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Genetics

The *Arabidopsis SKP1-LIKE1* gene is essential for male meiosis and may control homologue separation

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ABSTRACT **The yeast and human *SKP1* genes regulate the mitotic cell cycle but are not yet known to be required for meiosis. Nine *Arabidopsis SKP1* homologues have been uncovered and are named *ASK1* through *ASK9*. Here, we report the isolation and characterization of a male sterile *Arabidopsis* mutant and show that the mutant defect was caused by a *Ds* transposon insertion into the *ASK1* gene. In the *ask1-1* mutant, abnormal microspores exhibit a range of sizes. Furthermore, during mutant male meiosis, although homologous chromosome pairing appeared normal at metaphase I, chromosome segregation at anaphase I is unequal, and some chromosomes are abnormally extended. Therefore, in *ask1-1*, at least some homologues remain associated after metaphase I. In addition, immunofluorescence microscopy indicates that the mutant spindle morphology at both metaphase I and early anaphase I is normal; thus, the abnormal chromosome segregation is not likely caused by a spindle defect. Because the yeast Skp1p is required for targeting specific proteins for ubiquitin-mediated proteolysis, we propose that *ASK1* controls homologue separation by degrading or otherwise removing a protein that is required directly or indirectly for homologue association before anaphase I.**

Chromosome association and separation are two fundamental processes in cell division of all cellular organisms. In eukaryotic organisms, there are two different types of chromosome association and separation: those of sister chromatids during mitosis and meiosis II and those of homologous chromosomes during meiosis I. Normal meiotic chromosomal behavior is essential for segregation of genetic materials during eukaryotic sexual reproduction. Several proteins required for homologue pairing have been identified [see reviews (1, 2)]. However, it is not known what proteins are involved in controlling meiotic homologue separation.

Meiosis in plants has been studied for many years by using cytology and genetics (for review of early studies, see refs. 3 and 4; see also refs. 5–8). In recent years, cytological studies also have been performed to examine meiosis in *Arabidopsis thaliana* (7, 9–11), and *Arabidopsis* meiotic mutants have been identified (10–16). For example, the *syn1* mutant has defective male meiosis showing abnormal chromosome segregation and producing 5–8 variably sized spores (10). *SYN1* has been cloned recently, and it encodes a protein with similarity to the yeast *RAD21* gene (17). On the other hand, little is known about the molecular nature of genes controlling chromosome separation during meiosis.

Recently, the yeast and human *SKP1* genes have been found to regulate the mitotic cell cycle (18). The human SKP1 protein associates with the cyclin A–cdk2 complex that controls entry into the S phase (19). In yeast, the Skp1p protein interacts with cyclins, the Cdc4p protein, and a component of the mitotic kinetochore (20, 21). Mutant analysis indicates that the yeast *SKP1* gene is required for entry into the S phase and for completion of the M phase (20, 21). The yeast Skp1p protein is an essential component of the ubiquitin–ligase complex SCF that targets specific proteins for degradation (20–24). The SCF complexes consist of Skp1p, Cdc53p/cullin, and one of the F-box-containing proteins, which are receptors for specific targets of proteolysis (21–23). At M phase, Skp1p is associated with the kinetochore and may mediate the phosphorylation and degradation of another kinetochore component, allowing sister chromatids to separate (20, 24). Although *SKP1* is essential for the yeast mitotic cell cycle, it is not known whether it is also required for meiosis.

In *Arabidopsis*, a *SKP1* homologue, *Atskp1*, was recently shown to be highly expressed in dividing cells, such as those in meristems and organ primordia (25), consistent with an expected role in mitosis. Furthermore, *Arabidopsis* cDNA and genomic sequencing projects indicate that *Arabidopsis* has at least 9 *SKP1* homologues (26). To avoid confusion and to conform to *Arabidopsis* gene nomenclature conventions, we and others have renamed the *Atskp1* as *ASK1*, for *Arabidopsis SKP1-like 1*, and the other *Arabidopsis SKP1* homologues *ASK2* through *ASK9* (26). Previously, no mutant was available for any of the *ASK* genes; therefore, their *in vivo* functions are not clear.

In this report, we describe the isolation of an *Arabidopsis* male sterile mutant, *ask1-1*, that carries a *Ds* insertion in the *ASK1* gene. We demonstrate that this *Ds* insertion is the cause of the male sterility. We found that the *ask1-1* mutant is defective in male meiosis, exhibiting an uneven distribution of chromosomes and a failure in separation of at least some homologous chromosomes during male anaphase I. Fluorescence microscopy of wild-type and mutant male meiotic cells revealed no detectable difference in meiosis I spindles. Because the yeast protein Skp1p is an essential component of the ubiquitin–ligase complex (18), we propose that the *Arabidopsis* ASK1 protein regulates, directly or indirectly, the degradation and/or modification of a protein required for homologue association.

MATERIALS AND METHODS

Isolation of Mutant and Revertants. *A. thaliana* (ecotype Landsberg *erecta*-Ler) *Ds* transposon insertional lines were generated according to Sundaresan *et al.* (27). To obtain revertant sectors, mutant F_2 plants from the original *Ds* to *Ac* cross were grown to maturity. In mutant plants carrying the *Ac*

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Abbreviations: DAPI, 4,6-diamino-2-phenylindole dihydrochloride; DIC, differential interference contrast; MMC, microspore mother cell; SCF, Skp1p, Cdc 53p/cullin, F-box-protein; TAIL-PCR, thermal asymmetric interlaced PCR.

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element, remobilization of the *Ds* element resulted in the production of revertant sectors, which were recognized as single or multiple fertile seedpods. The plants from the seeds of individual revertant sectors segregated for both normal and mutant plants roughly in a 3:1 ratio, indicating that they did not result from fertilization by contaminating normal pollen.

Molecular Analysis. Thermal asymmetric interlaced (TAIL)–PCR was performed according to Liu *et al.* (28), using the degenerate primer AD2 (28) and nested *Ds* primers as described by Grossniklaus *et al.* (29). PCR with plant sequence primers (5′-AAGGTGATCGAGTATTGCAAGAG and 5′-GACATGTTAGAATCAAAGCAAGT) was performed with an annealing temperature of 60°C. For DNA gel blot analysis, the 301-bp *Eco*RI-*Stu*I fragment of the cDNA was used as the probe. For mRNA gel blot analysis, total RNA was extracted from floral buds of the wild-type and mutant plants. A 0.65-kilobase *Xho*I fragment including most of the *ASK1* cDNA was used as the probe.

Light Microscopy. For bright-field light microscopy, fresh anthers were dissected by using fine syringe needles to release the contents onto a slide. Samples were stained with 0.002% Toluidine Blue and were examined and photographed by using a Nikon compound microscope. For 4, 6-diamino-2-phenylindole dihydrochloride (DAPI) and anti-β-tubulin fluorescence microscopy, influorescences were fixed in Carnoy's fixative (6 ethanol:3 chloroform:1 acetic acid), and the anthers of individual fixed buds were dissected in a small drop of water as described above for fresh anthers. The dissected anthers were air-dried and stained with a drop of 1 μg of DAPI/ml of Vectorshield (Vector Laboratories) solution. Immunofluorecence microscopy was performed according to Bai *et al.* (17) with samples prepared as described in ref. 30. Propidium iodide (1 μg/ml of Vectorshield) was used for DNA staining in the anti-β-tubulin experiments. Samples were examined and photographed by using Zeiss or Nikon microscopes. Photographs were scanned and digitally enhanced by using PHOTOSHOP 5.0 (Adobe Systems, San Jose, CA).

RESULTS

Isolation of a Male Sterile Mutant. To identify mutants affected in meiosis and male reproduction, we first generated 550 *Arabidopsis Ds* insertional lines and screened them for sterile mutants. We found that one line, designated ET5223, was male sterile because of a nuclear recessive mutation (data not shown). Female fertility in this mutant appeared to be normal because it produced full sets of seeds (>30/sillique) when pollinated with wild-type pollen, and ≈200 of the resulting F_1 plants also had normal fertility.

Molecular Analysis of the Mutated Gene. We first tested whether the mutation was caused by a *Ds* insertion by looking for fertile revertant sectors in mutant plants. Among 63 mutant plants, 17 plants produced a total of 22 fertile sectors, suggesting that this mutation was indeed caused by a *Ds* insertion. To obtain the plant sequence adjacent to the *Ds* insertion, we generated a PCR product of ≈400 bp adjacent to the 3′ end of the *Ds* element by using the TAIL-PCR procedure (28) and *Ds*-specific primers. Database searches using the sequence of this PCR product revealed that it contains two regions exactly matching adjacent portions of the cDNA sequence of the *Arabidopsis ASK1* gene, a homologue of the yeast and human *SKP1* genes (20, 25). We designated the *Ds* insertional allele as *ask1-1* (Fig. 1*A*).

In the TAIL-PCR product between the two putative exons, there is a putative intron of 257 bp with canonical intron donor and acceptor sites. In addition, the *Ds* insertional site was found to be near the middle of the protein-coding region (Fig. 1*B*). When a primer with a sequence matching the cDNA sequence flanking the *Ds* insertion was paired with an appropriate 5′ or 3′ *Ds* primer, PCR products were obtained by using

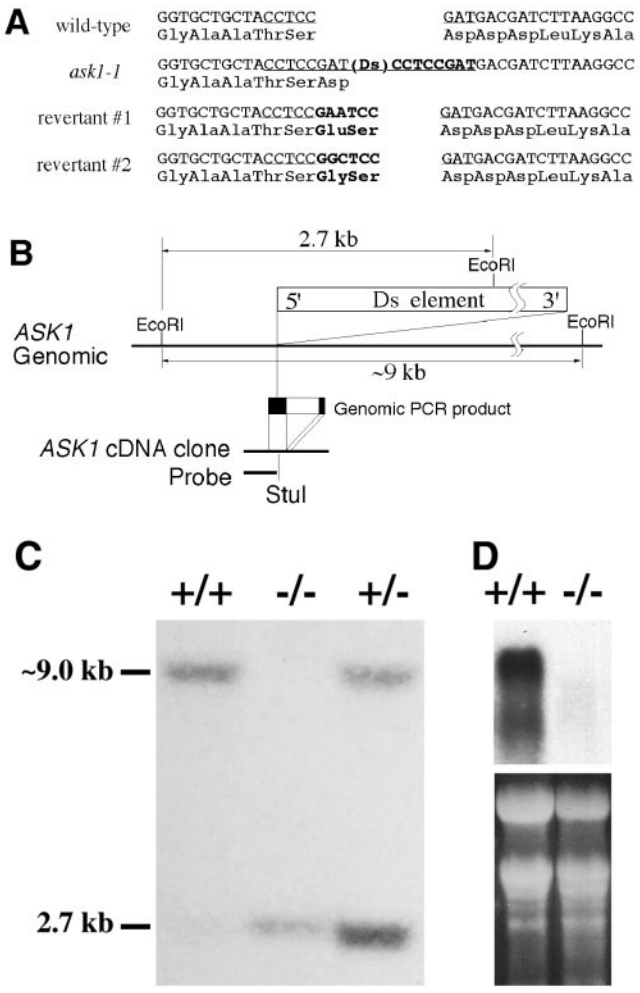

FIG. 1. Analysis of the *ASK1* locus. (*A*) Sequence near the *Ds* insertional site in the wild type, *ask1-1*, or two revertants. The duplicated sequence caused by the *Ds* insertion is underlined, and the altered sequences after *Ds* excision are in bold-face. (*B*) Map of the *ask1-1* locus showing the positions of the *Ds* insertion and *Eco*RI sites. Solid and open boxes in the genomic PCR product represent exon and intron sequences, respectively. Also indicated is the 301-bp region from the 5′ end to an *Stu*I site used as the probe for the Southern blot hybridization shown in *C*. (*C*) Genomic Southern hybridization analysis of the wild type (+/+), the mutant (−/−), and the heterozygous plant (+/−). (*D*) Northern hybridization analysis, showing that the wild type (+/+) contained a high level (4.5-hour radioactive exposure) of *ASK1* mRNA [0.8 kilobases (*Top*)] whereas it was not detectable in the mutant (−/−). The lower panel shows a photograph of the RNA gel stained with ethidium bromide.

DNA from the *ask1-1* mutant or *ASK1*/*ask1-1* heterozygous plants, but not from the wild-type DNA. Sequence analysis of these PCR products confirmed that the *Ds* element was inserted into the *ASK1* gene with an 8-nucleotide duplication, characteristic of a *Ds* insertion (Fig. 1*A*). This conclusion was supported by genomic Southern hybridization experiments (Fig. 1*C*; data not shown) using parts of the cDNA (obtained from the *Arabidopsis* Biological Resource Center at Ohio State University) (Fig. 1*B*) or the TAIL-PCR product as probes.

Furthermore, PCR using the two plant primers flanking the *Ds* insertional site produced a fragment of the expected size (457 bp, including the intron) from genomic DNA of the wild-type and heterozygous plants, but not from the *ask1-1* mutant DNA. Similar PCR with DNA from phenotypically normal progeny of 14 revertant sectors resulted in normal- or approximately normal-sized products (not shown). Sequence analysis indicated that 2 revertants had 6-bp nucleotide dupli-

cations without interrupting the ASK1 ORF (Fig. 1*A*) and 12 revertants had wild-type sequence (data not shown). These results indicate that the *Ds* insertion in *ASK1* is indeed the cause of the male sterile phenotype. *ASK1* is expressed in meristems and other dividing cells, including those in floral buds (25). Northern blot analysis showed that *ASK1* was expressed in the wild-type floral buds but not in the mutant floral buds (Fig. 1*D*), indicating that the *Ds* insertion had disrupted *ASK1* expression.

The *ask1-1* Mutant Produces Abnormal Microspores and Tetrads. To understand the *ask1-1* male sterile defect, we examined pollen development in both the wild type and the mutant. Normal pollen (Fig. 2*A*) develops from the microspore (Fig. 2*C*), which is the product of the male meiosis in the microspore mother cells (MMCs). Microspores and pollen grains normally have uniform sizes at a given stage (Fig. 2 *A* and *C*). However, in *ask1-1* flowers, no normal pollen grain was released from the anther. Inside the *ask1-1* anthers, abnormal pollen grains had already degenerated into "shells" (Fig. 2*B*). Furthermore, *ask1-1* microspores at any given stage had variable sizes (Fig. 2*D*). In young *ask1-1* anthers, tetrads contained a variable number of microspores of different sizes (Fig. 2*F*), unlike the normal tetrads (Fig. 2*E*). The distribution of spore number per mutant "tetrad" is shown in Fig. 2*G*. Although the spore size and number variation resembles some previously described *Arabidopsis* mutants (10–12), the *ask1-1* mutant is unique in having tetrads with fewer than four microspores.

***ask1-1* Is Defective in Homologous Chromosome Segregation.** The above observations suggest that the *ask1-1* mutant is defective in male meiosis. To further investigate this defect, wild-type and *ask1-1* MMCs were examined by using fluorescence microscopy with DAPI staining before and during male meiosis. To preserve cellular structures as much as possible, we analyzed meiosis in hundreds of intact MMCs. Meiotic stages were determined by observing nuclear and spindle characteristics in differential interference contrast (DIC) images of MMCs and by comparing their DAPI images here with previously published results (10, 31). In the wild type, the meiosis I prophase MMC had a clearly visible nucleus in the DIC image (Fig. 3 *A* and *B*). The DAPI images of prophase I revealed condensing chromosomes (Fig.3 *F* and *G*). At metaphase I, a spindle was observed, and the nuclear membrane had disappeared (Fig. 3*C*). At the same time, chromosomes were highly condensed and formed a tight band at the equatorial plane (Fig. 3*H*). At anaphase I, the spindle is present (Fig. 3*D*), and the homologous chromosomes had separated (Fig. 3*I*). As the cell progressed to telophase I (Fig. 3*E*), the spindle has elongated between the two separate groups of chromosomes (Fig. 3*J*). It is clear that there are no DAPI staining chromosomes between the two groups of chromosomes at anaphase I or telophase I.

In the *ask1-1* mutant, male meiosis I prophase and metaphase (Fig. 3 *K–M*) appeared to be similar to those in the wild type, and chromosomes condensed and aligned at the metaphase plate. In contrast, from anaphase I (Fig. 3 *N–P*) to telophase I (Fig. 3 *Q–T*) in *ask1-1* plants, all meiotic cells (>100) examined showed nonseparation of some chromosomal materials and/or uneven separation. Often, some chromosomes appeared to be spread along the long axis of the spindle (Fig. 3 *N–R*). These elongated chromosomal materials likely represented stretched chromosomes that remained associated while being pulled toward the two opposite poles by the spindle. Frequently, the distribution of chromosomes is unequal (Fig. 3 *Q* and *T*). It is noteworthy that, sometimes, most of the chromosomes are on one side of the equatorial plane (Fig. 3*S*). Furthermore, some chromosomes remained at or near the equatorial plane (Fig. 3 *N* and *S*).

The fact that, in the *ask1-1* mutant, meiosis I chromosomes aligned at the metaphase plate and then seemed to be pulled apart suggests that the meiosis I spindle is functionally similar to that of the wild type. To further determine the integrity of the *ask1-1* spindle, we performed immunofluorescence microscopy using an antibody against β-tubulin and found that

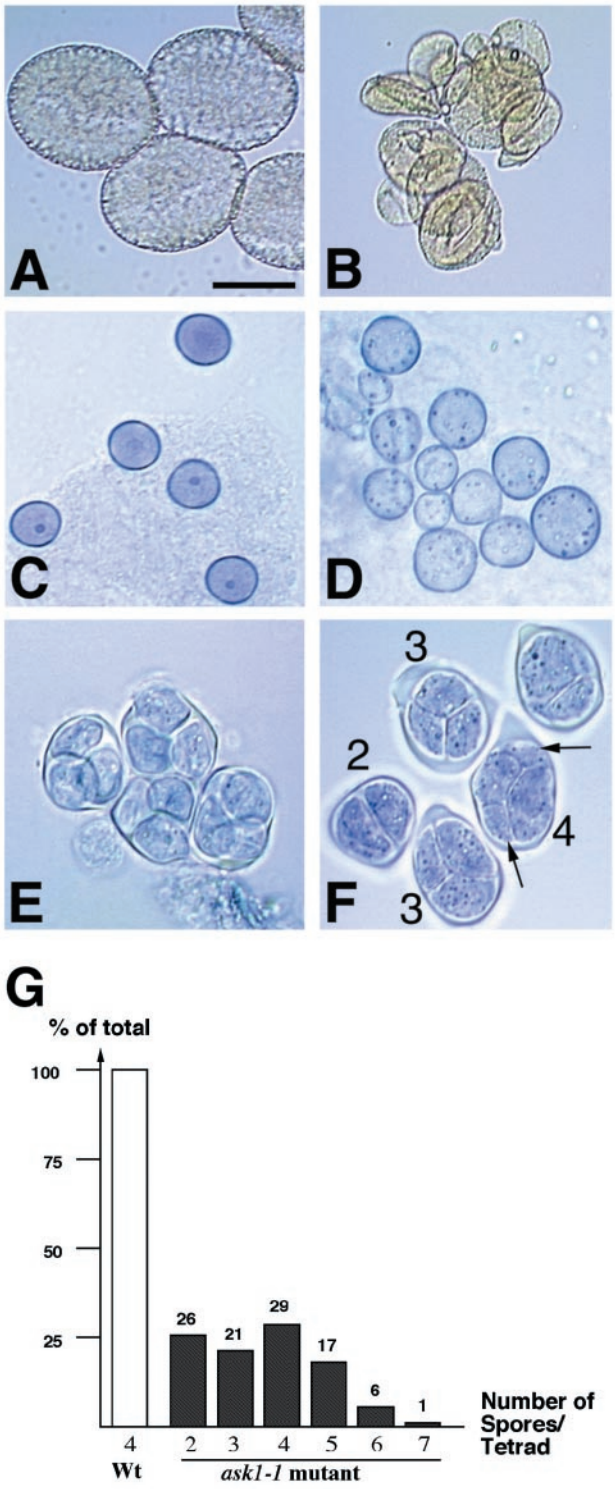

FIG. 2. Wild-type and *ask1-1* mutant pollen development. (*A*) Wild-type (Ler) pollen grains. (*B*) *ask1-1* pollen grains, which have degenerated. (*C*) Uniformly sized wild-type microspores. (*D*) *ask1-1* microspores of variable sizes from the same anther. (*E*) Wild-type tetrads, showing three of the four spores. (*F*) *ask1-1* tetrads, with the number of spores indicated. All panels have the same magnification. (Bar = 20 μm.) (*G*) Distribution of spore number in tetrads. More than 100 wild-type and 143 *ask1-1* tetrads were examined.

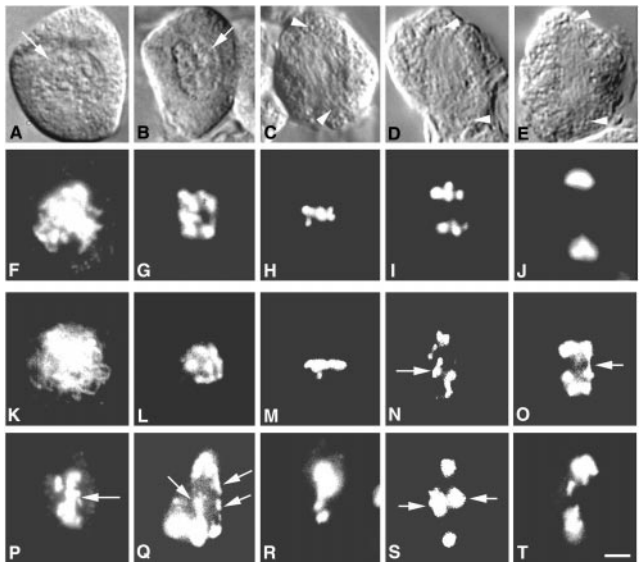

FIG. 3. Wild-type and mutant meiosis I. (*A–E*) DIC images of wild-type MMCs. (*F–J*) DAPI images of the cells shown in *A–E*, respectively. (*K–T*) DAPI fluorescent images of *ask1-1* MMCs. DIC images of *ask1-1* cells (not shown) are similar to those of wild-type cells at the same stage. (*A* and *B*) Prophase I and late prophase I, respectively. Both *A* and *B* had a nucleus at the center (arrow). (*C–E*) Metaphase I, anaphase I, and telophase I, respectively, showing spindles (approximate positions of the poles are indicated with arrowheads). (*F*) Chromosomes had begun to condense. (*G*) Condensed chromosomes can be recognized as brightly stained regions in the nucleus. (*H*) Chromosomes aligned at metaphase I. (*I*) Homologous chromosomes were separating in anaphase I. (*J*) Chromosomes moved to the two poles of the spindle. (*K–L*) The *ask1-1* prophase I seemed normal and showed progressive chromosome condensation, and the presence of a nucleus (not shown). (*M*) Highly condensed chromosome aligned at the metaphase plate. (*N–P*) At anaphase I in *ask1-1* cells, some chromosomes moved apart whereas others remained near the plate (arrows in *N* and *P*). Some extended DAPI staining was seen (*N*, arrow in *O*). (*Q–T*) Late anaphase or telophase I; some chromosomes remained at the equatorial plane persistently (arrows) whereas others reached the two poles. All panels have the same magnification. (Bar = 5 μm.)

ask1-1 spindle at male meiotic metaphase I (not shown) and early anaphase I (Fig. 4*C*) appeared to be the same as that in the wild type (Fig. 4*A*). This result indicates that the defect of meiotic chromosome segregation in the *ask1-1* mutant is not likely to be caused by a defect in the spindle.

During wild-type meiosis II, each phase had characteristic DAPI staining patterns and DIC nuclear/spindle features (Fig. 5). At normal prophase II, two nuclei were visible in the DIC image (Fig. 5*A*), and two brightly DAPI-stained chromosomal groups were separated by a lightly stained band of organelles (Fig. 5*F*), as also was observed by others (10, 31). On the other hand, in the *ask1-1* mutant at prophase II, some chromosomal materials continued to extend across the organelle band between the two chromosome groups (Fig. 5 *K*, *P*, and *U*). At metaphase II in the wild type, two spindles were observed in the DIC image (Fig. 5*B*), the DAPI-stained organelle band was parallel with the spindles, and two groups of condensed chromosomes resided at the equatorial planes (Fig. 5*G*). In contrast, in the *ask1-1* mutant, the distribution of chromosomes was more scattered (Fig. 5*L*) and uneven (Fig. 5 *Q* and *V*). Some chromosomes continued to straddle across the organelle band (Fig. 5*L*). Later in the wild type, meiotic II spindles appeared to have elongated (Fig. 5*C*) and chromatids separated (Fig. 5*H*) in anaphase II. At late telophase II, spindles had disappeared (Fig. 5*D*), and four groups of chromosomes were well separated from each other (Fig. 5*I*). In comparison, the *ask1-1* mutant meiosis after metaphase II

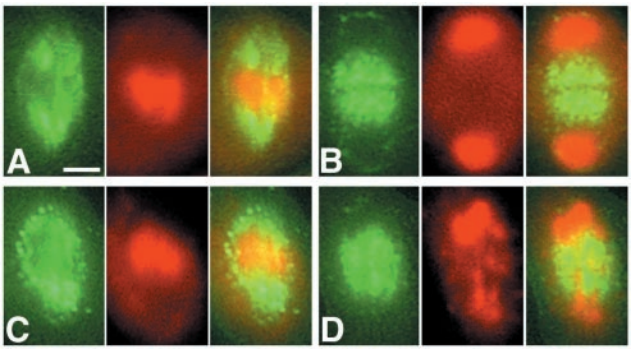

FIG. 4. Immunofluorescence microscopy of spindles and chromosomes. Each set of three panels are from a single representative meiotic cell. The left panel is an image of the fluorescein staining of the microtubule structure, the center panel is an image of the chromosomes stained with propidium iodide, and the right panel is a composite image of both. (*A*) A wild-type cell at early anaphase I. (*B*) A wild-type cell at telophase I, showing the new microtubule structure between the separated groups of chromosomes. (*C*) An *ask1-1* cell at early anaphase I; both the spindle and the chromosomes appear to be the same as those in the wild-type cell. (*D*) An *ask1-1* cell at telophase I. Although the microtubule structure is similar to the normal one, the chromosome separation is clearly not normal. All panels have the same magnification. (Bar = 5 μm.)

continued to exhibit abnormal chromosomal behavior. Some chromosomes seemed to remain connected, either parallel with or across the organelle band (Fig. 5 *M* and *R*). In addition, more than four areas of chromosomes could often be seen at approximately the time of telophase II (Fig. 5 *N*, *S*, and *X*). At the conclusion of normal meiosis, cytokinesis produced four microspores (three are visible in Fig. 5 *E* and *J*), each with an equal amount of DNA, as indicated by DAPI staining (Fig. 5*J*). However, in the *ask1-1* tetrads, number of microspores ranged from 2 to 7 (Fig. 3*G*; Fig. 5 *O*, *T*, and *Y*). Also, the sizes of spores were often different in *ask1-1* tetrads (Fig. 5 *T* and *Y*).

It is important to point out that, throughout normal meiosis II, there was no chromosomal connection, either across the organelle band or along the spindles. On the other hand, in *ask1-1* meiosis II, chromosomal connections were observed both across and parallel with the organelle band. In addition, closely spaced pairs of chromosomes often were seen in the mutant (double arrows in Fig. 5 *Q*, *V*, and *W*), suggesting that they may be homologues that fail to separate. In conclusion, meiosis in *ask1-1* was abnormal from as early as mid-anaphase I, showing defects in homologous chromosome segregation. Continued defects in subsequent steps of meiosis resulted in abnormal distribution of chromosomes to a variable number of chromosomal groups with different amounts of DNA and formation of microspores of different sizes.

DISCUSSION

Isolation of a *Ds* Insertion into the *ASK1* gene. It is known that *Arabidopsis* has at least nine different homologues (26) of the yeast and human *SKP1* genes. The lack of mutations in these *ASK* genes has made it difficult to study their *in vivo* functions, although preferential expression of *ASK1* in dividing cells (25) suggests that it also may be involved in the mitotic cell cycle, resembling the human and yeast *SKP1* genes (19–21). We have identified a *Ds* insertion into the *ASK1* gene. This new mutant now allows us to study the function of *ASK1* in *Arabidopsis*.

***ASK1* Is Essential for Male Meiosis in *Arabidopsis*.** The separation of homologous chromosomes during meiosis I is as important as the pairing of these chromosomes. Identification and analysis of genes involved in these processes are crucial to our understanding of the control of meiosis. Our results on the

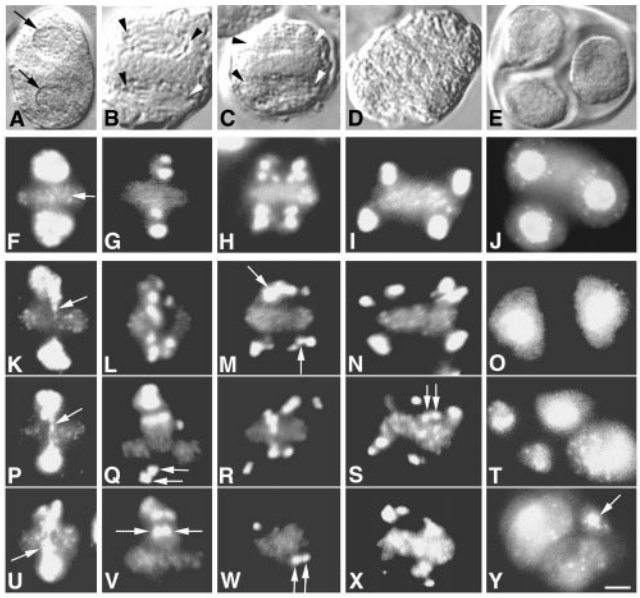

FIG. 5. Wild-type and mutant meiosis II. Images of similar stages are shown in the same column. (*A–E*) DIC images of wild-type MMCs. (*F–J*) DAPI images of the cells shown in *A–E*, respectively. (*K–Y*) DAPI fluorescent images of *ask1-1* MMC s. DIC images of *ask1-1* cells (not shown) are abnormal and not well defined. Therefore, assignment of stages for *ask1-1* cell relies largely on DAPI staining patterns. (*A*) Prophase II, with two nuclei (arrows). (*B*) Metaphase II, with two spindles; the arrowheads mark the positions of the spindle poles. (*C*) Anaphase II, with two spindles are marked as in *B*. (*D*) Telophase II, with spindles no longer present. (*E*) A tetrad, showing three of the four spores; the nuclei are visible. (*F*) Prophase II, with an organelle band (arrow) and two brightly stained groups of chromosomes. (*G*) Metaphase II, with two groups of highly condensed chromosomes, one on each side of the organelle band; only some chromosomes are visible in this plain of focus. (*H*) Anaphase II. The chromatids had separated, moving apart along the axis of the spindle. (*I*) Telophase II. Four brightly stained areas represent four groups of partially decondensed chromosomes. (*J*) Three stained nuclei of the tetrad. (*K*, *P*, and *U*) Three *ask1-1* cells at prophase II. Two brightly DAPI-staining regions were seen on either side of the organelle band, and some DAPI-staining materials were found across the organelle band (arrows). (*L*) A cell at metaphase II; the chromosomes were not localized to two regions. Some were near the organelle band. (*Q* and *V*) Two images of the same metaphase II cell at different focal planes. The area above the organelle band had much more staining than that below the band. (*M*) Approximately anaphase II. DNA seemed to be extended (arrows) parallel to the organelle band. (*R* and *W*) Two images of the same cell at approximately anaphase II, showing scattering of chromosomes. (*N*) Approximately telophase II, showing six regions of condensed chromosomes. (*S* and *X*) Two images of the same cell showing several regions of condensed chromosomes. Some chromosomes overlapped the organelle band. (*O*) A tetrad with two spores. (*T*) A tetrad with four spores, two of them are larger than the other two. (*Y*) An image of a tetrad showing three of its five spores. One of them (arrow) is extremely small. Double arrows in *Q*, *S*, *V*, and *W* indicate adjacent chromosomes. All panels have the same magnification. (Scale bar = 5 μm.)

ask1-1 mutant indicate that *ASK1* is essential for normal male meiosis. The *ask1-1* mutation causes the production of tetrads with a variable number of microspores that have different sizes and seem to have different amounts of DNA. Our analysis of the mutant meiosis suggests that the separation of homologous chromosomes is defective. At least some chromosomal pairs seemed to be stretched during anaphase I, as indicated by the extended DAPI-staining materials. It is striking that some homologues seemed to remain associated even at prophase II to anaphase II, suggesting that such association is very stable. In addition, the distribution of chromosomes is not equal after anaphase I. Quite often, the difference in amount of DAPI-staining between the two groups of chromosomes was great enough to suggest that one or more pairs of chromosomes have moved to one pole only. These abnormal chromosomal behaviors could be consequences of an abnormally prolonged association of homologues. The *ask1-1* mutant phenotype is similar to that described for the maize *mei025* mutant, which exhibits chromosome association beyond metaphase I (3). This maize mutant differs from *ask1-1* in that it is both male and female sterile, suggesting that it may be defective in another gene. Other maize "sticky-chromosome" mutations cause defects as early as prophase I and metaphase I (3), suggesting that they may affect different gene functions.

How may *ASK1* function in male meiosis, particularly in regulating homologous chromosome separation? Our immunofluorescence results indicate that the *ask1-1* meiotic I spindle appears to be normal. In addition, chromosomal behavior at metaphase I and early anaphase I suggests that the *ask1-1* spindle has normal functions in aligning and pulling the chromosomes. Therefore, the *ask1-1* defect in chromosomal segregation is not likely to be caused by an abnormally formed spindle during meiosis I. It is known that the yeast Skp1p is required for degradation of specific proteins, such as the cell cycle regulator Sic1p, by the ubiquitin-mediated proteolysis (20, 21). Yeast Skp1p also was found to be a kinetochore protein that mediates protein interactions for the phosphorylation of another kinetochore protein in yeast (20, 24). Thus, we propose that the *Arabidopsis* ASK1 protein is essential for the ubiquitin-mediated proteolysis, and/or protein phosphorylation, that is required (directly or indirectly) for disassembling a complex that holds homologues together. Although it is not known what the nature of the pairing structure is, it is not likely that ASK1 plays a major role in the degradation of the synaptonemal complex formed during prophase I because the chromosomal materials would be unstretchable by the spindle if the synaptonemal complexes were still intact. One possibility is that ASK1 mediates the breakdown of the chiasmata, structures that are thought to hold homologues together at metaphase I.

Possible Similarity of the Control of Chromosome Separation in Meiosis and Mitosis. In yeast, SCF and the anaphase promoting complex are known to be responsible for specifically targeting proteins for degradation through ubiquitin mediated proteolysis (18). It is also known that anaphase promoting complex is involved in release of sister-chromatid cohesion in yeast by proteolytic removal of an inhibitor for such release (32, 33). Our results support a proposed *Arabidopsis* SCF that functions in male homologue dissociation by mediating the degradation of a key protein regulator, which in turn controls the integrity of the pairing complex. This mechanism could be similar to that for release of sister-chromatid cohesion in yeast. It will be interesting to investigate whether anaphase promoting complex and SCF, respectively, regulate sister-chromatid cohesion and homologue separation in *Arabidopsis*, yeast, and other systems. Ubiquitin-mediated proteolysis also has been implicated in regulating other chromosomal behaviors, including telomere behavior in *Drosophila* (34) and disassembly of synaptonemal complex in mammalian systems (35). Therefore, ubiquitin-mediated proteolysis may be a major theme in multiple events concerning chromosome separation.

Diverse Functions of Plant *SKP1* Homologues. The fact that a single yeast *SKP1* gene is essential for mitosis and the *Arabidopsis ask1-1* mutant is viable and female fertile suggests that other *ASK* gene(s) is (are) likely to provide essential functions in other developmental processes such as vegetative growth and female homologous chromosome separation. The existence of multiple *SKP1* homologues is also true in another plant *Antirrhinum* (36). Thus, on one hand, it is likely that one or more of these genes are essential for mitosis and/or female meiosis whereas *ASK1* is essential for male homologue separation. On the other hand, *ASK1* also has a nonessential role

in processes other than male homologue separation because of its strong expression in many parts of the plant and the mild defect observed in the overall growth of the *ask1-1* plant (37). Therefore, in plants, duplicated *SKP1* homologous genes had diverged to allow different members of the gene family to acquire more specialized functions in mitosis and male or female meiosis while maintaining some overlapping functions. One known example of such gene divergence is the *Drosophila twine* and *string* genes: both are homologues of the fission yeast *cdc25* gene, with *twine* specialized in meiosis and *string* specialized in mitosis (38, 39). Further studies of other *ASK* genes, possibly as essential regulators of mitosis and female meiosis, should allow us to gain more insights into meiosis and mitosis and the roles of this gene family in controlling related cellular processes in the entire life cycle of *Arabidopsis*.

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