 (white), 2 (light gray), 3 (dark gray), 4 (black) and 5 (dark gray, hatched) branches.

also have altered leaf shapes (Reinholz, 1966; Leyser and Furner, 1992). Because leaf shape is mainly established during outgrowth of the leaf primordia (Fleming, 2002; Tsukaya, 2003), these observations suggest that CAF-1 function is also required in organ primordia and developing lateral organs. This conclusion is supported by the strong expression of *FAS1* in leaf primordia (Kaya et al., 2001). In contrast to the post-embryonic organs of a mature plant, hypocotyl and cotyledons are formed during embryogenesis independently of the SAM. During initial seedling development, very little cell division occurs, and this is restricted mainly to the cotyledons (Gendreau et al., 1997). Growth is controlled by cell elongation, which is often paralleled by DNA endoreduplication. We found that CAF-1 mutant seedlings have shorter hypocotyls in the dark and that this is mainly due to reduced cell number rather than altered cell size. Therefore, CAF-1 deficiency impairs normal development by interfering with cell proliferation as early as during embryogenesis or seedling development.

CAF-1 controls leaf epidermal development

Cell shape in leaf epidermis and parenchyme tissue was normal in CAF-1 mutants, suggesting that CAF-1 is not required for the correct differentiation of most leaf cells. By contrast, but similar to the effects in hypocotyls, loss of CAF-1 interfered with cell proliferation in rosette leaves, where the number of adaxial epidermal cells was reduced by half. In addition, cells in the adaxial epidermis of rosette leaves were significantly enlarged in CAF-1 mutants. This increase in cell size might be caused by a compensatory mechanism for the reduced cell numbers (Tsukaya, 2005). This could explain why, despite the greatly reduced numbers of epidermal cells, the leaf area is often only mildly reduced in *fas1-4* and *fas2-4* mutants (data not shown). Furthermore, altered cell number and cell size could potentially account for the change in trichome numbers on rosette leaves of CAF-1 mutants, because it was primarily the trichome density relative to leaf size, rather than the trichome density relative to epidermal cell number, that was reduced (data not shown). Thus, CAF-1 is needed for efficient cell proliferation in developing hypocotyls and rosette leaves, and compensation by altered cell size might occur preferentially in leaves but not in hypocotyls.

CAF-1 is required for trichome differentiation

Trichome morphogenesis is a well-studied developmental process, and many mutants with altered trichomes have been identified (Oppenheimer, 1998; Schnittger and Hülskamp, 2002; Larkin et al., 2003). The genetic analysis of branching mutants suggests that several redundant pathways control branch formation (Luo and Oppenheimer, 1999). One group of genes affecting trichome branching primarily controls the number of DNA endoreduplication cycles, which in turn determines branch number (Hülskamp et al., 1994; Perazza et al., 1999; Kirik et al., 2001). *GL3* is one member of this group and encodes a bHLH protein that positively regulates endoreduplication and branching events in trichomes (Payne et al., 2000). A second group of branching mutants affects branch number independently of DNA endoreduplication (Folkers et al., 1997; Luo and Oppenheimer, 1999; Qiu et al., 2002). In all CAF-1 mutants, trichome patterning was maintained and no trichome clusters were observed, indicating that CAF-1 is not required for commitment to the trichome cell fate. Similarly, trichomes in *fas1*, *fas2* and *msi1-as*, and in the double mutants we investigated, were single cells containing one nucleus, suggesting that most steps in trichome differentiation are completed normally even in the absence of CAF-1. However, all CAF-1 mutants had increased branch numbers. Interestingly, increased trichome branching was also observed in *Hosoba toge toge*, a deletion mutant lacking a 75.8-kb region encompassing 15 genes, including *FAS1* (Kaya et al., 2000). Because only *fas1* trichomes had a consistently increased DNA content, the increased branching is not strictly correlated with DNA endoreduplication. Therefore, CAF-1 functions either in a DNA endoreduplication-independent pathway or downstream of DNA endoreduplication to control trichome branching. This hypothesis is also supported by the fact that the *gl3-1 fas2-1* double mutants analysed have intermediate branch numbers and none of the two alleles was epistatic over the other. Most likely, CAF-1 functions in a DNA endoreduplication-independent pathway for trichome branching. In summary, CAF-1 is not generally needed for cell fate determination, but it is needed for normal cell proliferation and, in trichomes, even for proper differentiation.

CAF-1-independent functions of CAF-1 subunits

Mutants lacking any one of the three subunits of *Arabidopsis* CAF-1 display a range of similar phenotypes that is consistent with the loss of CAF-1 activity. Similarly, CAC1, CAC2 and CAC3 are all required for CAF-1 function in yeast (Game and Kaufman, 1999). However, some phenotypes caused by reduced FAS1, FAS2 or MSI1

levels differ, suggesting that CAF-1 subunits can function independently of CAF-1. For instance, only the loss of *FAS2* led to smaller hypocotyl epidermal cells independent of the genetic background. Likewise, *msi1-as* plants show unique features, in particular they often develop cotyledon epinasty. Because this trait was never observed in *fas1* or *fas2*, it most likely resulted from the lack of a CAF-1-independent MSI1 function. In addition, the previously described *msi1-cs* plants develop severe phenotypic defects, including homeotic changes of floral organ identity, which are not present in *fas1*, *fas2* and *msi1-as* (Hennig et al., 2003). Embryos of the *msi1-1* mutant abort early in development, but embryos of *fas1*, *fas2* and *msi1-as* show no obvious developmental defects (Kaya et al., 2001; Köhler et al., 2003; Guitton et al., 2004). Biochemical analyses suggest that MSI1-like proteins participate in several protein complexes acting on chromatin, including histone deacetylases, chromatin remodelling factors and Polycomb Group protein complexes (for a review, see Hennig et al., 2005). We found that *Arabidopsis* MSI1, in addition to FAS1, also interacts with fertilization-independent endosperm (FIE), histone deacetylase, and the retinoblastoma-related RBR protein (Ach et al., 1997; Hennig et al., 2003; Köhler et al., 2003) (data not shown). Further studies will reveal which of these partners participate(s) in CAF-1 complex-independent functions of MSI1 during plant development.

CAF-1 is needed for normal cell cycle progression during endoreduplication

The DNA content of CAF-1 mutant cells was increased in light- and dark-grown seedlings. In both cases, DNA endoreduplication occurs during normal development, but many mutant cells continued DNA endoreduplication for on average one extra round. This observation was surprising considering that RNAi knock-down of human p150 causes an accumulation of cells in S-phase (Hoek and Stillman, 2003). Because *Arabidopsis* CAF-1 mutants are viable, plant cells appear to be less dependent on CAF-1 function, thus revealing an additional role of CAF-1, i.e. to prevent excessive rounds of DNA endoreduplication. In yeast and humans, CAF-1 appears to be required for kinetochore formation (Sharp et al., 2002; Sharp et al., 2003). Interfering with kinetochore formation could potentially bypass mitosis and cause an increased DNA content. Alternatively, failure to exit the G2 phase of the cell cycle could cause an accumulation of cells with increased DNA content. Because the effects on DNA content were similar in the strong *fas1-1* and *fas2-4* alleles and in the weak *fas1-4* and *msi1-as* alleles, even a partial loss of CAF-1 activity can considerably affect cellular DNA content. However, CAF-1 is not absolutely essential for normal cell cycle progression in *Arabidopsis*, as the *fas2-1* allele in L*er* had a largely unchanged DNA content. This suggests that the L*er* accession contains genetic determinants that make CAF-1 largely dispensable for normal cell cycle progression. Because the developmental phenotypes of the two *fas2* null alleles *fas2-1* and *fas2-4* were similar in L*er* and Col, only the cell cycle function but not the developmental function of CAF-1 can be compensated in L*er*. In addition, this suggests that the developmental defects of CAF-1 mutants are not a direct consequence of defective cell cycle progression.

A role of CAF-1 in duplication of epigenetic information

CAF-1 has nucleosome assembly activity and functions by depositing histone H3-H4 dimers onto newly synthesized DNA, as it is recruited to places of DNA synthesis by PCNA (for a review see, Loyola and Almouzni, 2004). As CAF-1 facilitates replication-coupled chromatin assembly, it may be needed to faithfully duplicate epigenetic information in chromatin during mitosis (Enomoto and Berman, 1998; Ridgway and Almouzni, 2000; van Nocker, 2003; Henikoff et al., 2004). Indeed, CAF-1 is needed for the maintenance of gene silencing in yeast, mammals and plants (Enomoto and Berman, 1998; Tchenio et al., 2001; Takeda et al., 2004; Ono et al., 2006; Schönrock et al., 2006b). In *Arabidopsis*, CAF-1 contributes to the compaction of heterochromatin but is not essential for the silencing of most heterochromatic genes (Schönrock et al., 2006b). Nevertheless, several silent heterochromatic loci become stochastically activated in some cells in a fraction of *fas2* plants (Ono et al., 2006). In addition, the euchromatic *GLABRA2* gene has a cell-type-specific chromatin environment, which is lost in *fas2* mutants (Costa and Shaw, 2006). Interestingly, plants containing greatly reduced MSI1 levels suffer from a progressive loss of floral morphology, whereas none of the analyzed *fas1* or *fas2* mutant alleles showed a similar increase in phenotype severity (Hennig et al., 2003). In contrast to the morphological phenotype, the molecular phenotype of *fas2* mutants increased with plant age as the expression of *SCR::GFP* was disturbed more in older than in younger roots (Kaya et al., 2001), and the percentage of plants with de-repressed *CACTA* transposons increased from 20% to 65% (Ono et al., 2006). Some of the pleiotropic phenotypes of CAF-1 mutants occur stochastically (Leyser and Furner, 1992; Kaya et al., 2001) and are most likely caused by stochastic defects in the expression of developmental regulators. Thus, CAF-1 might be needed for normal development because it facilitates the faithful duplication of epigenetic information in chromatin during mitosis.

CAF-1 is required for the control of multiple developmental processes in plants

In yeast, double mutants defective in CAF-1 and HIR or ASF1 cause defects in cell proliferation (Tyler et al., 1999; Sharp et al., 2001; Sharp et al., 2002), and CAF-1 mutants in higher eukaryotes, including *Arabidopsis*, most likely suffer from delayed cell division as well (Hoek and Stillman, 2003; Schönrock et al., 2006b). Several of the less stochastic phenotypes of *Arabidopsis* CAF-1 mutants, including changes in embryonic and post-embryonic organ size (e.g. hypocotyl, rosette), and reduced growth rates, could be caused by defects in cell proliferation. Because CAF-1 is needed for both efficient S-phase progression (Hoek and Stillman, 2003) and mitosis (Sharp et al., 2002; Sharp et al., 2003), absence of CAF-1 activity can interfere with steps in development that are based on a sensitive balance between cell cycle progression, cell division and differentiation. In meristems, this balance is particularly important, and mutant phenotypes arising from imbalances in meristems are therefore very obvious. Our results demonstrate, however, that the function of CAF-1 is not restricted to meristems, but is also required during many other differentiation and developmental processes. Most strikingly, even the differentiation of single-celled trichomes, which is independent of cell division, depends on CAF-1 activity.

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Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/133/21/4163/DC1

References

Ach, R. A., Taranto, P. and Gruissem, W. (1997). A conserved family of WD-40 proteins binds to the retinoblastoma protein in both plants and animals. *Plant Cell* **9**, 1595-1606.

Alonso, J. M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H., Shinn, P., Stevenson, D. K., Zimmerman, J., Barajas, P., Cheuk, R. et al. (2003).

Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* **301**, 653-657.
Beemster, G. T., De Veylder, L., Vercruysse, S., West, G., Rombaut, D., Van Hummelen, P., Galichet, A., Gruissem, W., Inze, D. and Vuylsteke, M. (2005). Genome-wide analysis of gene expression profiles associated with cell cycle transitions in growing organs of Arabidopsis. *Plant Physiol.* **138**, 734-743.
Bulger, M., Ito, T., Kamakaka, R. T. and Kadonaga, J. T. (1995). Assembly of regularly spaced nucleosome arrays by Drosophila Chromatin Assembly Factor 1 and a 56-kDa histone-binding protein. *Proc. Natl. Acad. Sci. USA* **92**, 11726-11730.
Byrne, M. E. (2005). Networks in leaf development. *Curr. Opin. Plant Biol.* **8**, 59-66.
Costa, S. and Shaw, P. (2006). Chromatin organization and cell fate switch respond to positional information in Arabidopsis. *Nature* **439**, 493-496.
Enomoto, S. and Berman, J. (1998). Chromatin Assembly Factor 1 contributes to the maintenance, but not the re-establishment, of silencing at the yeast silent mating loci. *Genes Dev.* **12**, 219-232.
Enomoto, S., McCune-Zierath, P. D., Gerami-Nejad, M., Sanders, M. A. and Berman, J. (1997). RLF2, a subunit of yeast Chromatin Assembly Factor **1**, is required for telomeric chromatin function in vivo. *Genes Dev.* **11**, 358-370.
Fleming, A. J. (2002). The mechanism of leaf morphogenesis. *Planta* **216**, 17-22.
Fleming, A. J. (2005). The control of leaf development. *New Phytol.* **166**, 9-20.
Folkers, U., Berger, J. and Hülskamp, M. (1997). Cell morphogenesis of trichomes in Arabidopsis: differential control of primary and secondary branching by branch initiation regulators and cell growth. *Development* **124**, 3779-3786.
Gaillard, P. H., Martini, E. M., Kaufman, P. D., Stillman, B., Moustacchi, E. and Almouzni, G. (1996). Chromatin assembly coupled to DNA repair: a new role for Chromatin Assembly Factor 1. *Cell* **86**, 887-896.
Game, J. C. and Kaufman, P. D. (1999). Role of *Saccharomyces cerevisiae* Chromatin Assembly Factor-1 in repair of ultraviolet radiation damage in vivo. *Genetics* **151**, 485-497.
Gendreau, E., Traas, J., Desnos, T., Grandjean, O., Caboche, M. and Höfte, H. (1997). Cellular basis of hypocotyl growth in *Arabidopsis thaliana*. *Plant Physiol.* **114**, 295-305.
Guitton, A. E., Page, D. R., Chambrier, P., Lionnet, C., Faure, J. E., Grossniklaus, U. and Berger, F. (2004). Identification of new members of *FERTILISATION INDEPENDENT SEED* Polycomb group pathway involved in the control of seed development in *Arabidopsis thaliana*. *Development* **131**, 2971-2981.
Henikoff, S., Furuyama, T. and Ahmad, K. (2004). Histone variants, nucleosome assembly and epigenetic inheritance. *Trends Genet.* **20**, 320-326.
Hennig, L., Taranto, P., Walser, M., Schönrock, N. and Gruissem, W. (2003). Arabidopsis MSI1 is required for epigenetic maintenance of reproductive development. *Development* **130**, 2555-2565.
Hennig, L., Bouveret, R. and Gruissem, W. (2005). MSI1-like proteins: an escort service for chromatin assembly and remodeling complexes. *Trends Cell Biol.* **15**, 295-302.
Hoek, M. and Stillman, B. (2003). Chromatin Assembly Factor-1 is essential and couples chromatin assembly to DNA replication in vivo. *Proc. Natl. Acad. Sci. USA* **100**, 12183-12188.
Hülskamp, M., Misra, S. and Jürgens, G. (1994). Genetic dissection of trichome cell development in Arabidopsis. *Cell* **76**, 555-566.
Jiao, R., Bachrati, C. Z., Pedrazzi, G., Kuster, P., Petkovic, M., Li, J. L., Egli, D., Hickson, I. D. and Stagljar, I. (2004). Physical and functional interaction between the Bloom's syndrome gene product and the largest subunit of Chromatin Assembly Factor-1. *Mol. Cell. Biol.* **24**, 4710-4719.
Kaufman, P. D., Kobayashi, R. and Stillman, B. (1997). Ultraviolet radiation sensitivity and reduction of telomeric silencing in *Saccharomyces cerevisiae* cells lacking Chromatin Assembly Factor-1. *Genes Dev.* **11**, 345-357.
Kaya, H., Sato, S., Tabata, S., Kobayashi, Y., Iwabuchi, M. and Araki, T. (2000). *Hosoba toge toge*, a syndrome caused by a large chromosomal deletion associated with a T-DNA insertion in Arabidopsis. *Plant Cell Physiol.* **41**, 1055-1066.
Kaya, H., Shibahara, K., Taoka, K., Iwabuchi, M., Stillman, B. and Araki, T. (2001). *FASCIATA* genes for Chromatin Assembly Factor-1 in Arabidopsis maintain the cellular organization of apical meristems. *Cell* **104**, 131-142.
Kirik, V., Bouyer, D., Schobinger, U., Bechtold, N., Herzog, M., Bonneville, J. M. and Hülskamp, M. (2001). *CPR5* is involved in cell proliferation and cell death control and encodes a novel transmembrane protein. *Curr. Biol.* **11**, 1891-1895.
Köhler, C., Hennig, L., Bouveret, R., Gheyselinck, J., Grossniklaus, U. and Gruissem, W. (2003). Arabidopsis MSI1 is a component of the MEA/FIE Polycomb group complex and required for seed development. *EMBO J.* **22**, 4804-4814.
Koornneef, M., Dellaert, L. W. and van der, V. e. J. (1982). EMS- and radiation-induced mutation frequencies at individual loci in *Arabidopsis thaliana* (L.) Heynh. *Mutat. Res.* **93**, 109-123.
Krawitz, D. C., Kama, T. and Kaufman, P. D. (2002). Chromatin Assembly Factor 1 mutants defective for PCNA binding require ASF1/HIR proteins for silencing. *Mol. Cell. Biol.* **22**, 614-625.
Krude, T. and Keller, C. (2001). Chromatin assembly during S phase: contributions from histone deposition, DNA replication and the cell division cycle. *Cell Mol. Life Sci.* **58**, 665-672.
Larkin, J. C., Brown, M. L. and Schiefelbein, J. (2003). How do cells know what they want to be when they grow up? Lessons from epidermal patterning in Arabidopsis. *Annu. Rev. Plant Biol.* **54**, 403-430.
Laux, T., Mayer, K. F., Berger, J. and Jurgens, G. (1996). The *WUSCHEL* gene is required for shoot and floral meristem integrity in Arabidopsis. *Development* **122**, 87-96.
Leyser, H. M. and Furner, I. J. (1992). Characterisation of three shoot apical meristem mutants of *Arabidopsis thaliana*. *Development* **116**, 397-403.
Loyola, A. and Almouzni, G. (2004). Histone chaperones, a supporting role in the limelight. *Biochim. Biophys. Acta* **1677**, 3-11.
Luo, D. and Oppenheimer, D. G. (1999). Genetic control of trichome branch number in Arabidopsis: the roles of the *FURCA* loci. *Development* **126**, 5547-5557.
Mello, J. A. and Almouzni, G. (2001). The ins and outs of nucleosome assembly. *Curr. Opin. Genet. Dev.* **11**, 136-141.
Mello, J. A., Sillje, H. H., Roche, D. M. D., B., Kirschner Nigg, E. A. and Almouzni, G. (2002). Human ASF1 and CAF-1 interact and synergize in a repair-coupled nucleosome assembly pathway. *EMBO Rep.* **3**, 329-334.
Moggs, J. G., Grandi, P., Quivy, J. P., Jonsson, Z. O., Hubscher, U., Becker, P. B. and Almouzni, G. (2000). A CAF-1-PCNA-mediated chromatin assembly pathway triggered by sensing DNA damage. *Mol. Cell. Biol.* **20**, 1206-1218.
Monson, E. K., de Bruin, B. and Zakian, V. A. (1997). The yeast CAC1 protein is required for the stable inheritance of transcriptionally repressed chromatin at telomeres. *Proc. Natl. Acad. Sci. USA* **94**, 13081-13086.
Murzina, N., Verreault, A., Laue, E. and Stillman, B. (1999). Heterochromatin dynamics in mouse cells: interaction between Chromatin Assembly Factor-1 and HP1 proteins. *Mol. Cell* **4**, 529-540.
Nabatiyan, A. and Krude, T. (2004). Silencing of Chromatin Assembly Factor-1 in human cells leads to cell death and loss of chromatin assembly during DNA synthesis. *Mol. Cell. Biol.* **24**, 2853-2862.
Ono, T., Kaya, H., Takeda, S., Abe, M., Ogawa, Y., Kato, M., Kakutani, T., Scheid, O. M., Araki, T. and Shibahara, K. (2006). Chromatin assembly factor 1 ensures the stable maintenance of silent chromatin states in Arabidopsis. *Genes Cells* **11**, 153-162.
Oppenheimer, D. G. (1998). Genetics of plant cell shape. *Curr. Opin. Plant Biol.* **1**, 520-524.
Payne, C. T., Zhang, F. and Lloyd, A. M. (2000). *GL3* encodes a bHLH protein that regulates trichome development in Arabidopsis through interaction with GL1 and TTG1. *Genetics* **156**, 1349-1362.
Perazza, D., Herzog, M., Hülskamp, M., Brown, S., Dorne, A. M. and Bonneville, J. M. (1999). Trichome cell growth in *Arabidopsis thaliana* can be derepressed by mutations in at least five genes. *Genetics* **152**, 461-476.
Qiu, J. L., Jilk, R., Marks, M. D. and Szymanski, D. B. (2002). The Arabidopsis *SPIKE1* gene is required for normal cell shape control and tissue development. *Plant Cell* **14**, 101-118.
Quivy, J. P., Grandi, P. and Almouzni, G. (2001). Dimerization of the largest subunit of Chromatin Assembly Factor 1, importance in vitro and during Xenopus early development. *EMBO J.* **20**, 2015-2027.
Reese, B. E., Bachman, K. E., Baylin, S. B. and Rountree, M. R. (2003). The methyl-CpG binding protein MBD1 interacts with the p150 subunit of Chromatin Assembly Factor-1. *Mol. Cell. Biol.* **23**, 3226-3236.
Reinholz, E. (1966). Radiation induced mutants showing changed inflorescence characteristics. *Arabid. Inf. Serv.* **3**, 19-20.
Ridgway, P. and Almouzni, G. (2000). CAF-1 and the inheritance of chromatin states: at the crossroads of DNA replication and repair. *J. Cell Sci.* **113**, 2647-2658.
Sarraf, S. A. and Stancheva, I. (2004). Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. *Mol. Cell* **15**, 595-605.
Scheres, B., van den Toorn, H. and Heidstra, R. (2004). Root genomics: towards digital in situ hybridization. *Genome Biol.* **5**, 227.
Schnittger, A. and Hülskamp, M. (2002). Trichome morphogenesis: a cell-cycle perspective. *Philos. Trans. R. Soc. London Ser. B* **357**, 823-826.
Schönrock, N., Bouveret, R., Leroy, O., Borghi, L., Köhler, C., Gruissem, W. and Hennig, L. (2006a). Polycomb-group proteins repress the floral activator *AGL19* in the *FLC*-independent vernalization pathway. *Genes Dev.* **20**, 1667-1678.
Schönrock, N., Exner, V., Probst, A., Gruissem, W. and Hennig, L. (2006b). Functional genomic analysis of CAF-1 mutants in *Arabidopsis thaliana*. *J. Biol. Chem.* **281**, 9560-9568.
Serrano-Cartagena, J., Robles, P., Ponce, M. R. and Micol, J. L. (1999). Genetic analysis of leaf form mutants from the Arabidopsis information service collection. *Mol. Gen. Genet.* **261**, 725-739.
Sessions, A., Burke, E., Presting, G., Aux, G., McElver, J., Patton, D., Dietrich, B., Ho, P., Bacwaden, J., Ko, C. et al. (2002). A high-throughput Arabidopsis reverse genetics system. *Plant Cell* **14**, 2985-2994.

Sharp, J. A., Fouts, E. T., Krawitz, D. C. and Kaufman, P. D. (2001). Yeast histone deposition protein ASF1p requires HIR proteins and PCNA for heterochromatic silencing. *Curr. Biol.* **11**, 463-473.
Sharp, J. A., Franco, A. A., Osley, M. A. and Kaufman, P. D. (2002). Chromatin Assembly Factor 1 and HIR proteins contribute to building functional kinetochores in *S. cerevisiae*. *Genes Dev.* **16**, 85-100.
Sharp, J. A., Krawitz, D. C., Gardner, K. A., Fox, C. A. and Kaufman, P. D. (2003). The budding yeast silencing protein SIR1 is a functional component of centromeric chromatin. *Genes Dev.* **17**, 2356-2361.
Shibahara, K. and Stillman, B. (1999). Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. *Cell* **96**, 575-585.
Smith, J. S., Caputo, E. and Boeke, J. D. (1999). A genetic screen for ribosomal DNA silencing defects identifies multiple DNA replication and chromatin-modulating factors. *Mol. Cell. Biol.* **19**, 3184-3197.
Smith, S. and Stillman, B. (1989). Purification and characterization of CAF-1, a human cell factor required for chromatin assembly during DNA replication in vitro. *Cell* **58**, 15-25.
Taddei, A., Roche, D., Sibarita, J. B., Turner, B. M. and Almouzni, G. (1999). Duplication and maintenance of heterochromatin domains. *J. Cell Biol.* **147**, 1153-1166.
Takeda, S., Tadele, Z., Hofmann, I., Probst, A. V., Angelis, K. J., Kaya, H., Araki, T., Mengiste, T., Scheid, O. M., Shibahara, K. et al. (2004). BRU1, a novel link between responses to DNA damage and epigenetic gene silencing in Arabidopsis. *Genes Dev.* **18**, 782-793.
Tchenio, T., Casella, J. F. and Heidmann, T. (2001). A truncated form of the human CAF-1 p150 subunit impairs the maintenance of transcriptional gene silencing in mammalian cells. *Mol. Cell. Biol.* **21**, 1953-1961.
Tsukaya, H. (2003). Organ shape and size: a lesson from studies of leaf morphogenesis. *Curr. Opin. Plant Biol.* **6**, 57-62.
Tsukaya, H. (2005). Leaf shape: genetic controls and environmental factors. *Int. J. Dev. Biol.* **49**, 547-555.
Tyler, J. K., Adams, C. R., Chen, S. R., Kobayashi, R., Kamakaka, R. T. and Kadonaga, J. T. (1999). The RCAF complex mediates chromatin assembly during DNA replication and repair. *Nature* **402**, 555-560.
van Nocker, S. (2003). CAF-1 and MSI1-related proteins: linking nucleosome assembly with epigenetics. *Trends Plant Sci.* **8**, 471-473.
Verreault, A., Kaufman, P. D., Kobayashi, R. and Stillman, B. (1996). Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. *Cell* **87**, 95-104.
Walker, J. D., Oppenheimer, D. G., Concienne, J. and Larkin, J. C. (2000). *SIAMESE*, a gene controlling the endoreduplication cell cycle in *Arabidopsis thaliana* trichomes. *Development* **127**, 3931-3940.
Worsdell, W. C. (1905). Fasciation: its meaning and origin. *New Phytol.* **4**, 55-74.
Zhang, Z., Shibahara, K. and Stillman, B. (2000). PCNA connects DNA replication to epigenetic inheritance in yeast. *Nature* **408**, 221-225.