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Disruption of the *Arabidopsis* SMC4 gene, *AtCAP-C*, compromises gametogenesis and embryogenesis

Received: 12 July 2005 / Accepted: 31 August 2005 / Published online: 16 February 2006
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Abstract In higher eukaryotes, the condensin complex is a multisubunit apparatus that plays a pivotal role in the coordinated condensation of chromatin during mitosis. The catalytic subunits, CAP-E and CAP-C, members of the SMC family of ATPases, form a heterodimer, the activity of which is controlled by the non-SMC subunits CAP-D2, CAP-G and CAP-H. Here, we report the characterization of a T-DNA insertion mutant of the *Arabidopsis* CAP-C gene. Analysis of the progeny of selfed heterozygotes revealed that the homozygous null genotype is embryo lethal, with arrest occurring at or before the globular stage of development. Patterning defects associated with altered planes of cytokinesis were found in both the embryo and the suspensor. Crosses of heterozygotes with wild type plants revealed both male and female gametophytic defects. Stretched chromatin was observed between segregating mitotic chromosomes in pollen produced by selfed heterozygotes. Additionally, some plants heterozygous for the T-DNA insertion exhibited loss of apical dominance and mild fasciation, indicating a semi-dominant effect of the mutation. These results reveal a critical role for *AtCAP-C* during cell division and, unlike our previous studies on the *AtCAP-E* genes, suggest that no redundant factors for *AtCAP-C* exist in the *Arabidopsis* genome.

Keywords Condensin · Embryogenesis · Cytokinesis · Chromatin · Pollen

Abbreviations SMC: Structural maintenance of chromosomes · CAP: Chromosome associated

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protein · BASTA: Glufosinate ammonium · DAPI: 4',
6-Diamidino-2-phenylindole · MS: Murashige and
Skoog medium

Introduction

Proper development of multicellular organisms depends on coordination between two complex processes, the regulation of the cell cycle and the commitment of cells to differentiation. In the last decade, cell cycle studies have revealed a multitude of different regulatory molecules which influence cell cycle progression by orchestrating both nuclear and cytoplasmic processes (Dewitte and Murray 2003; Wignall et al. 2003). A successful cell cycle requires that the genetic material be faithfully duplicated and accurately segregated to opposite spindle poles, and during these processes chromatin undergoes dramatic structural changes. Interphase chromatin is relatively decondensed, facilitating events of DNA metabolism, but spatial and topological constraints require significant compaction of chromatin for mitosis. Compaction is accomplished in part by the condensin complex, a multisubunit enzyme that acts together with topoisomerase II to condense chromatids and disentangle them during prophase of mitosis. The catalytic subunits of the complex are members of the evolutionarily conserved structural maintenance of chromosomes (SMC) family whose members participate in a variety of processes including condensation, sister chromatid cohesion, repair/recombination and dosage compensation (see Losada and Hirano 2005 for a recent review). The SMC2/4 members (also referred to as CAP-E and CAP-C, respectively) form a heterodimer that appears to be designed as a flexible clamp, the opening and closing of which may be associated with ATP dependent supercoiling of DNA and thus compaction (Hirano et al. 2001; Bazett-Jones et al. 2002). The heterodimer is guided by three non-SMC proteins, CAP-D2, CAP-G and CAP-H, one or more of which may be phosphorylated by a cyclin dependent kinase to synchronize their

activity with other cell cycle controls (Kimura et al. 1998; Sutani et al. 1999).

In plants, several *SMC* genes have been cloned from *Arabidopsis*. Mengiste et al. (1999) reported the characterization of a RAD18-like *SMC* protein that plays a role in recombinational DNA repair. Three different *SMC* genes are represented by *titan* mutants, which derive their name from the dramatic enlargement of endosperm nuclei (Tzafrir et al. 2002). *ttn3*, encoding an *SMC2* protein, exhibits aberrant endosperm nuclei and giant mitotic figures, but there are no significant defects in the embryo proper nor any post-embryonic defects. Two other *titan* mutants (*ttn7* and *ttn8*, encoding *SMC3* and *SMC1*, respectively) differ from *ttn3* in that in addition to endosperm defects, embryo development is compromised, and embryos arrest very early in development (Liu et al. 2002). The lethality of these mutants parallels that found for orthologous gene mutations in other organisms. The viability of *ttn3* (*SMC2/AtCAP-E1*) is due to functional redundancy conferred by a second, very similar gene, *AtCAP-E2*. Mutant analysis of these *AtCAP-E* genes revealed both male and female gametophytic defects, and in the double null mutant embryo arrest occurs at or before the globular stage and is characterized by defects in the organization of both the embryo and the suspensor (Siddiqui et al. 2003). Furthermore, reduction of *AtCAP-E* levels by antisense technology gave rise to plants exhibiting meristem structure and function defects, slow growth and precocious differentiation of certain cell types. Thus, the *AtCAP-E* proteins collectively are essential for embryogenesis, and reduction in protein levels perturb the normal structure and function of meristems.

To further research on the condensin complex in *Arabidopsis*, we screened for mutants in the *SMC4* (*AtCAP-C*) gene, which was known to exist through the efforts of genome sequencing, but for which no mutant had been described. Here, we report the characterization of such a mutant that displays phenotypes similar to those exhibited by the *AtCAP-E* mutants, including altered segregation of alleles during gametogenesis and embryo lethality of null mutants.

Materials and methods

Isolation of an *AtCAP-C* T-DNA insertion mutant and complementation of the mutant phenotype

A BLAST search in the SAIL (Syngenta Arabidopsis Insertion Library) database with the *AtCAP-C* (encoding for the putative *Arabidopsis SMC4* ortholog) genomic sequence (Accession number: NM_124236) revealed a line (86-D2) with a T-DNA insertion near the middle of the gene. The seeds of this line were germinated on Murashige and Skoog (MS) plates containing BASTA (10 µg/ml, Crescent Chemicals) to select for plants carrying a T-DNA insertion. Genomic DNA was isolated

from BASTA resistant plants and the integration site of the T-DNA was determined by sequencing of a PCR amplification product generated by employing a gene-specific primer (*SMC4*-forward 5'-AGCCGGTCTGGA GTTGTGCAAATGG-3') and the T-DNA left border primer (LB3-5'-TAGCATCTGAATTCATAACCA TCTCGATACAC-3'). The insertion was found to be near the end of the seventh intron in the *AtCAP-C* gene. A total of 32 BASTA resistant plants were transferred to soil, and genotyping was conducted with two sets of primers, one employing a gene specific primer and the LB3 T-DNA primer (see above) to confirm the presence of a T-DNA insertion. The second set of gene specific primers (*SMC4*-forward, described above, and *SMC4*-backward 5'-GAGCTGTTTCTGAGCATCTGTGAA GG-3'), was employed with amplification of a 1,542 bp fragment being indicative of the presence of a wild type copy of the gene.

For mutant rescue, the wild type *AtCAP-C* gene was first amplified by PCR, using three sets of primers: *SMC4pro* forward 5'-TTGAAGTCGACAATTCTAG CTTGATGAAAACC-3' and *SMC4* 22333 Bam 5'-CACTTAATACATATAGAGGATCCAAACAAAA TTAC-3' to amplify a 3.1 kb fragment containing promoter sequences; *SMC4* 22343 Bam backward 5'-TT TGGATCCTCTATATGTATTAAGTGTTACATGT GTT-3' and *SMC4* 25613 Bam Backward 5'-TAAAG AAGCAGTGGGATAAGCTAATTAACCTC-3' to amplify a 3.3 kb fragment containing the proximal promoter region and the first eleven exons of the gene; *SMC4* 25584 Forward 5'-CTTGCTCGTCTTCAAA AGAATTTCTAGAGAGG-3' and *SMC4* 30321 backward 5'-AATTTGGCGCGCCATAGTTAGTGTTAA TCCCAGCAT-3' to amplify a 4.7 kb fragment containing the remainder of the coding sequence (underlined nucleotides correspond to engineered restriction sites). The 4.7 kbp product was cut with *XbaI* and *AscI* and introduced into pHPT-link, a modified binary vector derived from pGVPT-HPT (Becker et al. 1992). Because of non unique restriction enzyme sites the first two PCR products were cloned into pBluescript (Stratagene) and a 6.4 kbp *Sall/XbaI* fragment was released and mobilized into the *Sall/XbaI* sites of the intermediate construct. This reconstituted the wild type gene with the exception of engineered *Sall* (at -3.8 kbp relative to the beginning of the first exon), *BamHI* (at -686), and *AscI* (110 bp after the stop codon) sites. The rescue construct was used to transform *Agrobacterium* GV3101 and kanamycin/gentamycin resistant cells were used to transform 86D2 plants by the method of Clough and Bent (1998). Transgenic plants were selected on MS plates containing 15 µg/ml hygromycin and 10 µg/ml BASTA.

RNA analysis

RNA was prepared from various tissues of Columbia wild type and 86D2 plants using the Trizol protocol

(Invitrogen) or a hot phenol/LiCl protocol (Verwoerd et al. 1989). RT-PCR was conducted using approximately 1 μ g of total RNA, using oligo dT as a primer according to the product literature supplied with Superscript III reverse transcriptase (Invitrogen). Ten microliters of each reaction was diluted to 50 μ l, mixed well, and 2 μ l aliquots were used as templates for PCR with two sets of primers, one designed to amplify *AtCAP-C* cDNA and the other to amplify actin cDNA sequences. The *AtCAP-C* primers were 5'-GTCCCCA-TGATGAAGGTTTCTTG-3' (exon 5) and 5'-CTTT-ATATCAGCTTTCCAAGAGGTAG-3' (exon 12), which generate a 1.8 kb product. The actin primers were: 5'-TGTTGAGGATATTCAGCCACTTGTC-3' and 5'-TGTGAGATCCCGACCCGCAAGATCA-3', and generate a 642 bp product. Conditions for PCR were: 1 min at 94°C, followed by 35 cycles of 94°C for 40 s, 53°C for 30 s, and 72°C for 2 min. A final extension cycle of 72°C for 5 min was carried out. Aliquots of each reaction were subjected to electrophoresis and based on the relative abundance of the actin gene amplification products, loading of the *AtCAP-C* reactions was adjusted accordingly.

Cytological analyses

Inflorescences were allowed to develop until the opening of the first flower bud. The entire inflorescence was then fixed in 3:1 ethanol and acetic acid. Fixation was done for 24–36 h, with the fixative being removed and replaced after 3 h. After fixation the buds were rinsed three times in 70% ethanol, and then either used immediately or stored at 4°C in 70% ethanol.

For squash preparations, an individual bud was placed in 45% acetic acid for 2 min, then transferred to a drop of water on a microscope slide. The anthers were removed from the bud, and all other organs were discarded. The water was then wicked away from the anthers and one drop of Vectamount with DAPI (Vector Labs) was added. Anthers were then sliced in half, and the contents were squashed out of the anther halves with the flat end of a spear. Anther debris was removed and a coverslip was added. The edges of the coverslip were sealed with fingernail polish. Microspore nuclei were examined using a 100 \times lens of a Zeiss Axiophot fluorescence microscope. Images were captured with a Sony video camera and converted to digital images using Northern Eclipse software.

To examine embryos, hand pollinations were performed and at specific times after fertilization, siliques were harvested and fixed for 4 h in 3:1 ethanol and acetic acid. The fixative was replaced with 70% ethanol and several changes were made over the next 4 h. To decolorize, the tissue was placed in an 8:2:1 solution of chloral hydrate and glycerol and water for one to several days. Siliques were dissected in this solution and embryos imaged by DIC microscopy.

Results

Identification of an Arabidopsis SMC4 gene and analysis of expression

Independent BLAST searches of the Arabidopsis genome database with the SMC4 sequence from humans and *Xenopus* indicated the presence of a single SMC4 ortholog in the Arabidopsis genome on chromosome V (TAIR Accession: 2152525; Locus: At5g48600). Annotation of the gene by AGI revealed that it is approximately 7.2 Kb in length, having 28 exons. To conform to the nomenclature used for *SMC4* genes in higher eukaryotes, this gene was named *AtCAP-C* (Arabidopsis thaliana Chromosome Associated Protein-C).

A T-DNA insertion mutant in the *AtCAP-C* gene was identified in the Syngenta T-DNA mutant collection as line 86D2 (McElver et al. 2001). Sequencing of the PCR fragment amplified using the T-DNA specific left border primer and the gene specific primer revealed that the T-DNA insertion lies near the end of the seventh intron (Fig. 1a).

To examine wild type mRNA levels, we conducted RT-PCR with RNA from cauline leaves, inflorescences, roots, rosette leaves, seedlings, siliques, and stems of

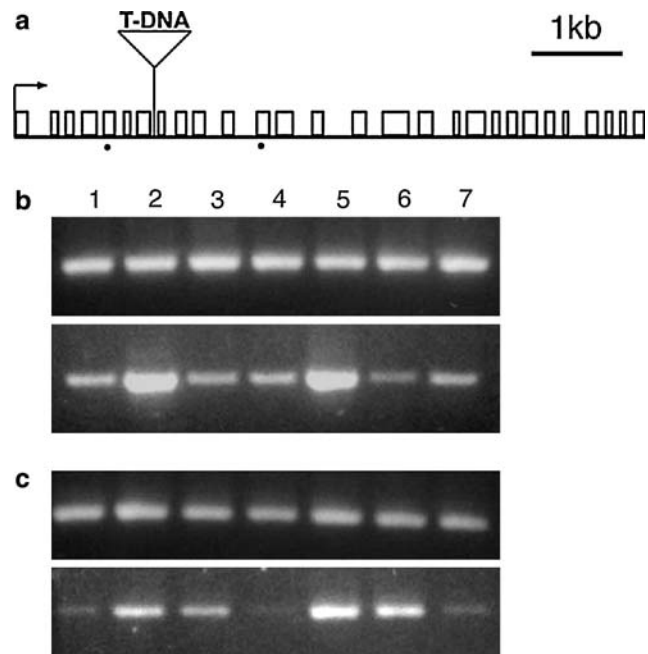


Fig. 1 *AtCAP-C* gene structure and expression. **a** Intron/exon map showing the 28 exons (open boxes). The position of the Syngenta 86D2 T-DNA insertion is shown. The dots below exons 5 and 12 are the approximate positions of primer binding sites used for genotyping. **b** RT-PCR analysis using RNA from wild type cauline leaves, inflorescences, roots, rosette leaves, seedlings, siliques and stems (lanes 1–7, respectively), actin gene specific primers (top) and *AtCAP-C* gene specific primers (bottom). **c** RT-PCR analysis of RNA from the 86D2 mutant. Lane designations are the same as in **b**. Top panel, actin signal; bottom panel, *AtCAP-C* heterozygote signal

Columbia plants. Total RNA was prepared and reverse transcribed with oligo dT, then aliquots were used for PCR amplification of both *AtCAP-C* and actin gene fragments. Expression of *AtCAP-C* is highest in seedlings and inflorescences, and significantly lower but detectable in other tissues examined (Fig. 1b). This expression profile is similar to that observed for *AtCAP-E* (Siddiqui et al. 2003) and may be generally correlated with the mitotic indices of the tissues examined. To gauge *AtCAP-C* mRNA levels in 86D2 heterozygotes, we conducted RT-PCR as described above. Figure 1c shows that, while actin mRNA levels were similar to that observed in wild type, the intensity of *AtCAP-C* bands is reduced in all 86D2 samples, as might be expected since only one copy of the gene has suffered a mutation (see below).

Analysis of the *AtCAP-C* mutant phenotype

In order to identify mutant plants homozygous for the insertion at the *AtCAP-C* locus, we screened seeds generated from a segregating population of the *AtCAP-C* mutant line; 32 BASTA resistant plants were genotyped by PCR using primers flanking the T-DNA insertion site. Selection on BASTA ensures that resistant plants have at least one copy of the T-DNA insert. No homozygous mutant plants were identified among the 32 BASTA resistant plants that were genotyped. Consistently, all BASTA resistant plants were heterozygous for the T-DNA insertion. DNA gel blot analysis of genomic DNA from *AtCAP-C*^{+/-} plants showed that the T-DNA was only inserted at the *AtCAP-C* locus in a tandem array of at least two copies of the T-DNA (data not shown). The inability to obtain a homozygous mutant of *AtCAP-C* strongly suggests that disruption of both copies of *AtCAP-C* is lethal and that *AtCAP-C*^{-/-} individuals are lost early in development.

Embryogenesis is compromised in *AtCAP-C* mutants

The developing siliques of selfed *AtCAP-C*^{+/-} plants were examined for aberrant seed phenotypes. We observed a large number of defective unfertilized ovules (compared to the wild type) that appeared as empty spaces between the apparently normal sibling seeds (Fig. 2a, b). Amongst the fertilized ovules, both green (harboring normally developing embryos) as well as white (containing arrested embryos) seeds were observed (Fig. 2b). Although DNA gel blotting revealed only one insertion site for the T-DNA in this line, to ensure that this defect is due to an insertion in the *AtCAP-C* gene, we transformed the 86D2 line with a wild type gene and following selection of T2 plants, found that the defective seed phenotype was rescued by the transgene (Fig. 2c).

Next, embryo development in both the normal and defective seeds was examined using differential interference contrast microscopy. While the wild type sibling



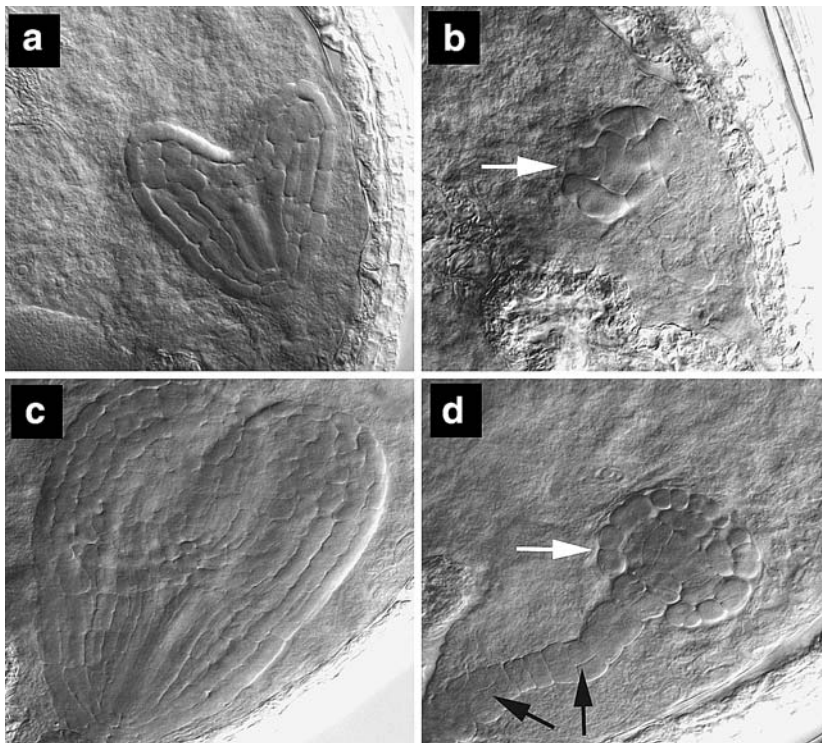
Fig. 2 Silique morphology of wild type and mutant self crosses. **a** Mid mature silique of a wild type Columbia plant, illustrating normal morphology of developing seeds. **b** Silique of a selfed *AtCAP-C*^{+/-} plant showing empty spaces and aborted seeds (arrows) segregating with normally developing siblings. **c** Silique of 86D2 plants (T2 generation) transformed with a wild type *AtCAP-C* gene showing rescue of the defective seed phenotype

seeds harbored embryos at about the heart stage of development (Fig. 3a), the mutant embryos were developmentally delayed and often arrested at what appears to be the globular stage (Fig. 3b). Occasionally, the embryo would proceed past this stage, but proper patterning was disrupted. Figure 3c shows such an embryo in which the cotyledons failed to separate. Aberrant development was associated with altered planes of cell division in both the embryo and the suspensor (Fig. 3b, d), a defect also observed for *AtCAP-EI/2* mutant embryos (Siddiqui et al. 2003). Thus, as might be expected for the heterodimer partner of *AtCAP-E*, mutations in *AtCAP-C* results in patterning defects and embryonic lethality.

Genetic analysis of the *AtCAP-C* mutation

We screened seeds generated from self-crosses of *AtCAP-C*^{+/-} plants to determine what percentage of seeds are resistant to BASTA. If all resulting genotypes are viable at the gametophyte stage we would expect the genotypic ratio in the sporophytes to be 1 *AtCAP-C*^{-/-} (BASTA resistant): 2 *AtCAP-C*^{+/-} (BASTA resistant): 1 *AtCAP-C*^{+/+} (BASTA sensitive). This genotypic ratio would lead to a 3:1 ratio of BASTA resistance: BASTA sensitivity if the *AtCAP-C*^{-/-} plants are viable. Since we had evidence of embryo lethality in the null mutant plants we expected to see a ratio of less than 3:1. In the case of embryo lethality, one would expect the ratio of 2:1. Ratios lower than 2:1 indicate embryo lethality in homozygous mutants and failure in either female or male gametogenesis; ratios lower than 1:1 are indicative of inviability of both female and male gametophytes (Pagnussat et al. 2005). We observed a ratio of 1:0.18 ($n=1,546$; Chi squared tests revealed a *P* value of less than 0.005, data not shown), strongly suggesting gametogenic lesions in *AtCAP-C*⁻ gametophytes. However, an alternative possibility is that the BASTA gene

Fig. 3 Cytological analysis of embryos and suspensors. Plants heterozygous for the T-DNA insertion were allowed to self and developing seeds were examined by Normarski optics to examine embryos and suspensors. **a** Normal sibling embryo at the heart stage of development. **b** Abnormal sibling arrested at approximately the globular stage of embryogenesis. **c** Abnormal sibling at approximately the heart stage that exhibits fused cotyledons. **d** Abnormal sibling arrested at the globular stage. Arrows point to altered planes of cytokinesis in suspensors and embryos



underwent silencing without T-DNA loss. To investigate this, we explanted 12 BASTA sensitive seedlings to soil, then genotyped each by PCR using a T-DNA left border primer and the gene specific primer, as well as with primers flanking the T-DNA insertion site. No fragment was amplified with the T-DNA specific and gene specific primers, however, a fragment of the expected size (for the wild type gene) was amplified with the gene specific primers flanking the T-DNA insertion site. This strongly suggested that the extreme skewing of the ratio against recovery of BASTA resistant plants was not due to the silencing or inactivation of the BASTA resistance gene on the T-DNA, but rather was due to a reduction in the recovery or viability of *AtCAP-C*^{+/-} heterozygous seeds.

To examine male and female gametophytic affects separately, progeny of reciprocal genetic crosses between heterozygous *AtCAP-C*^{+/-} individuals and wild type plants were performed and the resulting F1 seeds were scored for the segregation of the T-DNA locus conferring BASTA resistance to the progeny. Since *AtCAP-C*^{+/-} plants would produce equal numbers of wild type and mutant gametes (C⁺, C⁻, respectively), 50% of the F1 progeny would be expected to be heterozygous for the T-DNA insertion (BASTA resistant) while the other 50% would be expected to be wild type (BASTA sensitive), if there were no sources of inviability.

When *AtCAP-C*^{+/-} plants were used as the female parent, only 19.8% of the F1 progeny were BASTA resistant (*n*=164). This indicated that at most, 40% (19.8% observed versus 50% expected) of the mutant megaspores were able to combine with wild type pollen to produce viable, BASTA resistant embryos. Thus 60%

of the mutant megaspores failed either in megasporogenesis or embryogenesis. If the skewing of the ratio against recovery of resistant plants were due exclusively to failure of embryogenesis in heterozygotes, without failure in gametogenesis, then the skewed ratios should have been the same in our reciprocal crosses. While we did see extensive skewing against recovery of BASTA resistant plants in both crosses, the effect was more dramatic when *AtCAP-C*^{+/-} plants were used as the male parent, an even greater skewing against BASTA resistance was observed; only 4.8% of the F1 progeny were BASTA resistant (*n*=156). Thus there is clear evidence that *AtCAP-C*⁻ microspores fail in microsporogenesis.

Pollen development in *AtCAP-C*^{+/-} plants

The genetic data indicated that male gametogenesis is compromised in *AtCAP-C* mutant microspores. To look for the origin of lethality we examined pollen grain mitoses for aberrant segregation. In the Syngenta T-DNA collection, all mutants were generated in a background for the *quartet* mutation (Preuss et al. 1994). Therefore, we examined pollen from both 86D2 *AtCAP-C* heterozygotes and from control *quartet* plants (*AtCAP-C*^{+/+}). Of 43 pollen grains from three different *quartet* plants, all appeared to undergo the pollen grain mitoses normally. We examined 46 pollen grain mitoses from two *AtCAP-C* heterozygotes in a *quartet* background and found 21 nuclei exhibiting obvious chromosome defects. The most obvious defect was failure of the chromosomes sets to properly separate at anaphase

as indicated by the presence of chromatin bridges that connected the two chromatin bodies (Fig. 4). As the 86D2 parent plants were necessarily heterozygous, this frequency is near the 50% value expected if the microspores are compromised by a defective *AtCAP-C* allele, and supports a critical role for *AtCAP-C* in chromosome condensation and segregation during mitosis.

Defects in *AtCAP-C*^{+/-} heterozygotes

The chromosome bridges observed in the microspore mitosis can create chromosome fragments that likely compromise gametogenesis, and could cause abnormalities in heterozygous embryos and plants. We examined embryos from the *AtCAP-C*^{+/-} plants crossed to wild type at 4–7 days after fertilization, but found very few aberrant embryos (data not shown).

However, many of the recovered *AtCAP-C*^{+/-} heterozygotes did exhibit aspects of fasciation, most commonly manifested as supernumary shoots and phyllotaxy defects. To insure that these altered phenotypes were not due to any effect of the selection media but rather correlate with the interruption of the *AtCAP-C* gene, we planted unselected seeds to soil and screened the resulting plants for both phenotypic defects and the segregation of the *AtCAP-C* associated T-DNA. In all cases where phenotypic defects were observed, PCR

reactions confirmed the existence of a T-DNA within the *AtCAP-C* gene (data not shown). Thus it is possible that some *AtCAP-C*^{+/-} heterozygotes fail in embryogenesis, but at stages later than 4–7 days after fertilization.

We conclude that the *AtCAP-C*^{-/-} genotype produces embryo lethality, and that gametogenesis is compromised to a variable extent in *AtCAP-C*⁻ gametophytes. *AtCAP-C*⁻ gametophytes that do survive might have some chromosome fragmentation due to mitotic abnormalities that occur in the gametophytic mitoses. These abnormalities may cause subsequent aberrations in heterozygous embryos and fasciation in the limited number of recovered mature heterozygous plants.

Discussion

Condensins are ubiquitous central components of the chromosome condensation machinery in eukaryotic cells (Losada and Hirano 2005). Interaction of these proteins with various partner proteins leads to the assembly of complexes that sponsor a variety of other cellular processes (Hagstrom and Mayer 2003).

We previously characterized the *AtCAP-E* genes of *Arabidopsis* (Siddiqui et al. 2003), and as might be expected for its heterodimer partner, many aspects of *AtCAP-C* expression and mutant phenotypes that are reported here are strikingly similar. Both *AtCAP-C* and *AtCAP-E* are expressed in all tissues, but expression levels are highest in young tissues where cells are continuing to cycle and have not yet reached the cell expansion phase when mitotic indices are much lower. Reducing the levels of either protein results in developmental defects, manifest as reduction in growth rate, loss of apical dominance, and phyllotaxy defects. Spores carrying null alleles exhibited a severe decline in viability and this we correlated with defects in chromosome segregation during cell division. In situations where spores participated in successful fertilization, developmental defects were observed in mutant embryos. These were manifest as slow growth, disorganization of embryos and/or suspensors, altered planes of cell division and ultimately, embryonic death. Based on the statistics of self crosses and reciprocal crosses with wild type, these defective mutants are likely *AtCAP-C* nulls. Lastly, heterozygous plants exhibit substantially normal development, but in some instances loss of apical dominance and phyllotaxy defects were observed.

In plants, perturbation of genes that coordinate different aspects of chromatin organization and cell cycling give rise to developmental defects similar to those we observed (Kaya et al. 2001; Hemerly et al. 2000; Hennig et al. 2003; Stevens et al. 2004). In the case of the *fasciata* mutants, which represent defects in chromatin assembly factors, structural and functional meristem defects could be correlated with altered expression of the *WUSCHEL* (*WUS*) and the *SCARECROW* (*SCR*) genes, which govern shoot and root meristem function,

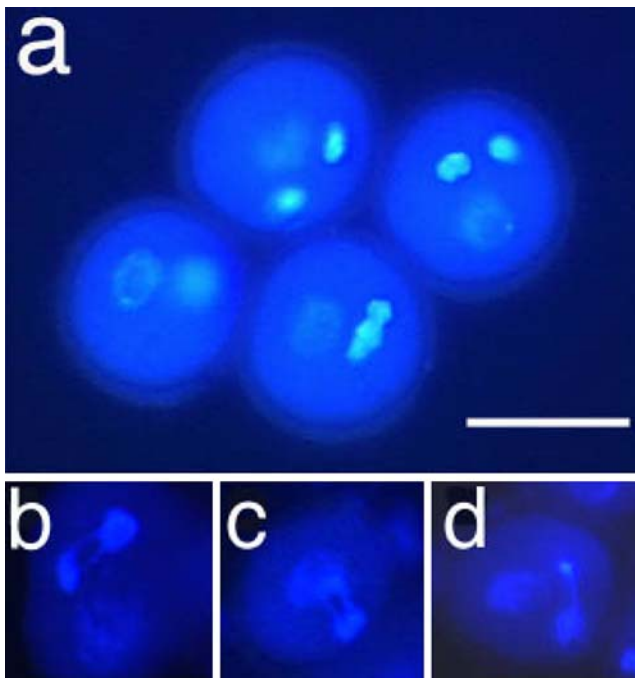


Fig. 4 Cytological analysis of pollen grain mitoses. **a** Tetrad from a selfed 86D2 (*AtCAP-C*^{+/-}) plant, illustrating two apparently normal mitoses (top), a chromatin bridge (lower right), and a fourth pollen grain out of the plane of focus (lower left). **b–d** Examples of other pollen grains with chromatin bridges in *AtCAP-C*⁻ segregants. Bar = 15 μ m

respectively (Kaya et al. 2001). Mutants in the *AtCAP-E* genes cause developmental anomalies (Siddiqui et al. 2003) and it has recently been shown that these mutants express RNAs that are normally transcriptionally silent (Takeda et al. 2004). Similarly, when the MSI1 protein is depleted by cosuppression, various developmental defects were observed and molecular analyses revealed that the temporal and spatial expression patterns of two homeotic genes, *AGAMOUS* and *APETALA2*, were altered (Hennig et al. 2003). Analyses of MSI1 in various systems suggests that the protein serves to guide heterochromatin formation and maintain some genes in repressed states. Lastly, mutations in genes involved in regulating other aspects of DNA metabolism and cell cycle progression also condition aberrant developmental phenotypes (Bundock and Hooykaas 2002; Takahashi et al. 2002; Stevens et al. 2004). Taken together, these studies provide strong evidence that changes in chromatin dynamics can alter gene expression, and serve to reinforce the importance of epigenetic regulation of genes in controlling development.

How altered expression of these developmental regulators underpins the observed phenotypic defects in the mutants remains a mystery. Defects in cell cycling, due to improper chromatin assembly or delays in replication or packaging, may simply activate checkpoint controls that lead to slower growth and reductions in organ size as is observed for some of the mutants. Fasciation and phyllotaxy defects are more difficult to decipher, but implicate differentiation programs and alterations in the machinery that specifies the proper planes of cytokinesis. We have demonstrated that the tunica/corpus organization of the SAM is perturbed in *AtCAP-E* antisense plants (Siddiqui et al. 2003) and that periclinal divisions in suspensors occur in both *AtCAP-E* and *AtCAP-C* compromised plants. It has been shown that the position of spindle poles influences the orientation of cytokinesis (O'Connell and Wang 2000; Kusch et al. 2003), and given the requirements for condensin in centromere organization and proper spindle assembly in animal cells (Hagstrom et al. 2002; Wignall et al. 2003) it seems likely that altered condensation of chromosomes leads to changes in the relative positioning of spindles to result in random patterning changes. In plants the spatial orientation of cell division depends on phragmoplast formation and a number of factors having roles in cytokinesis have been identified (Nacry et al. 2000). Phragmoplastin, a dynamin-like protein, can influence the orientation of cytokinesis as constitutive expression (Geisler-Lee et al. 2002) and localized expression (Wyrzykowska and Fleming 2003) can lead to alterations in cell division. There are at least 16 different dynamin-related proteins in *Arabidopsis*, and the four that have been characterized all interact with phragmoplastin (Hong et al. 2003). One of these (*ADL1*) is required for embryogenesis and seedling development (Kang et al. 2003). These dynamin-related proteins are thus prime targets to investigate as potential mediators of the cytokinetic defects in condensin deficient plants. The growing spectrum of

annotated genes and technical advances in transcription profiling will permit the testing of this hypothesis and the identification of additional components of the pathways linking chromosome organization with alterations in cell fate and morphogenesis.

Acknowledgements The authors wish to thank Dr. Nancy Dengler for advice and assistance with microscopy, Dr. Thomas Berleth for helpful discussions and Mr. David Doua for assistance with genotyping. We acknowledge the *Arabidopsis* Biological Resource Center for seeds of the quartet mutant, and Syngenta for providing the 86D2 lines. N.U.S. was supported by an Ontario Graduate Scholarship and a University of Toronto Research Fellowship. This research was supported by grants from the Natural Sciences and Engineering Research Council to C.A.H. and C.D.R.

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