Arabidopsis Separase Functions beyond the Removal of Sister Chromatid Cohesion during Meiosis[1[OA]]

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Separase is a capase family protease that is required for the release of sister chromatid cohesion during meiosis and mitosis. Proteolytic cleavage of the α-kleisin subunit of the cohesin complex at the metaphase-to-anaphase transition is essential for the proper segregation of chromosomes. In addition to its highly conserved role in cleaving the α-kleisin subunit, separase appears to have acquired additional diverse activities in some organisms, including involvement in mitotic and meiotic anaphase spindle assembly and elongation, interphase spindle pole body positioning, and epithelial cell reorganization. Results from the characterization of Arabidopsis (*Arabidopsis thaliana*) separase (*ESP*) demonstrated that meiotic expression of *ESP* RNA interference blocked the proper removal of cohesin from chromosomes and resulted in the presence of a mixture of fragmented chromosomes and intact bivalents. The presence of large numbers of intact bivalents raised the possibility that separase may also have multiple roles in Arabidopsis. In this report, we show that meiotic expression of *ESP* RNA interference blocks the removal of cohesin during both meiosis I and II, results in alterations in nonhomologous centromere association, disrupts the radial microtubule system after telophase II, and affects the proper establishment of nuclear cytoplasmic domains, resulting in the formation of multinucleate microspores.

The proper segregation of chromosomes during mitosis and meiosis is dependent on the systematic formation and subsequent removal of sister chromatid cohesion, which is required for homologous chromosome pairing, recombination, and repair (for review, see Onn et al., 2008; Peters et al., 2008). It is also required for the pairwise alignment of chromosomes on the metaphase I spindle and for the generation of tension across centromeres, thereby ensuring their bipolar attachment. In mitosis, cohesion is maintained by the cohesin complex, which consists of four evolutionally conserved proteins: Sister Chromatid Cohesion1 (SCC1), SCC3, Structural Maintenance of Chromosome1 (SMC1), and SMC3 (for review, see Nasmyth and Haering, 2005). During meiosis, SCC1 is largely replaced by its meiotic homolog REC8.

The establishment of sister chromatid cohesion in yeast involves a multistep process (Milutinovich et al., 2007) that begins during telophase of the previous cell cycle when cohesin subunits associate with the chromatin, ultimately becoming enriched at discrete loci termed cohesin-associated regions (Blat and Kleckner, 1999; Laloraya et al., 2000). Cohesion is established during S-phase in a process that requires the Chromosome Transmission Fidelity protein (Ctf7), which is also known as Eco1 (Skibbens et al., 1999; Toth et al., 1999) and involves the replication fork (Kenna and Skibbens, 2003; Lengronne et al., 2006). In budding yeast (*Saccharomyces cerevisiae*) and fission yeast (*Schizosaccharomyces pombe*), cohesin complexes remain on the chromosomes until mitotic anaphase (Uhlmann et al., 1999, 2000; Tomonaga et al., 2000). In contrast, in vertebrates, most cohesin complexes are released from the chromosomes during prophase in a separase-independent process (Waizenegger et al., 2000; Losada et al., 2002). The small fraction of cohesin that remains primarily in centromeric regions is released to start anaphase (Sumara et al., 2000). The release of chromosome cohesion at the metaphase-to-anaphase transition is triggered by the Cys protease, separase (ESP1), which specifically cleaves the α-kleisin subunit (Ciosk et al., 1998; Uhlmann et al., 1999, 2000; Buonomo et al., 2000; Hauf et al., 2001). Prior to the metaphase-to-anaphase transition, securin inhibits the protease activity of separase. At the onset of anaphase, securin is degraded by the anaphase-promoting complex/cyclosome freeing separase, which cleaves SCC1, facilitating the release of cohesion and chromosome separation (Cohen-Fix et al., 1996; Ciosk et al., 1998).

Studies on the distribution of cohesin proteins during meiosis in a number of organisms, including yeast, *Caenorhabditis elegans*, mammals, and Arabidopsis (*Arabidopsis thaliana*), have shown that similar to the situation during mitosis in animal cells, a significant

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amount of cohesin is either removed from or redistributed on prophase chromosomes in a separase-independent process (Pasierbek et al., 2001; Cai et al., 2003; Eijpe et al., 2003; Lee et al., 2003; Yu and Koshland, 2005). The final resolution of chiasmata, formed as the result of homologous chromosome recombination, and the separation of homologous chromosomes depends on separase cleavage of the meiotic α-kleisin subunit, REC8, along chromosome arms at anaphase I (Buonomo et al., 2000; Kitajima et al., 2003). Centromeric cohesion is protected by the conserved SGO family of proteins until anaphase II when separase cleavage of REC8 facilitates the separation of sister chromatids (Rabitsch et al., 2003; Katis et al., 2004; McGuinness et al., 2005).

In addition to its highly conserved role in cleaving the α-kleisin subunit, separase appears to have acquired additional diverse activities in different organisms (Queralt and Uhlmann, 2005). For example, separase plays a role in DNA repair by promoting the redistribution of cohesin complexes to sites of DNA damage during mitotic interphase in budding and fission yeast (Nagao et al., 2004; Strom et al., 2004). Separase is also important for mitotic anaphase spindle assembly and elongation (Jensen et al., 2001; Papi et al., 2005; Baskerville et al., 2008), interphase spindle pole body positioning (Nakamura et al., 2002), and spindle formation during meiosis in yeast (Buonomo et al., 2003). It is also important for the proper positioning of the centrosomes during the first asymmetric mitotic division, eggshell development in *C. elegans* (Siomos et al., 2001; Rappleye et al., 2002), and for epithelial cell reorganization and dynamics in *Drosophila melanogaster* (Pandey et al., 2005). In zebra fish, a separase mutation causes genome instability and increased susceptibility to epithelial cancer (Shepard et al., 2007).

Results from the characterization of Arabidopsis separase suggested that the protein also has multiple roles in plants (Liu and Makaroff, 2006). Seeds homozygous for a T-DNA insert in Arabidopsis *ESP* exhibited embryo arrest at the globular stage with the endosperm exhibiting a weak *titan*-like phenotype. Furthermore, expression of *ESP* RNA interference (RNAi) from the meiosis-specific *DMC1* promoter disrupted the proper removal of the SYN1 cohesin protein from chromosomes during meiosis and resulted in the presence of a mixture of fragmented chromosomes and intact bivalents. The presence of large numbers of intact bivalents led the authors to suggest that in addition to its requirement for the removal of cohesin, ESP may also be required for either the proper attachment of the kinetochores to the spindle or spindle function. These findings, along with the observations that separase appears to have multiple roles in other organisms, led us to conduct a detailed characterization of meiosis in *ESP* RNAi plants.

In this report, we show that meiotic expression of *ESP* RNAi blocks the release of sister chromatid cohesion during both meiosis I and II, results in nonhomologous centromere association, disrupts the radial microtubule system (RMS) after telophase II, and affects the proper establishment of nuclear cytoplasmic domains. Unlike the large majority of plant meiotic mutants that have been characterized to date, reduction of ESP levels during meiosis leads to the formation of multinucleate microspores.

RESULTS

ESP Is Essential for Sister Chromatid Separation during Anaphase II

We previously showed that *DMC1*-driven expression of *ESP*-RNAi interferes with the separation of chromosomes during anaphase I and II (Liu and Makaroff, 2006). The apparent failure of sister chromatids to separate during anaphase II was predicted to be due to the continued presence of centromere cohesion; however, we could not rule out the possibility that the observed defects during meiosis II were due in part to the failure of homologous chromosomes to separate during meiosis I. In order to investigate this possibility and more thoroughly characterize the effect of reduced ESP levels on meiotic chromosome behavior, we introduced the *DMC1-ESP* RNAi construct into *ask1-1* plants, in which homologous chromosome pairing is altered (Yang et al., 2006), and conducted a detailed analysis of meiosis in plants defective in either one or both of the proteins.

The 4′,6-diamino-phenylindole (DAPI)-stained spreads of meiotic chromosomes from *ESP* RNAi, *ask1-1*, and *ESP* RNAi; *ask1-1* plants were prepared and analyzed. Chromosome condensation and the pairing of homologous chromosomes appeared relatively normal during leptotene, zygotene (data not shown), and pachytene (Fig. 1A) in *ESP* RNAi plants. However, we did observe that most pachytene-staged meiocytes in *ESP* RNAi plants contained fewer bright DAPI-stained areas than wild-type meiocytes at the same stage. Usually four to six brightly stained heterochromatin regions, including five centromeric and one nucleolar organizer region, are observed in wild-type meiocytes during pachytene. In contrast, we typically observed between one and three heterochromatin regions in *ESP* RNAi meiocytes at the same stage (Fig. 1A), suggesting that there is a higher frequency of nonhomologous centromere association in these cells. However, five bivalents were observed at diakinesis (Fig. 1B) and metaphase I (Fig. 1C), indicating that the association of nonhomologous centromeres is transient. During anaphase I, homologous chromosomes failed to separate properly, resulting in the presence of chromosomes stretched between the two spindle poles in most cells (Fig. 1D). Cells containing five bivalents were frequently observed at metaphase II (Fig. 1M). Stretched bivalents were again observed at anaphase II (Fig. 1N). A number of cells were observed containing nonseparated bivalents at

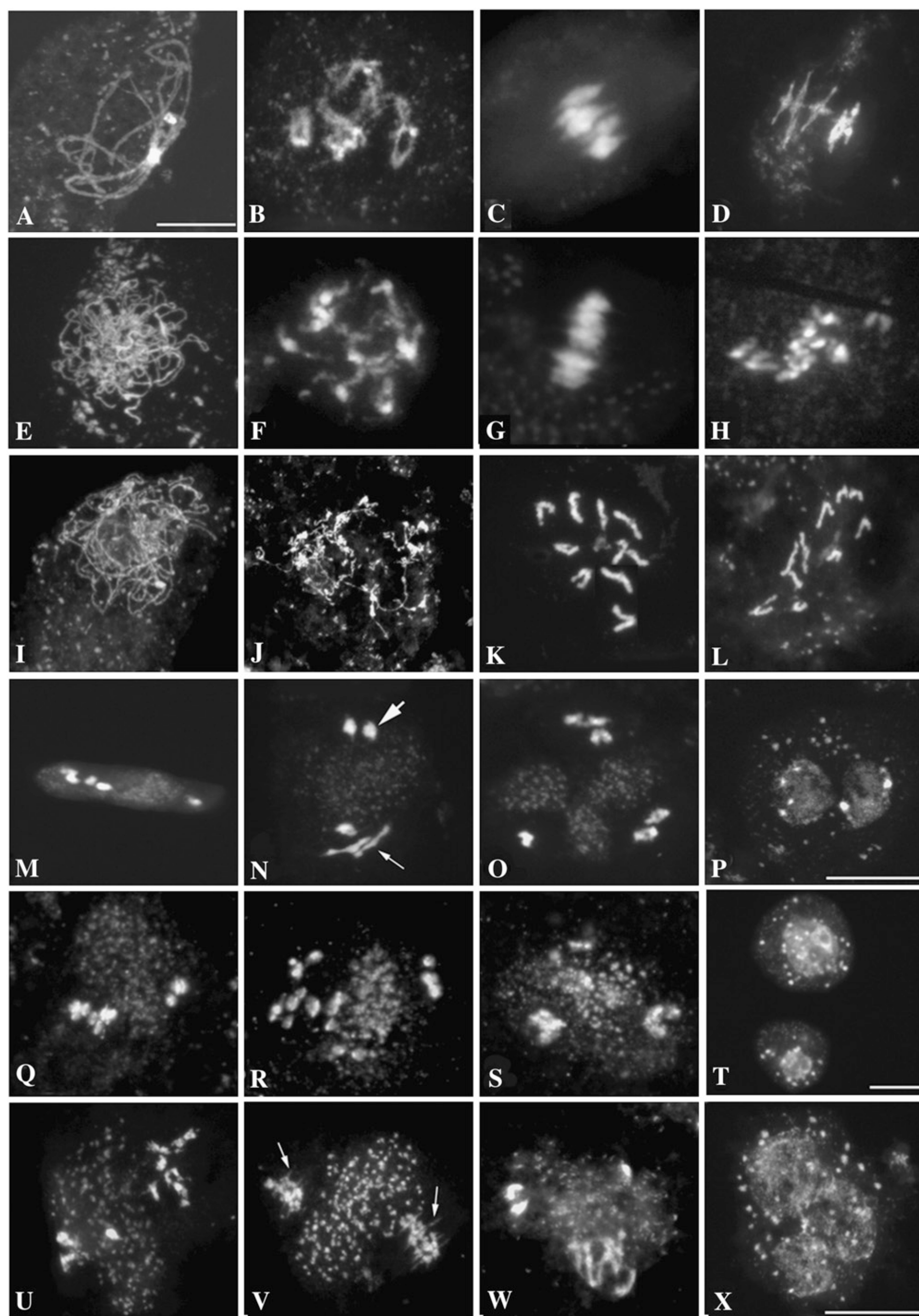

Figure 1. Chromosome analysis of *ask1-1*, *ESP* RNAi, and *ESP* RNAi; *ask1-1* plants is shown. Meiotic chromosome spreads of *ESP* RNAi (A–D and M–P), *ask1-1* (E–H and Q–T), and *ESP* RNAi; *ask1-1* plants (I–L and U–X). A, E, and I, Early pachytene, note the absence of homologous pairing in E and I. B, F, and J, Early diakinesis. C, G, and K, Metaphase I. D, H, and L, Anaphase I, showing nonsegregation of bivalents in *ESP* RNAi plants and random segregation of univalents in the *ask1-1* and *ESP* RNAi; *ask1-1* plants. M, Q, and U, Metaphase II. Note the nonsegregated bivalents and uneven segregation of univalents in the mutants. N, R, and V, Early anaphase II showing nonsegregated bivalents (big arrow in N) and stretched chromosomes (small arrows in N and V). O, S, and W, Telophase II. P, T, and X, Microspores with single (T) and multiple nuclei (P and X). Bars = 10 μm. Unless otherwise shown, the images correspond to the size bar shown in A.

telophase II (Fig. 1O), suggesting that the homologous chromosomes either never attached to the spindle or were pulled to one side of the cell due to the persistent presence of sister chromatid cohesion. Polyads, some of which appeared to contain microspores with multiple nuclei (Fig. 1P), were produced after cytokinesis.

Meiotic chromosomes in *ESP* RNAi; *ask1-1* plants generally resembled the *ask1-1* mutant prior to anaphase II. Homologous chromosomes failed to pair properly during prophase I in *ask1-1* (Fig. 1, E and F) and *ESP* RNAi; *ask1-1* plants (Fig. 1, I and J), with 10 univalents typically observed during metaphase I in both plants (Fig. 1, G and K). Homologous chromosomes randomly segregated to opposite poles during anaphase I in both *ask1-1* and *ESP* RNAi; *ask1-1* meiocytes (Fig. 1, H and L), resulting in an uneven distribution of chromosomes at metaphase II (Fig. 1, Q and U). Chromosome bridges were not observed in meiocytes of *ask1-1* or *ESP* RNAi; *ask1-1* plants at anaphase I. In contrast to the random segregation of chromosomes during anaphase II in *ask1-1* plants (Fig. 1R), meiocytes of *ESP* RNAi; *ask1-1* plants contained

stretched sister chromatids at anaphase II (Fig. 1V), indicating that persistent cohesion blocked sister chromatid segregation. This phenotype is similar to what is observed in *ESP* RNAi plants (Fig. 1D). At telophase II, variable groups of chromosomes were observed in both *ask1-1* and *ESP* RNAi; *ask1-1* mutant meiocytes (Fig. 1, S and W). A number of polyads containing microspores with multiple nuclei were produced after cytokinesis in *ESP* RNAi; *ask1-1* plants (Fig. 1X). Microspores with multiple nuclei were never observed in the *ask1-1* mutant (Fig. 1T). These results confirm that ESP is required for the removal of cohesin during both meiosis I and II and demonstrate that *ask1-1* is epistatic to *ESP* prior to anaphase II. They also suggest that expression of *ESP* RNAi affects meiotic heterochromatin during prophase I and ultimately the proper establishment of nuclear domains following cytokinesis.

Meiotic Expression of *ESP* RNAi Increases Nonhomologous Centromere Joining during Prophase I

The observation that meiocytes expressing *ESP* RNAi exhibited a reduced number of heterochromatin regions suggested that ESP may function earlier than anaphase I. In order to further investigate this possibility, fluorescence in situ hybridization (FISH) experiments were conducted using the 180-bp repetitive centromere fragment as a probe on meiocytes of wild-type, *ask1-1*, *ESP* RNAi, and *ESP* RNAi; *ask1-1* plants, and the number of CEN signals at pachytene were compared. Two to five CEN signals were observed in wild-type meiocytes at pachytene, with most cells containing four or five signals (Fig. 2). Pachytene cells with four (27.5%) or five (66.7%) CEN signals accounted for >94% of the wild-type meiocytes observed (n = 69), and cells with a single CEN signal were never observed. Pachytene stage meiocytes in *ESP* RNAi transgenic plants were also found to have between two and five CEN signals; however, most of the cells contained less than three signals (Fig. 2). Meiocytes with one (21%), two (44%), and three (20%) CEN signals accounted for 85% of the total *ESP* RNAi cells observed (n = 61). Meiocytes of *ask1-1* plants, which exhibit defects in homologous chromosome pairing, contained approximately eight (three to 10) centromere signals throughout prophase I (Fig. 2). Interestingly, *ESP* RNAi; *ask1-1* plants most closely resembled *ESP* RNAi plants. While meiocytes with between one and eight CEN signals were observed, almost 80% of the cells had four or fewer signals (average = 4.1, n = 79). Meiocytes with five (6%), six (3%), seven (5%), and eight (5%) CEN signals were also observed.

These results confirm that meiotic expression of *ESP* RNAi results in the nonspecific association of centromeres during pachytene. Furthermore, this association is not dependent on homologous chromosome pairing. This suggests that ESP may play a role in centromeric heterochromatin structure/formation during early meiosis.

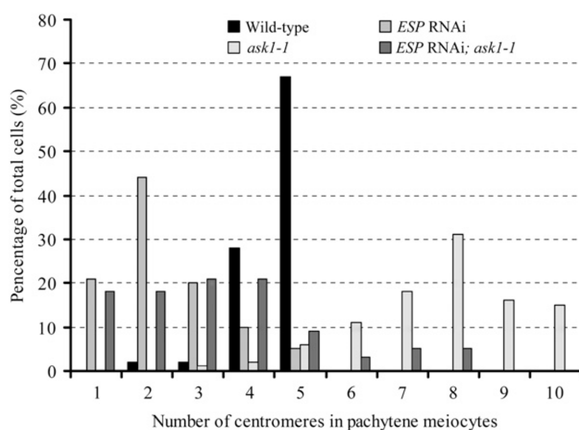

Figure 2. Distribution of pachytene CEN FISH signals in wild-type, *ESP* RNAi, *ask1-1*, and *ESP* RNAi; *ask1-1* plants.

Meiotic Expression of *ESP* RNAi Results in the Stretching of Pericentromeric Heterochromatin during Anaphase I

Five CEN signals are typically observed during pachytene in wild-type meiocytes, with the number of signals gradually increasing during diplotene/diakinesis as the homologous chromosomes desynapse (data not shown; Zhao et al., 2006). Most mid to late diakinesis cells contain five pairs of signals (total 10), with the homologous centromeres usually present as pairs at both ends of the aligned bivalents during metaphase I (Fig. 3A). The centromeres initially separate, followed by the chromosome arms, as homologous chromosomes are pulled to opposite sides of the cell by the spindle during anaphase I (Fig. 3B), resulting in two groups of sister chromatids with five CEN signals each during late anaphase I (Fig. 3C). During meiosis II, sister chromosomes segregate, producing a tetrad of microspores each containing five CEN signals after cytokinesis (Fig. 3, I–L).

In *ESP* RNAi meiocytes, the centromeres of homologous chromosomes typically remained closely associated at metaphase I (Fig. 3E). During anaphase I, *ESP* RNAi meiocytes exhibit severe chromosome stretching. We had initially assumed that the stretched regions represented homologous chromosome arms that had failed to resolve due to persistent cohesion. However, lines of discrete centromere signals were detected along the stretched chromosomes (Fig. 3, F and G), and the DNA remaining in the center of the cells was not labeled with the CEN probe, indicating that the chromosome arms remain tightly joined in the center of the cell, while the pericentromeres stretch and unravel as they are pulled to opposite poles. Consistent with this observation, it was recently shown that pericentromeric chromatin is an elastic element of the spindle

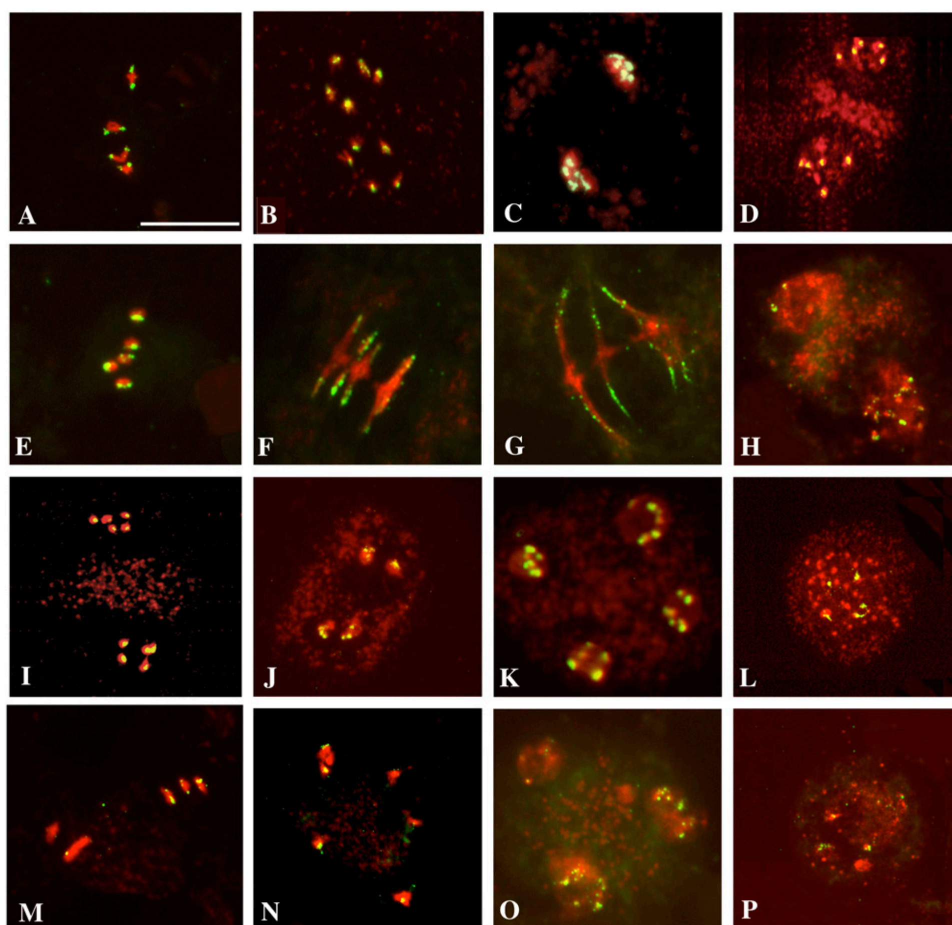

Figure 3. Centromere FISH on wild-type and *ESP* RNAi meiocytes. Meiotic chromosomes from wild-type (A–D and I–L) and *ESP* RNAi (E–H and M–P) plants stained with DAPI (red) and hybridized with the pAL1 centromere probe (green) are shown. A and E, Metaphase I. Homologous CENs are not fully resolved in *ESP* RNAi meiocytes. B and F, Anaphase I. Chromosomes are severely stretched and CEN signals are broken along the stretched chromosomes in *ESP* RNAi meiocytes. C and G, Late anaphase I. Stretched chromosomes become more severe, and broken CEN signals are more distinctive in *ESP* RNAi meiocytes. D and H, Telophase I. The broken CEN signals are unevenly segregated in *ESP* RNAi meiocytes, indicating that some chromosomes do not have CENs. I and M, Metaphase I. Unseparated bivalents and decreased CEN signals are observed on some *ESP* RNAi bivalents. J and N, Late anaphase II. K and O, Telophase II. Uneven distribution of CEN signals is observed in *ESP* RNAi meiocytes. L and P, Microspores. Wild-type spores have a single nucleus and five CENs, while the *ESP* RNAi spore has three nuclei (one large and two small) containing many broken CEN signals. Bar = 10 μm.

that is stretched proportionally to the force applied to it (Bouck and Bloom, 2007).

Later in meiosis, a mixture of large, strongly labeled and small, weakly labeled centromere signals were observed distributed throughout the cells (Fig. 3H). These most likely represent broken centromeres. Consistent with this assumption are the presence of chromosome fragments with no CEN signals (Fig. 3M). Bivalents containing a pair of closely spaced signals are also observed in some cells consistent with our prediction that some bivalents fail to separate (Fig. 2, M and N). Polyads and released microspores with irregular numbers and intensities of CEN signals were observed later in meiosis (Fig. 3, O and P). Therefore, failure to release arm cohesion during meiosis I results in stretching, unwinding, and breakage of the pericentromeric regions.

Depletion of *ESP* Results in Polyads Containing Multiple Nuclei

It was previously reported that the depletion of *ESP* results in the production of polyads containing two to nine microspores (Liu and Makaroff, 2006). Upon further analysis, we found that some meiocytes from *ESP* RNAi plants appeared to contain microspores with multiple nuclei (Fig. 1P). We therefore determined if this is a common phenotype of *ESP* RNAi meiocytes. A single nucleus is present in each microspore at the end of meiosis in both wild-type (Fig. 4A)

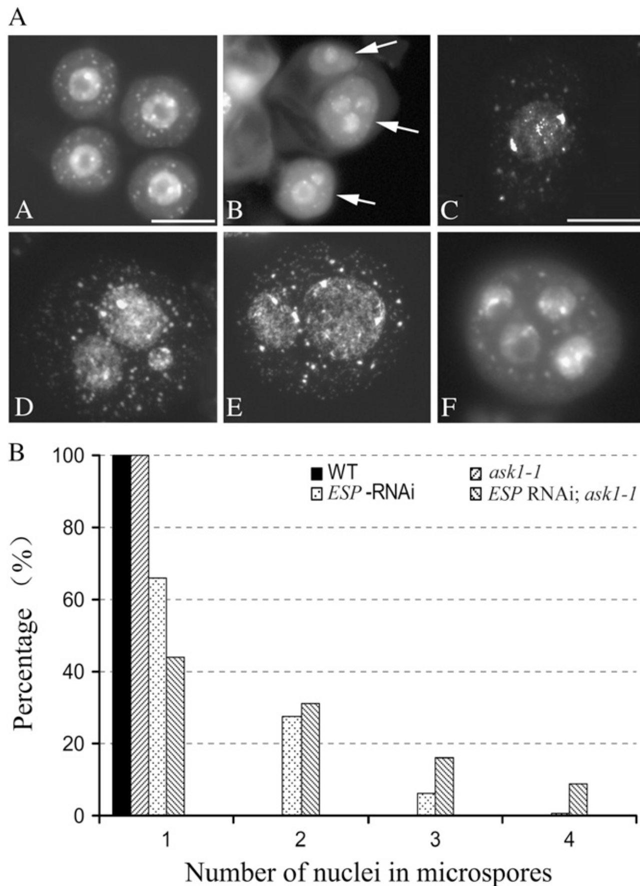

Figure 4. Meiotic expression of *ESP* RNAi results in abnormal numbers of nuclei in microspores. A, DAPI-stained postmeiotic products of wild-type (category A) and *ESP* RNAi (categories B–F) plants. Category A, Just released wild-type microspores with single nuclei. Category B, Tetrad with spores containing 1, 2, and 3 nuclei (arrows). Categories C to F, Just released mutant spores with 1, 2, 3, and 4 nuclei, respectively. B, Percentage of microspores with single or multiple nuclei in wild-type (WT), *ask1-1*, *ESP* RNAi, and *ESP* RNAi; *ask1-1* plants. Bars = 10 μm.

and *ask1-1* (data not shown) plants. In contrast, microspores with one (66%), two (27%), three (6%), and four (2%) nuclei were observed in *ESP* depleted plants (Fig. 4, A and B–F). Interestingly, although multiple nuclei were never observed in the microspores of *ask1-1* plants (Fig. 4B), microspores of *ESP* RNAi; *ask1-1* plants contained multiple nuclei and actually had a higher percentage of microspores with multiple nuclei than *ESP* RNAi plants. Approximately 56% of microspores contained two to four nuclei in *ESP* RNAi; *ask1-1* plants compared to 34% in *ESP* RNAi plants (Fig. 4B).

A transmission electron microscopy (TEM) analysis was conducted on meiocytes from *ESP* RNAi plants to confirm that the microspores do in fact contain multiple intact nuclei and to possibly gain further insight into why multiple nuclei are present in some cells. Wild-type microsporocytes contain four haploid nuclei located at the cell periphery in a tetrahedral arrangement after telophase II (Fig. 5A). Each nucleus is surrounded both by a nuclear membrane and endoplasmic reticulum (ER; Fig. 5A). The onset of cytokinesis is first observed as small ingrowths of callose that are deposited between the nuclei demarking future microspore domains (Fig. 5B). After cytokinesis has taken place, the four haploid microspores are tetrahedrally arranged and encased in a thick callose wall. Usually only two or three microspores with their respective nuclei are visible in a section (Fig. 5C). After callose wall dissolution, the microspores are released, increase in size, and start depositing a sporopollenin wall. At this stage the nucleus is centrally positioned (Fig. 5D).

Following telophase II and prior to the onset of cytokinesis in *ESP* RNAi meiocytes, the coenocytic microsporocytes contain an abnormal arrangement of nuclei (Fig. 5, E and F). In many cases, instead of the nuclei localizing to the cell periphery in a tetrahedral arrangement, the nuclei were observed to group together. In all instances, adjacent nuclei had their own nuclear membranes, but shared a common layer of ER (Fig. 5, E–H). Ingrowths of the callose wall again marked the onset of cytokinesis, but in many instances, these did not correspond to a single nuclear domain. After cytokinesis, microspores containing multiple nuclei, which were always in close proximity, were observed (Fig. 5I). The multiple nuclei in newly released microspores remained encircled with a common ER network (Fig. 5J).

ESP Is Important for Microtubule Array Formation after Telophase II

The presence of bivalents followed by the presence of mispositioned nuclei after telophase II raised the possibility that reduction/elimination of ESP in meiocytes may affect the spindle and/or microtubule network. In order to investigate this possibility, we performed immunolocalization studies on wild-type and *ESP* RNAi meiocytes using an antibody against β-tubulin. No significant differences were observed in β-tubulin staining patterns between wild-type and *ESP* RNAi plants prior to metaphase I. At metaphase I, the spindle structures in *ESP* RNAi meiocytes exhibited a typical bipolar configuration similar to those of wild-type cells (data not shown; Lam et al., 2005). At metaphase II, wild-type meiocytes contain two bipolar spindles (Fig. 6A). *ESP* RNAi meiocytes at metaphase II typically contained between two and four bipolar spindles, each associated with a chromatin mass (Fig. 6, A and C). Prior to cytokinesis in wild-type cells, the bipolar spindle was replaced by a RMS that formed from microtubules emanating from the surface of each nucleus (Fig. 6, A and B). In contrast, in *ESP* RNAi meiocytes, the RMS was generally very weak or absent. In some cells, microtubules appeared to surround nuclei more or less randomly (Fig. 6, A and D), whereas, in other cells, structures resembling phragmoplasts formed (Fig. 6, A, E, and F). Therefore, expression of *ESP* RNAi results in alterations in the RMS during late telophase II and tetrad stages.

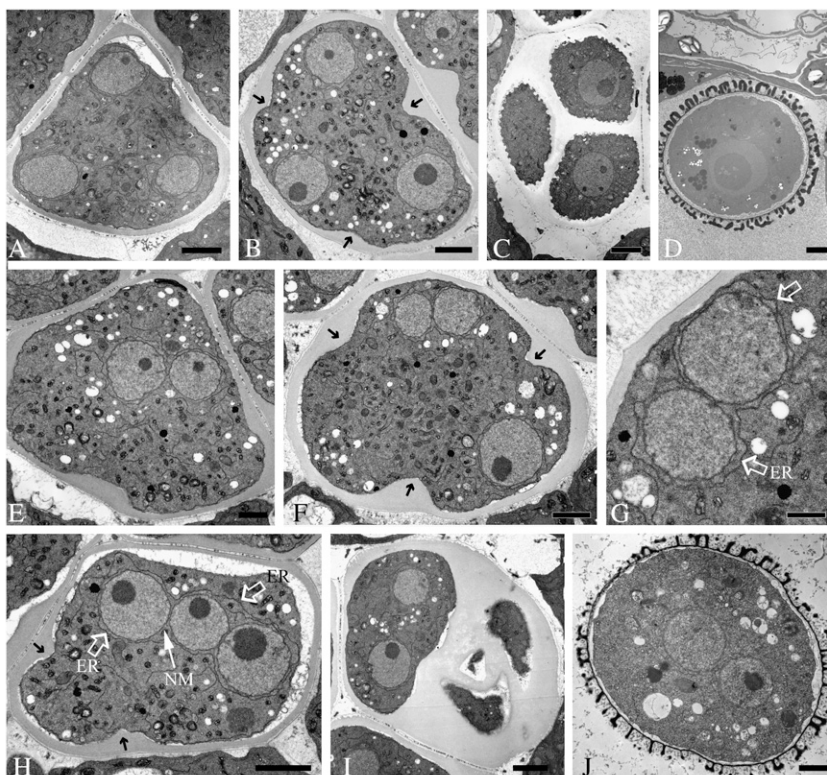

Figure 5. TEM micrographs of wild-type and *ESP* RNAi anther cross sections. A to D, The wild type. E to J, *ESP* RNAi mutant plants. A, The three visible nuclei are positioned in opposite poles. B, Incipient cell wall formation between the nuclei (solid arrows) demarks future microspore domains. C, Tetrahedral microspore tetrad with single nuclei in each cell. D, Young microspore with one nucleus centrally positioned. E, Two visible nuclei positioned close to one another and not directly in one pole. F, The incipient cell wall formation (solid arrows) delimits the future microspore domains and is indicative of how many nuclei each microspore will have; note the top pole with two adjacent nuclei. G, Detail of F showing ER (hollow arrows) wrapped around both nuclei. H, The three contiguous nuclei are situated in one pole and are surrounded by a continuous ER sheet (hollow arrows) and individual nuclear membrane (NM); early cell wall formation indicates that they might be packaged into one microspore. I, Microspore tetrad showing one microspore with two nuclei. J, Free microspore with two adjacent nuclei. Bars = 2 mm in A to F, H, and I, 1 mm in J, and 0.8 mm in G.

DISCUSSION

Separase and Chromosome Disjunction

We previously showed that depletion of ESP in wild-type plants interferes with the normal segregation of homologous chromosomes during anaphase I and sister chromatids at anaphase II (Liu and Makaroff, 2006). The presence of bivalents in *ESP* RNAi transgenic plants after anaphase I is similar to what was seen in *S. pombe* cells expressing a noncleavable form of Rec8p, where little to no segregation of either homologs or sister chromatids was observed (Kitajima et al., 2003). Similarly, inactivation of separase in yeast *sep1-1* mutants and *C. elegans* embryos using *sep-1* RNAi also blocked the normal disjunction of homologous chromosomes during meiosis I (Buonomo et al., 2000; Siomos et al., 2001). In mice, about 70% of oocytes overexpressing securin, the separase inhibitor, arrest at metaphase II with a mixture of univalents and bivalents (Terret et al., 2003). In all cases, the bivalents were typically found together in either the center of the cell or in one quadrant of the cell. In separase-depleted Arabidopsis lines, the bivalents were typically found distributed throughout the cell. Cellular arrest was not observed, and the cells progressed through meiosis II and cytokinesis. This is in contrast to the metaphase I or metaphase II arrest reported in separase-inhibited cells of most other organisms (Buonomo et al., 2000; Siomos et al., 2001; Kitajima et al., 2003; Terret et al., 2003). The lack of meiotic arrest in *ESP* RNAi meiocytes supports the hypothesis that meiotic checkpoint controls typical of metazoans are absent in plants (Armstrong and Jones, 2003).

It is generally assumed that separase is required for the release of cohesion at both metaphase I and II. However, it is possible that defects in sister chromatid segregation during meiosis II could result in part from the aberrant homolog separation that occurs at anaphase I. We show in this report that depletion of ESP in *ask1-1* plants, which are defective in homologous chromosome synapsis and contain a mixture of univalents and abnormal bivalents at metaphase I, blocks sister chromatid separation during anaphase II (Fig. 4V), thereby clearly demonstrating the involvement of ESP in the release of cohesion during both anaphase I and II.

While studies in a wide range of organisms have shown that separase is essential for the release of sister chromatid cohesion, differences in the removal of cohesin complexes have also been observed (for review, see Queralt and Uhlmann, 2005; Losada, 2007; Onn et al., 2008; Peters et al., 2008). In Arabidopsis, cohesion is released from meiotic chromosomes in a three-step process. A large portion of arm cohesin is released from the chromosomes in an ESP-independent process during late prophase I (Cai et al., 2003; Liu and Makaroff, 2006). Residual arm cohesion is released by ESP during anaphase I to allow the disjunction of homologous chromosomes, followed by ESP-dependent removal of cohesin from the centro-

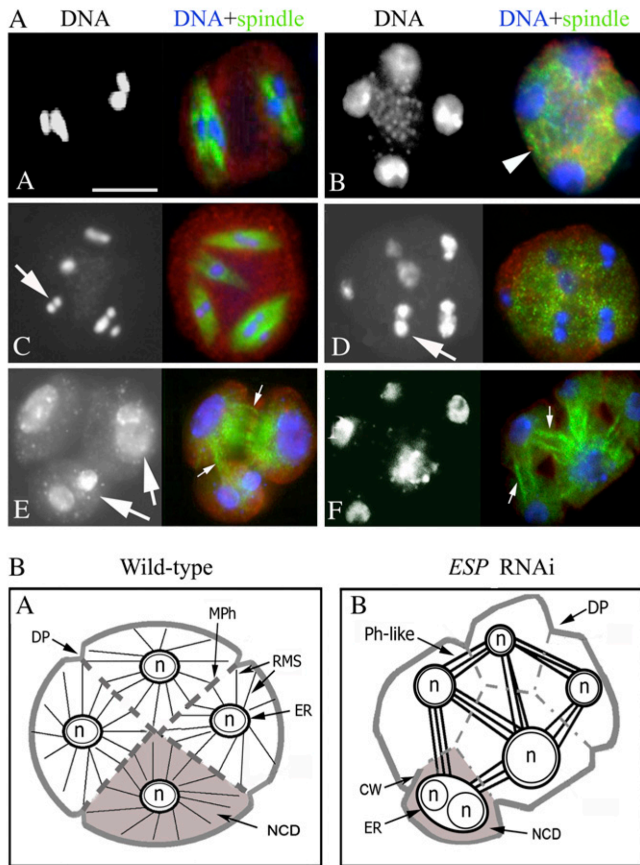

Figure 6. The RMS is altered in *ESP*-RNAi meiocytes. A, Wild-type (categories A and B) and *ESP* RNAi (categories C–F) meiocytes stained with DAPI and hybridized with anti-β-tubulin antibody. Categories A and C, Metaphase II. Four spindles are present in the mutant. Categories B and D, Telophase II. E and F, Meiocytes at cytokinesis. The RMS is present in wild-type cells (large arrowhead in category B), while multinucleate spores (big arrows) and phragmoplast-like microtubules are present in *ESP*-RNAi meiocytes (small arrows). B, Illustration of microtubule network during cytokinesis in wild-type and *ESP* RNAi transgenic plants. Category A, RMS in wild-type tetrad, modified from Otegui and Staehelin (2004). Category B, Altered microtubules lacking RMS in *ESP*-RNAi polyad. DP, division plane; MPh, miniphragmoplast; NCD, nuclear cytoplasmic domain; Ph-like, phragmoplast like microtubule; cw, cell wall; n, nucleus.

meric regions of sister chromatids at early anaphase II. Cohesin complexes are removed in a separase independent process, which involves a polo-like kinase, during mitotic prophase in vertebrates (Waizenegger et al., 2000; Hauf et al., 2005). This suggests that the separase-independent release of cohesin in Arabidopsis may also involve a kinase-dependent pathway.

Separase Is Required for Multiple Processes in Arabidopsis

While separase appears to be universally responsible for chromosome disjunction at anaphase, a number of organism-specific separase-dependent functions have also been reported. These include mitotic anaphase spindle assembly and elongation (Jensen et al., 2001; Papi et al., 2005; Baskerville et al., 2008), interphase spindle pole body positioning (Nakamura et al., 2002), and spindle formation during meiosis (Buonomo et al., 2003) in yeast and the proper positioning of centrosomes in *C. elegans* (Siomos et al., 2001; Rappleye et al., 2002).

In this report, we show that reduction/elimination of ESP during meiosis results in a number of cellular defects in addition to blocking chromosome disjunction. Specifically, we observe that meiotic expression of *ESP* RNAi (1) results in alterations in nonhomologous centromere association, (2) disrupts the RMS and in some telophase II meiocytes converts it to phragmoplast-like structures, and (3) affects the proper establishment of nuclear cytoplasmic domains that leads to the formation of multinucleate microspores.

DAPI-stained spreads of meiotic chromosomes and centromere FISH experiments both demonstrated a prolonged, nonspecific association of centromeres during prophase in *ESP* meiocytes. In wild-type meiocytes, a transient clustering and aggregation of chromocenters is typically observed during zygotene (Armstrong et al., 2001). However, wild-type pachytene meiocytes typically contain four to five centromere signals, and meiocytes with a single centromere signal are very rare. In contrast, 85% of pachytene meiocytes in *ESP* RNAi plants contain three or fewer centromere signals. This association of nonhomologous centromere regions appears to be independent of the homologous chromosome search and pairing process, as the phenotype is also observed in *ESP* RNAi; *ask1-1* plants. Meiocytes from *ask1-1* plants, which display an abnormal association of the chromosomes with the nuclear envelope, also exhibit the nonspecific association of chromosomes during zygotene (Yang et al., 2006). However, an average of 7.8 centromere signals were observed by pachytene in *ask1-1* meiocytes, while meiocytes in *ESP* RNAi; *ask1-1* plants contain on average 4.1 centromere signals. Therefore, it is possible that ESP plays a role in controlling/releasing the transient centromere association that occurs during zygotene in Arabidopsis (Armstrong et al., 2001).

In contrast to the situation in yeast, separase is not required for normal meiotic spindle formation in Arabidopsis. With the exception of the presence of multiple spindles in some meiocytes, spindle formation appeared relatively normal in *ESP* RNAi meiocytes. While some broadening of the spindles was observed, which presumably results from alterations in chromosome segregation caused by persistent cohesion, relatively normal spindles were observed during meiosis I and II in *ESP* RNAi plants. This is in contrast to the alterations in spindle formation observed during meiosis in yeast (Jensen et al., 2001; Papi et al., 2005; Baskerville et al., 2008).

Although separase is not required for meiotic spindle formation in Arabidopsis, it is necessary for proper

formation of the RMS, which is formed as part of the reorganization of the spindle at the end of meiosis II. The RMS is a microtubule network that emanates from the surface of each microspore nucleus and extends throughout each nuclear cytoplasmic domain of the nascent tetrad during cytokinesis (Fig. 6B). The RMS plays a pivotal role in defining cytoplasmic boundaries and the position of cell wall formation (Brown and Lemmon, 2001; Otegui and Staehelin, 2004). In some *ESP* RNAi meiocytes, the RMS is very poorly defined and fails to fully extend to the cytoplasmic boundries (Fig. 6, A and D). In other meiocytes, the microtubules were more pronounced and narrowly focused. They often originated from only a limited portion of the nuclei instead of radiating out in all directions from the surfaces of adjacent nuclei (Fig. 6, A, E, and F). This arrangement of microtubules resembles phragmoplasts formed prior to cytokinesis in mitotic cells. These observations implicate separase in the proper establishment and organization of the radial system of microtubules typical of wild-type tetrads and suggest that ESP may be required for the proper interaction of microtubules with the nuclear envelope.

The RMS alterations that we observe in ESP-RNAi plants are likely related, either directly or indirectly, to the generation of microspores with multiple nuclei. During the normal progression of meiosis and cytokinesis, a nuclear membrane and sheet of ER surround each of the four clusters of chromosomes, which are arranged in a tetrahedral organization. Each nucleus then establishes a radial network of microtubuli that coalesce forming miniphragmoplasts that define the division planes and demark the locations for microspore cell wall formation (Yang et al., 2003a; Otegui and Staehelin, 2004). Subsequently, a postmeiotic cell plate, made up of a complex matrix of vesicles, putative kinesin proteins, and miniphragmoplast microtubules, is deposited at this division plane (Fig. 6, A and B). The vesicles fuse with each other to form tubular networks, which ultimately fuse with the plasma membrane and trigger the synthesis of callose. The accumulation of callose in the tubular network then induces its transformation into convoluted sheets, which merge and fuse into sheet-like projections across the cell plates to form the intersporal cell wall enclosing the tetrad of microspores.

In *ESP* RNAi meiocytes, multiple groups of partially dispersed chromosomes are present with a nuclear membrane forming around each of them. Some of the nuclei remain adjacent to one another and share a common sheet of ER. These adjacent nuclei act as a unit and are packaged into a single microspore. Adjacent nuclei may arise if a spindle did not form between two groups of chromosomes during meiosis II or if the chromosomes can't be separated by the spindle. While relatively normal looking spindles are found in *ESP* RNAi meiocytes, we did observe that spindles do not form on some chromosomes (Fig. 6, C and E). Adjacent nuclei often exhibit the greatest alterations in the RMS. Therefore, it is possible that the absence of ESP affects spindle formation on some chromosomes, which ultimately results in multinucleate microspores.

In yeast, separase is known to stabilize the dynamics of spindle microtubules at the onset of anaphase and to participate in the FEAR mitotic exit pathway (Stegmeier et al., 2002; Sullivan and Uhlmann, 2003). These activities are independent of its protease activity and depend, at least in part, on separase binding and regulating the activity of the cdc14 phosphatase (Higuchi and Uhlmann, 2005). However, more recently a catalytic role for Esp1 in anaphase spindle elongation that is independent of Mcd1 cleavage has also been identified (Baskerville et al., 2008).

Very little is currently known about the factors that control microtubule pattern formation during meiosis. A mutation in the Arabidopsis kinesin gene, *TETRASPORE* (*TES*)/*STUD*/*NACK2* has been described that results in microtubules that do not organize into a radial system, do not extend to the surface of adjacent nuclei, and fail to establish stable boundaries for nuclear cytoplasmic domains (Yang et al., 2003a; Tanaka et al., 2004). Microtubules in *tes* meiocytes accumulate around the nuclear surface, resulting in plants that produce tetranucleate microspores due to a complete failure of cytokinesis (Yang et al., 2003a). In contrast, cytokinesis is altered, but not eliminated, in *ESP* RNAi plants such that at the tetrad stage, polyads containing variable numbers of nuclei are observed. A mutation in *TAM*, which is required to maintain the normal pace and synchrony of cell division during male meiosis, also results in microspores that sometimes contain more than one nucleus (Magnard et al., 2001). At this time, it is not clear if meiocytes in *tam* plants also exhibit alterations in the RMS. Finally, while many Arabidopsis mutations result in the random distribution of chromosomes and chromosome fragments during meiosis, very few mutants have been found to produce microspores with multiple nuclei, and demonstrated alterations in the RMS are rare. For example, both *syn1* and *ask1-1* plants produce polyads; however, neither of the mutants produce microspores with multiple nuclei, and neither has been found to exhibit alterations in the RMS (Peirson et al., 1997; Yang and Ma, 2001).

In summary, ESP appears to play a number of roles during meiosis in Arabidopsis in addition to its highly conserved role in the release of sister chromatid cohesion. It is required for the proper establishment and organization of the RMS and defining nuclear/cytoplasmic boundaries. It also appears to play a role in the association of centromere regions of nonhomologous chromosomes. At this time, it is not clear if ESP plays a direct or indirect role in these processes, if the defects we observe are the result of the absence of separase protease activity, or if ESP has other activities. Additional studies are necessary to investigate these questions and further explore the roles of separase in plant cells.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of wild-type Arabidopsis (*Arabidopsis thaliana*), *ask1-1*, *ESP* RNAi, and *ESP* RNAi; *ask1-1* plants were sterilized in 70% ethanol and grown on a commercial potting mix in a growth chamber at 22°C with a 16-h-light/8-h-dark cycle. Buds at appropriate stages during meiosis were collected from prebolting plants, fixed, and analyzed as described below. *ESP* RNAi transgenic plants were initially identified by BASTA selection, and the presence of the transgene was then confirmed by PCR.

Plants defective in both *ASK1* and *ESP* expression were generated by crossing *ask1-1* homozygous plants with *ESP* RNAi transgenic plants. F2 plants from the crosses were screened with Basta, and *ask1-1* plants containing the RNAi construct were identified by performing PCR analysis on sterile plants using primers specific for *ASK1*, *ask1-1*, and the *ESP* RNAi construct.

Cytology

Meiosis in the transgenic lines was examined and compared to that in wild-type plants using DAPI-stained meiotic chromosome spreads (Ross et al., 1996). Immunolocalization was conducted on meiotic chromosome spreads as previously described (Yang et al., 2003b; Zhao et al., 2006). Poly-L-Lys-coated slides containing staged anthers that had been squashed and treated with 1.4% (w/v) GUS, 0.3% (w/v) cytohelicase, 0.3% (w/v) pectolyase, and 0.3% (w/v) cellulase were blocked (1× PBS and 5% [w/v] BSA) and then incubated overnight at 4°C in a moist chamber with primary antibody (1:500 dilution). After washing, the slides were incubated with Alexa Fluor 488-labeled goat anti-rabbit (1:500) or Alexa Fluor 594-labeled goat anti-mouse secondary antibody (1:500) overnight at 4°C and washed and the chromosomes stained with DAPI. Slides were viewed with an Olympus epifluorescence microscope system equipped with a Spot RT imaging system. Meiotic stages were assigned based on chromosome and cellular morphology.

Staged buds, which were fixed in acetic alcohol (ethanol:acetic acid, 3:1 [v/v]) for 2 h at room temperature and stored at −20°C, were subjected to FISH as previously published (Yang et al., 2006). The pAL1 clone containing a pericentromeric 180-bp repeat was used to detect centromere sequences (Martinez-Zapater et al., 1986). PCR amplification of pAL1 was followed by random primer labeling in the presence of Fluorescin-High Prime labeled dUTP (Roche). The labeled probe was used in hybridization solution at 5 μg/mL. Slides were counterstained with DAPI, mounted, and viewed as above.

TEM

A TEM analysis of microsporogenesis was conducted as described (Owen and Makaroff, 1995). Inflorescences were fixed at room temperature in freshly mixed fixative (4% [w/v] paraformaldehyde and 2.5% [w/v] glutaraldehyde in sodium phosphate [$NaPO_4$] plus 5 mM CaCl, pH 7.2, buffer) for 4 h and then transferred to fresh fixative at 4°C for 24 h. The inflorescences were rinsed with fixation buffer and postfixed in the fixation buffer with 1.5% (v/v) osmium tetroxide (OsO_4) for 4 h at room temperature. The samples were treated overnight at room temperature with 0.5% uranyl acetate, rinsed in double distilled water, dehydrated in an ascending acetone series, and infiltrated and embedded in Spurr's resin. Selected flower buds were ultrathin sectioned (approximately 75 nm), collected on 200 mesh Cu grids, and stained with 2% uranyl acetate (10 min) and 0.5% lead citrate (10 min). The material was viewed using a JEOL 100 S transmission electron microscope and photographed using TEM film (Kodak 4489).

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession number AY823256.

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LITERATURE CITED

Armstrong S, Franklin F, Jones G (2001) Nucleolus-associated telomere clustering and pairing preceed meiotic chromosome synapsis in *Arabidopsis thaliana*. J Cell Sci **114:** 4207–4217

Armstrong S, Jones G (2003) Meiotic cytology and chromosome behavior in wild-type *Arabidopsis thaliana*. J Exp Bot **54:** 1–10

Baskerville C, Segal M, Reed SI (2008) The protease activity of yeast separase (Esp1) is required for anaphase spindle elongation independently of its role in cleavage of cohesin. Genetics **178:** 2361–2372

Blat Y, Kleckner N (1999) Cohesins bind to preferential sites along yeast chromosome III, with differential regulation along arms verses the centric region. Cell **98:** 249–259

Bouck DC, Bloom K (2007) Pericentric chromatin is an elastic component of the mitotic spindle. Curr Biol **17:** 741–748

Brown RC, Lemmon BE (2001) The cytoskeleton and spatial control of cytokinesis in the plant life cycle. Protoplasma **215:** 35–49

Buonomo SBC, Clyne RK, Fuchs J, Loidl J, Uhlmann F, Nasmyth K (2000) Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell **103:** 387–398

Buonomo SBC, Rabitsch KP, Fuchs J, Gruber S, Sullivan M, Uhlmann F, Petronczki M, Toth A, Nasmyth K (2003) Division of the nucleolus and its release of CDC14 during anaphase of meiosis I depends on separase, SPO12, and SLK19. Dev Cell **4:** 727–739

Cai X, Dong F, Edelmann RE, Makaroff CA (2003) The Arabidopsis SYN1 cohesin protein is required for sister chromatid arm cohesion and homologous chromosome pairing. J Cell Sci **116:** 2999–3007

Ciosk R, Zachariae W, Michaelis C, Shevchenko A, Mann M, Nasmyth K (1998) An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. Cell **93:** 1067–1076

Cohen-Fix O, Peters JM, Kirschner MW, Koshland D (1996) Anaphase initiation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. Genes Dev **10:** 3081–3093

Eijpe M, Offenberg H, Jessberger R, Revenkova E, Heyting C (2003) Meiotic cohesin REC8 marks the axial elements of rat synaptonemal complexes before cohesins SMC1 beta and SMC3. J Cell Biol **160:** 657–670

Hauf S, Roitinger E, Koch B, Dittrich CM, Mechtler K, Peters JM (2005) Dissociation of cohesin from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2. PLoS Biol **3:** 419–432

Hauf S, Waizenegger IC, Peters JM (2001) Cohesin cleavage by separase is required for anaphase and cytokinesis in human cells. Science **293:** 1320–1323

Higuchi T, Uhlmann F (2005) Stabilization of microtubule dynamics at anaphase onset promotes chromosome segregation. Nature **433:** 171–176

Jensen S, Segal M, Clarke DJ, Reed SI (2001) A novel role of the budding yeast separin Esp1 in anaphase spindle elongation: evidence that proper spindle association of Esp1 is regulated by Pds1. J Cell Biol **152:** 27–40

Katis VL, Galova M, Rabitsch KP, Gregan J, Nasmyth K (2004) Maintenance of cohesin at centromeres after meiosis I in budding yeast requires a kinetochore-associated protein related to MEI-S332. Curr Biol **14:** 560–572

Kenna MA, Skibbens RV (2003) Mechanical link between cohesion establishment and DNA replication: Ctf7p/Eco1p, a cohesion establishment factor, associates with three different replication factor C complexes. Mol Cell Biol **23:** 2999–3007

Kitajima TS, Miyazaki Y, Yamamoto M, Watanabe Y (2003) Rec8 cleavage by separase is required for meiotic nuclear divisions in fission yeast. EMBO J **22:** 5643–5653

Laloraya S, Guacci V, Koshland D (2000) Chromosomal addresses of the cohesin component Mcd1p. J Cell Biol **151:** 1047–1056

Lam WS, Yang XH, Makaroff CA (2005) Characterization of *Arabidopsis thaliana* SMC1 and SMC3: evidence that ASMC3 may function beyond chromosome cohesion. J Cell Sci **118:** 3037–3048

Lee J, Iwai T, Yokota T, Yamashita M (2003) Temporally and spatially selective loss of Rec8 protein from meiotic chromosomes during mammalian meiosis. J Cell Sci **116:** 2781–2790

Lengronne A, McIntyre J, Katou Y, Kanoh Y, Hopfner KP, Shirahige K, Uhlmann F (2006) Establishment of sister chromatid cohesion at the *S. cerevisiae* replication fork. Mol Cell **23:** 787–799

Liu Z, Makaroff CA (2006) Arabidopsis separase AESP is essential for

embryo development and the release of cohesin during meiosis. Plant Cell **18:** 1213–1225

Losada A (2007) Cohesin regulation: fashionable ways to wear a ring. Chromosoma **116:** 321–329

Losada A, Hirano M, Hirano T (2002) Cohesin release is required for sister chromatid resolution, but not for condensin-mediated compaction, at the onset of mitosis. Genes Dev **23:** 3004–3016

Magnard JL, Yang M, Chen YCS, Leary M, McCormick S (2001) The Arabidopsis gene *Tardy Asynchronous Meiosis* is required for the normal pace and synchrony of cell division during male meiosis. Plant Physiol **127:** 1157–1166

Martinez-Zapater JM, Estelle MA, Somerville CR (1986) A highly repeated DNA sequence in *Arabidopsis thaliana*. Mol Gen Genet **204:** 417–423

McGuinness BE, Hirota T, Kudo NR, Peters JM, Nasmyth K (2005) Shugoshin prevents dissociation of cohesin from centromeres during mitosis in vertebrate cells. PLoS Biol **3:** 433–449

Milutinovich M, Unal E, Ward C, Skibbens RV, Koshland D (2007) A multi-step pathway for the establishment of sister chromatid cohesion. PLoS Genet **3:** 146–157

Nagao K, Adachi Y, Yanagida M (2004) Separase-mediated cleavage of cohesin at interphase is required for DNA repair. Nature **430:** 1044–1048

Nakamura T, Nagao K, Nakaseko Y, Yanagida M (2002) Cut1/separase C-terminus affects spindle pole body positioning in interphase of fission yeast: pointed nuclear formation. Genes Cells **7:** 1113–1124

Nasmyth K, Haering CH (2005) The structure and function of SMC and kleisin complexes. Annu Rev Biochem **74:** 595–648

Onn I, Heidinger-Pauli JM, Guacci V, Unal E, Koshland DE (2008) Sister chromatid cohesion: a simple concept with a complex reality. Annu Rev Cell Dev Biol **24:** 105–129

Otegui MS, Staehelin LA (2004) Electron tomographic analysis of post-meiotic cytokinesis during pollen development in Arabidopsis thaliana. Planta **218:** 501–515

Owen HA, Makaroff CA (1995) Ultrastructure of microsporogenesis and microgametogenesis in *Arabidopsis thaliana* (L) *heynh* ecotype Wassilewskija (*Brassicaceae*). Protoplasma **185:** 7–21

Pandey R, Heidmann S, Lehner CF (2005) Epithelial re-organization and dynamics of progression through mitosis in Drosophila separase complex mutants. J Cell Sci **118:** 733–742

Papi M, Berdougo E, Randall CL, Ganguly S, Jallepalli PV (2005) Multiple roles for separase auto-cleavage during the G2/M transition. Nat Cell Biol **7:** 1029–1044

Pasierbek P, Jantsch M, Melcher M, Schleiffer A, Schweizer D, Loidl J (2001) A *Caenorhabditis elegans* cohesion protein with functions in meiotic chromosome pairing and disjunction. Genes Dev **15:** 1349–1360

Peirson BN, Bowling SE, Makaroff CA (1997) A defect in synapsis causes male sterility in a T-DNA-tagged *Arabidopsis thaliana* mutant. Plant J **11:** 659–669

Peters JM, Tedeschi A, Schmitz J (2008) The cohesin complex and its roles in chromosome biology. Genes Dev **22:** 3089–3114

Queralt E, Uhlmann F (2005) More than a separase. Nat Cell Biol **7:** 930–932

Rabitsch KP, Petronczki M, Javerzat JP, Genier S, Chwalla B, Schleiffer A, Tanaka TU, Nasmyth K (2003) Kinetochore recruitment of two nucleolar proteins is required for homolog segregation in meiosis I. Dev Cell **4:** 535–548

Rappleye CA, Tagawa A, Lyczak R, Bowerman B, Aroian RV (2002) The anaphase-promoting complex and separin are required for embryonic anterior-posterior axis formation. Dev Cell **2:** 195–206

Ross KJ, Fransz P, Jones GH (1996) A light microscopic atlas of meiosis in *Arabidopsis thaliana*. Chromosome Res **4:** 507–516

Shepard JL, Amatruda JF, Finkelstein D, Ziai J, Finley KR, Stern HM, Chiang K, Hersey C, Barut B, Freeman JL, et al (2007) A mutation in separase causes genome instability and increased susceptibility to epithelial cancer. Genes Dev **21:** 55–59

Siomos MF, Badrinath A, Pasierbek P, Livingstone D, White J, Glotzer M, Nasmyth K (2001) Separase is required for chromosome segregation during meiosis I in *Caenorhabditis elegans*. Curr Biol **23:** 1825–1835

Skibbens R, Corson L, Koshland D, Hieter P (1999) Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. Genes Dev **13:** 307–319

Stegmeier F, Visintin R, Amon A (2002) Separase, polo kinase, the kinetochore protein Slk19 and Spo12 function in a network that controls Cdc14 localization during early anaphase. Cell **108:** 207–220

Strom L, Lindroos HB, Shirahige K, Sjogren C (2004) Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. Mol Cell **16:** 1003–1015

Sullivan M, Uhlmann F (2003) A non-proteolytic function of separase links the onset of anaphase to mitotic exit. Nat Cell Biol **5:** 249–254

Sumara I, Vorlaufer E, Gieffers C, Peters BH, Peters JM (2000) Characterization of vertebrate cohesin complexes and their regulation in prophase. J Cell Biol **151:** 749–761

Tanaka H, Ishikawa M, Kitamura S, Takahashi Y, Soyano T, Machida C, Machida Y (2004) The *AtNACK1/HINKEL* and *STUD/TETRASPORE/AtNACK2* genes, which encode functionally redundant kinesins, are essential for cytokinesis in Arabidopsis. Genes Cells **9:** 1199–1211

Terret ME, Wassmann K, Waizenegger I, Maro B, Peters JM, Verlhac MH (2003) The meiosis I-to-meiosis II transition in mouse oocytes requires separase activity. Curr Biol **13:** 1797–1802

Tomonaga T, Nagao K, Kawasaki Y, Furuya K, Murakami A, Morishita J, Yuasa T, Sutani T, Kearsey SE, Uhlmann F, et al (2000) Characterization of fission yeast cohesin: essential anaphase proteolysis of Rad21 phosphorylated in the S phase. Genes Dev **14:** 2757–2770

Toth A, Ciosk R, Uhlmann F, Galova M, Schleiffer A, Nasmyth K (1999) Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. Genes Dev **13:** 320–333

Uhlmann F, Lottspeich F, Nasmyth K (1999) Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. Nature **400:** 37–42

Uhlmann F, Wernic D, Poupart MA, Koonin EV, Nasmyth K (2000) Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. Cell **103:** 375–386

Waizenegger IC, Hauf S, Meinke A, Peters JM (2000) Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. Cell **103:** 399–410

Yang CY, Spielman M, Coles JP, Li Y, Ghelani S, Bourdon V, Brown RC, Lemmon BE, Scott RJ, Dickinson HG (2003a) *TETRASPORE* encodes a kinesin required for male meiotic cytokinesis in Arabidopsis. Plant J **34:** 229–240

Yang M, Ma H (2001) Male meiotic spindle lengths in normal and mutant Arabidopsis cells. Plant Physiol **126:** 622–630

Yang X, Makaroff CA, Ma H (2003b) The *Arabidopsis Male Meiocyte Death1* gene is required for male meiosis and encodes a novel putative chromatin remodeling factor. Plant Cell **15:** 1281–1295

Yang X, Timofejeva L, Ma H, Makaroff CA (2006) The *Arabidopsis* SKP1 homologue ASK1 controls meiotic chromosome remodeling and release of chromatin from the nuclear membrane and nucleolus. J Cell Sci **19:** 4025–4032

Yu HG, Koshland D (2005) Chromosome morphogenesis: condensin-dependent cohesin removal during meiosis. Cell **123:** 397–407

Zhao DZ, Yang XH, Quan L, Timofejeva L, Rigel NW, Ma H, Makaroff CA (2006) ASK1, a SKP1 homolog, is required for nuclear reorganization, presynaptic homolog juxtaposition and the proper distribution of cohesin during meiosis in Arabidopsis. Plant Mol Biol **62:** 99–110