Homolog interaction during meiotic prophase I in *Arabidopsis* requires the *SOLO DANCERS* gene encoding a novel cyclin-like protein

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Interactions between homologs in meiotic prophase I, such as recombination and synapsis, are critical for proper homolog segregation and involve the coordination of several parallel events. However, few regulatory genes have been identified; in particular, it is not clear what roles the proteins similar to the mitotic cell cycle regulators might play during meiotic prophase I. We describe here the isolation and characterization of a new *Arabidopsis* mutant called *solo dancers* that exhibits a severe defect in homolog synapsis, recombination and bivalent formation in meiotic prophase I, subsequently resulting in seemingly random chromosome distribution and formation of abnormal meiotic products. We further demonstrate that the mutation affects a meiosis-specific gene encoding a novel protein of 578 amino acid residues with up to 31% amino acid sequence identity to known cyclins in the C-terminal portion. These results argue strongly that homolog interactions during meiotic prophase I require a novel meiosis-specific cyclin in *Arabidopsis*.

Keywords: *Arabidopsis*/cyclin/meiosis/recombination/synapsis

Introduction

Meiosis is required for eukaryotic sexual reproduction and provides an important mechanism for generating genetic diversity among individuals of a species. Cytological studies indicate that the meiotic prophase I is a long and complex stage that differs from the mitotic prophase (see reviews by Ashley and Plug, 1998; Dawe, 1998; Zickler and Kleckner, 1999). During prophase I, chromosomes condense and homologous chromosomes (homologs) pair. The prophase I continuum is divided into five substages based on chromosomal characteristics: first, in the leptotene stage, chromosomes begin to condense and can

be seen as thin thread-like structures. Homolog pairing initiates in leptotene and continues extensively in the zygotene stage. In the pachytene stage, fully synapsed homologs are observed as thick thread-like structures under a light microscope. The synaptonemal complex (SC) forms at this stage and can be observed in detail using electron microscopy. During the diplotene stage, homologs become desynapsed along much of their length, but remain attached through chiasmata. Finally, during the diakinesis stage, chromosomes contract lengthwise to produce highly condensed bivalents. Therefore, an important outcome of prophase I is the formation of bivalents. Although the mechanisms may differ, it is universal that homologs are attached until the onset of anaphase I.

Molecular genetic and biochemical studies in yeasts, *Caenorhabditis elegans* and *Drosophila* have provided many of the molecular insights into homolog interactions during prophase I (Roeder, 1997; Dernburg *et al.*, 1998; Orr-Weaver, 1999; Zickler and Kleckner, 1999). Such interactions include pairing, synapsis and recombination, which are intimately associated with each other. Meiotic recombination is thought to begin in late leptotene or zygotene stages after homolog pairing has initiated, and continues in the pachytene stage while the homologs are synapsed. In budding yeast, double-stranded DNA breaks (DSBs) are generated by the Spo11p protein and are required for both recombination and SC formation. In *C. elegans*, DSBs also initiate homologous recombination, which is not required for SC formation (Dernburg *et al.*, 1998). In budding yeast, the *recA* homologs Dmc1p and Rad51p are required for recombination and SC formation (Bishop *et al.*, 1992; Rockmill *et al.*, 1995; Shinohara *et al.*, 1997).

In addition, sister chromatids are closely associated due to sister chromatid cohesion during prophase I (Klein *et al.*, 1999; Hirano, 2000). In budding yeast, sister chromatid cohesion depends on the cohesin complex, which contains at least four proteins: Smc1p, Smc3p, Scc1p and Scd3p (Michaelis *et al.*, 1997; Nasmyth, 1999). Rec8p, a meiosis-specific Scc1p homolog, from both fission and budding yeasts, is essential for normal meiosis (Molnar *et al.*, 1995; Orr-Weaver, 1999; Stoopmyer and Amon, 1999; Watanabe and Nurse, 1999). During meiotic prophase I and metaphase I, sister chromatid cohesion along the chromosomal arms is required to maintain bivalents. The separation of sister chromatids along the arms at the metaphase I–anaphase I transition allows the homologs to separate. Sister chromatid cohesion at the centromere serves to maintain sister association until anaphase II, when the sisters separate. Therefore, both the recombinational crossover and sister chromatid cohesion contribute to the formation of chiasmata. Clearly, regulation of both recombination and sister chromatid cohesion is critical for proper segregation of homologs, although very little is

known about such regulation at the molecular level, including the identity of genes that play regulatory roles.

Numerous studies have been conducted to understand the molecular control of the mitotic cell cycle, particularly in animal and fungal systems (Futcher, 1991; Reed, 1991; Pines, 1993; Roberts *et al.*, 1994). Among known mitotic cell cycle regulators, cyclins and cyclin-dependent protein kinases (CDKs) play central roles in controlling all major phases of the cell cycle, such as the G₂/M and G₁/S transitions (Murray, 1994; Andrews and Measday, 1998; Gitig and Koff, 2000). Cyclins were first identified as proteins showing a cyclical pattern of accumulation and destruction during early embryonic development in marine invertebrates (Evens *et al.*, 1983; Swenson *et al.*, 1986). In budding yeast, genetic analysis with various combinations of mutations in the B-type cyclins CLB1, CLB2, CLB3 and CLB4 indicates that CLB1 and CLB4 are required for meiosis II, but not for meiosis I (Grandin and Reed, 1993; Dahmann and Futcher, 1995). Furthermore, the budding yeast B-type cyclins CLB5 and CLB6 are required for pre-meiotic DNA replication, which seems indirectly to affect the initiation of recombination and SC formation (Dirick *et al.*, 1998; Stuart and Wittenberg, 1998; Smith *et al.*, 2001). Also, mouse cyclin A1 is required for the progression from pachytene to diplotene (Liu *et al.*, 1998). However, whether there is a more direct requirement for cyclins in homolog synapsis and bivalent formation during prophase I is not known.

Plants have been one of the most important sources of our knowledge about meiosis at the cytological level (Dawe, 1998; Zickler and Kleckner, 1999). Mutants with defects in homolog interactions have been described in several plants (Kaul and Murphy, 1985; Dawe, 1998). In particular, a wheat asynaptic mutant seemed to have normal leptotene but unpaired/unattached homologs throughout prophase I (La Cour and Wells, 1970). In addition, the maize *desynaptic* (*dy*) mutant was shown to form an SC, but could not fully maintain the SC at late pachytene; bivalents separate into univalents at a variable frequency during diplotene and diakinesis (Nelson and Clary, 1952; Maguire, 1978). However, the molecular nature of these mutant defects is as yet unknown.

Recently, normal male meiosis in *Arabidopsis* has been described using fluorescence microscopy (Ross *et al.*, 1996; Peirson *et al.*, 1997), paving the way for molecular genetic analysis of *Arabidopsis* meiosis. For example, the *Arabidopsis* *SYN1* gene (also called *DIF1*) encoding a protein similar to the yeast Rec8p cohesin subunit is essential for normal meiotic chromosome condensation and homolog interactions (Bai *et al.*, 1999; Bhatt *et al.*, 1999). In addition, disruption of an *Arabidopsis* *SPO11* homolog (*AtSPO11-1*) causes defects in meiotic recombination, SC formation and bivalent formation (Grelon *et al.*, 2001). Furthermore, *recA* homologs *AtDMC1* and *AtRAD51* are also required for meiosis in *Arabidopsis* (Klimyuk and Jones, 1997; Couteau *et al.*, 1999), and the Rad51 protein has also been implicated in meiosis of maize and lily (Anderson *et al.*, 1997; Franklin and Cande, 1998; Franklin *et al.*, 1999).

We have now isolated a meiotic mutant (*solo dancers*, or *sds*) that is severely defective in interactions between homologs in prophase I. Furthermore, we show that the *SDS* gene is expressed specifically in male and female

meiocytes and encodes a protein that is similar to known cyclins. Our results demonstrate for the first time that a meiosis-specific novel cyclin-like protein is critical for normal homolog synapsis and bivalent formation, suggesting that a CDK may play a key role in controlling the normal chromosomal events during meiotic prophase I in *Arabidopsis*.

Results

Isolation and genetic characterization of a meiotic mutant

In higher plants, male meiosis occurs inside the anther portion of the male reproductive organ, the stamen. To identify genes important for male meiosis, we screened for *Ds* insertional mutants with reduced fertility (see Materials and methods). Wild-type *Arabidopsis* plants (Figure 1A) produce large seedpods with dozens of seeds. We found that the progeny of one of the *Ds* lines segregated for normal and nearly sterile plants that had small seedpods (Figure 1B); furthermore, the normal and mutant plants were in a ratio of ~3:1, suggesting that the parental plant was heterozygous for a recessive mutation. When the mutant plants also expressed the *Ac* transposase, they could produce fertile sectors due to the excision of the *Ds* element (Figure 1C). Whereas normal flowers produce many pollen grains (Figure 1D), the mutant flowers produced little or no normal pollen (Figure 1E). Nevertheless, the mutant was able to produce some seeds when pollinated with wild-type pollen, indicating that it was female fertile, although the seed set was much lower than normal. In addition, progeny of a cross from such a pollination were normal, confirming that the mutation was indeed recessive. We have named this new mutant *solo dancers* (*sds*) for its meiotic phenotype (see below).

The *sds* mutant appeared to be normal in vegetative and flower development, producing normal floral organs (Figure 1E; other data not shown). Wild-type *Arabidopsis* anthers in an unopened floral bud contain normal pollen grains (Figure 1F). However, the *sds* mutant anthers had many abnormal pollen grains with variable sizes (Figure 1I). The defective pollen grains degenerated before the flower opened (data not shown). Analysis of immature anthers showed that the mutant microspores also had different sizes (Figure 1J), unlike normal microspores (Figure 1G). Further examination revealed that whereas a normal meiosis always produces four microspores of equal size in a tetrad (Figure 1H), the mutant male meiosis produced 2–8 microspores of variable sizes (Figure 1K shows a 'hexad' with six spores). From 565 *sds* meioses, we found six (1.1%) diads, 20 (3.5%) triads, 293 (51.9%) tetrads, 141 (25%) pentads, 80 (14.2%) hexads, 21 (3.7%) heptads and four (0.7%) octads. Therefore, the *sds* mutant is defective in male meiosis.

The sds mutant is defective in homolog synapsis and bivalent formation in meiotic prophase I

To determine meiotic defect(s) of the *sds* mutant, we compared wild-type and mutant male meiosis using a chromosome spread and 4',6-diamino-2-phenylindole dihydrochloride (DAPI) staining procedure (Ross *et al.*, 1996). During leptotene (Figure 2A) (Ross *et al.*, 1996),

Fig. 1. Wild-type and *sds* mutant plant, flower and pollen development. (A) A wild-type plant with normal seedpods (arrowheads). (B) An *sds* mutant plant, with small seedpods (arrows). (C) An *sds* mutant plant with small seedpods (arrows) and two large revertant sectors that have large seedpods (arrowheads). (D) A close-up view of the top portion of a wild-type flower, showing many pollen grains (p) on the stigma and along the side of the pistil. (E) Top of an *sds* flower, showing anthers and the stigma (s) that lack pollen. (F) A portion of a wild-type anther with functional pollen grains that stained red. (G) Wild-type microspores. (H) A normal tetrad with four spores (numbered). (I) A portion of an *sds* anther, showing many abnormal pollen grains stained blue. Several pollen grains are stained red and presumably are functional (arrows); some are larger than normal. (J) Microspores from an *sds* anther, showing a range of sizes. (K) Six spores from a meiosis in the *sds* mutant. Bar = 10 mm in (A–C), 250 μm in (D) and (E), 20 μm in (F), (G), (I) and (J), and 10 μm in (H) and (K).

chromosomes begin to condense and form visible thin lines. Chromosomes continue to condense and begin synapsis at zygotene (Figure 2B); at pachytene (Figure 2C), homologs have completed their synapsis, as

indicated by the appearance of thick lines. In late diplotene (Figure 2D), homologs desynapse along much of the chromosome, leaving only limited association at the chiasmata; the chromosomes are also condensed further.

Fig. 2. Male meiosis I in wild-type and the *sds* mutant. Shown are images of DAPI-stained chromosomes. (A–E) Wild-type prophase I at leptotene, zygotene, pachytene, diplotene and diakinesis, respectively; note that (E) shows five brightly stained entities, representing five attached pairs of condensed homologs. (F–J) The *sds* mutant prophase I at similar stages; note that in (J), 10 staining bodies can be seen, indicating that the condensed homologs formed univalents. The arrows, as well as the two pairs of arrowheads, point to two strands of a partially separated chromosome, suggesting precocious separation of the arms of sister chromatids. (K–N) Wild-type early metaphase I, late metaphase I, anaphase I and telophase I, respectively. Chromosomes align at the equator (K), and are elongated presumably due to the forces of the spindle (L). Homologs separate (M) and form two clusters (N). (O–R) Meiosis I images from the *sds* mutant after prophase I. Chromosomes condense further (O), similarly to normal metaphase I chromosomes, but they did not all align at the equator. Chromosomes in (P) are elongated similarly to those in (L). Chromosomes are elongated further in (Q), suggesting that they might be pulled by the spindle as normal homologs are at anaphase I. Some chromosomes more distant from the center seem to have decondensed (R), resembling those in normal telophase I.

At late diakinesis (Figure 2E), chromosomes are very highly condensed and the five bivalents can be easily recognized.

In *sds* mutant cells, chromosomal patterns similar to the wild-type leptotene through pachytene stages were observed (Figures 2F–H). Although it is difficult to determine whether diplotene in the *sds* mutant (Figure 2I) was normal, it was obvious that by diakinesis the homologs were not attached in *sds* cells, as indicated by the observation of 10 univalents (Figure 2J). In addition, we noticed that the *sds* cells with chromosome images similar to that in Figure 2H were very infrequent, suggesting a defect in synapsis. To determine the distribution of prophase I cells, we examined hundreds of wild-type and *sds* meiotic cells. Because male meiosis in *Arabidopsis* is slightly asynchronous, a population of male meiotic cells from a single flower can cover a few adjacent meiotic stages or substages of the long prophase I. When data from samples containing only or largely prophase I cells were summarized (Figure 3), we found that among the wild-type prophase I cells (three samples, 717 cells), nearly half were pachytene cells, and relatively few were leptotene cells, with the other stages having moderate

Fig. 3. Distribution of prophase I cells in wild type and the *sds* mutant. Chromosome spreads were examined as shown in Figure 2. The number of images at each stage is shown above the appropriate bar. The *sds* leptotene, zygotene, pachytene and diplotene stages were assigned on the basis of chromosome condensation and overall morphology, as shown in Figure 2. The *sds* zygotene and diplotene stages were not based on pairing or bivalents, respectively. The *sds* diakinesis images all had 10 univalents.

Fig. 4. Female meiosis I in wild type and the *sds* mutant. Shown here are DAPI-stained images of chromosome spreads from the wild type (A–C) or the *sds* mutant (D–I). (A) A diakinesis image showing five bivalents. (B) At metaphase I, the five bivalents are aligned in parallel. (C) At anaphase I, homologs had segregated and formed two groups of five chromosome each. (D) At diakinesis, 10 univalents can be seen. (E) A metaphase I cell with 10 univalents. (F) An anaphase I cell with one pair of chromosomes elongated (arrowheads), suggesting that they were from a bivalent. (G) A diakinesis nucleus with one bivalent (arrows) and eight univalents (arrows point to two of them). (H) A metaphase I cell with two bivalents (arrows) and six univalents. (I) An anaphase I cell with three pairs of separating chromosomes that were probably from three bivalents. Three (arrowheads) of the four univalents were on one side of the equator, probably resulting in spores that have four or six chromosomes.

numbers of cells. In particular, pachytene was also the most frequent class in each individual sample (data not shown). In contrast, the *sds* mutant showed a dramatically different distribution for prophase I substages (six samples, 1241 cells); the number of cells at the leptotene and zygotene stages was higher than normal, whereas cells at the pachytene stage were very rare. In this case, pachytene-like cells were the least frequent in each sample. The *sds* distribution of prophase I stages further supports the idea that synapsis is defective in the mutant.

Therefore, the *sds* mutant had a clear defect in homolog synapsis and bivalent formation in male meiosis, suggesting that normal homolog synapsis could not be achieved in the absence of *SDS* gene function, and chromosomes form univalents at the end of prophase I. On the other hand, chromosome condensation seems to be unaffected by the *sds* mutation, unlike the *syn1* mutant (Bai *et al.*, 1999; Bhatt *et al.*, 1999). We likened the wild-type chromosome interactions and condensation to a highly choreographed duet dance in which homologs ‘dance’ in pairs. Therefore, the mutant had a clear phenotype of homologs behaving as ‘solo dancers’.

Next, we compared wild-type and *sds* meiosis from metaphase I to telophase I for possible additional mutant defects. In wild-type cells at metaphase I (Figure 2K and

L), the five bivalents align at the equatorial plane. At anaphase I, homologs separate (Figure 2M), and move towards the opposite pole of the spindle (Figure 2N). In contrast, the univalents in the *sds* mutant did not align completely; some of them were quite far from the equator (Figure 2O and P). The abnormal distribution seemed to persist (Figure 2Q and R). It seemed that usually more than two clusters of chromosomes were formed. We examined chromosome distribution at late meiosis I. For wild-type cells at anaphase I, 62 out of 66 cells (93.9%) showed a 5:5 even distribution of chromosomes; the other four cells had <10 chromosomal DAPI spots, most probably due to a superimposition of some chromosomes. On the other hand, *sds* male meiotic cells exhibited many abnormal distribution patterns. Among 71 *sds* cells at anaphase I, 46 cells (64.8%) had two groups of chromosomes with the following distributions: 5:5 (nine cells), 6:4 (16 cells), 7:3 (14 cells), 8:2 (six cells) and 9:1 (one cell). The remaining cells had one (14 cells; 19.7%), two (eight cells; 11.3%) or three (three cells; 4.2%) chromosomes at the equator, with various distributions of other chromosomes (data not shown) on either side of the equator.

Because the *sds* mutant also had reduced female fertility, we examined female meiosis in the wild-type and the *sds* mutant. As shown in Figure 4, wild-type

Fig. 5. Male meiosis II in wild type and the *sds* mutant. (A–O) Images obtained from chromosome spreads. (A–D) Wild-type meiosis II at prophase, metaphase, anaphase and telophase, respectively. The arrows in these panels indicate the characteristic organellar band present during meiosis II. The DAPI staining patterns suggest the following events during normal meiosis II. First chromosomes condense (A) and move to the equatorial plane (B). Then sister chromatids separate (C) and move apart (D). The well-separated chromosomes form four nuclei (E). In the *sds* mutant, meiosis II seems to follow the normal course of events, although the distribution of initial groups of chromosomes is abnormal due to defects in meiosis I. Arrows in (F–H) and (K–N) indicate the organellar band. This band is also present in (I), but it is faint. Chromosomes condense (F and K) but they do not align into tight plates (G and L). Nevertheless, subsequent separation of sister chromatids seems normal (H, I, M and N). In some cases, even an isolated chromosome can separate into two sisters (arrowheads in H and M). The separated chromosomes then form clusters. In (I), there are two pairs of clusters with four chromosomes each, and a pair of clusters each with two chromosomes (arrowheads); in (N), one pair has four chromosomes in each cluster (arrowheads) and the other pair has six chromosomes in each cluster. These clusters then form nuclei (eight in J and six in O).

prophase I at diakinesis displays five bivalents (Figure 4A), which become highly condensed at metaphase I (Figure 4B). The homologs separate at anaphase I, showing two groups with five chromosomes each (Figure 4C). In contrast, female meiosis in the *sds* mutant at diakinesis showed a variable number of univalents. Among 48 nuclei observed, 23 (47.9%) had only univalents (e.g. Figure 4D), similar to the *sds* male meiosis. Seven (14.6%) and eight (16.7%) nuclei had one (Figure 4G) or two bivalents (data not shown), respectively, with additional chromosomes forming univalents. Only a very small number of nuclei had four (2/48, or 4.2%) or five (2/48) bivalents (data not shown). These 8% of female meioses probably accounted for most of the female fertility when normal pollen was used. At metaphase I in the *sds* mutant, as expected, many female meioses had only univalents (Figure 4E) and others had some bivalents (Figure 4H). At anaphase I, one or more pairs of chromosomes were often observed, suggesting that they were homologs that had been associated in bivalents (Figure 4F and I). Other anaphase I images are consistent with an absence of bivalents (data not shown). These results indicate that in the *sds* mutant, female meiosis is defective in a way similar to male meiosis, but to a lesser extent.

During meiosis II in the wild-type male meiocytes (Figure 5), the two groups of chromosomes are separated by a band of organelles (arrows in Figure 5A–D). Partially condensed chromosomes were observed during prophase II (Figure 5A); at metaphase II, they congressed at the two equators (Figure 5B). Sister chromatids then separated at

anaphase II (Figure 5C), moved to opposite poles at telophase II (Figure 5D) and decondensed to form four nuclei (Figure 5E). In the *sds* mutant, chromosome distribution was abnormal, most probably due to a defective meiosis I, but chromosome condensation and sister chromatid separation seemed to be quite normal. Chromosomes condensed at prophase II (Figure 5F and K), but alignment was abnormal (Figure 5G and L). Then sister chromatids separated at anaphase II (Figure 5H and M), and moved apart at telophase II (Figure 5I and N). Distributions of meiotic cells from metaphase I to the end of meiosis II were not dramatically different between the wild type and the *sds* mutant (data not shown).

Our results indicate that the *sds* mutant is defective in homolog synapsis and bivalent formation in prophase I. In addition, there was no obvious defect during later stages of meiosis I or during meiosis II other than those that are probable consequences of the failure to form bivalents. Furthermore, clear separation of sister chromatids at meiosis II indicates that meiotic DNA replication was not obviously affected in the mutant. Therefore, the *sds* mutant is specifically defective in homolog interaction during prophase I.

The *sds* mutant is defective in meiotic recombination

The phenotypes of the *sds* mutant were similar to those of the recombination mutants *Atspo11* and *Atdmc1*, suggesting that *sds* might also be defective in recombination. To test such a defect in the *sds* mutant, we generated *sds/sds*

Table I. Meiotic recombination in normal and *sds* mutant plants

Markers	<i>sds</i> mutant				Normal plants ^a				Map distance ^d
	Genotypes ^b			Recombination frequency ^c	Genotypes ^b			Recombination frequency ^c	
	P	R	DR		P	R	DR		
Chrom. II nga1126 and AthBIO26	91	0	0	0	109	45	2	17.0%	25.46
Chrom. V nga76 and nga106	92	9	0	4.5%	88	38	1	16.5%	35.05

^aPlants included both *SDS/SDS* (chromosome II) and *SDS/sds* (chromosome V) genotypes.

^bP (parental) = L1L2/L1L2 + L1L2/C1C2 + C1C2/C1C2; R (recombinant) = L1L2/L1C2 + L1L2/C1L2 + L1C2/C1C2 + C1L2/C1C2; DR (double recombinant) = L1C2/L1C2 + C1L2/C1L2; where L1 and L2 are Ler alleles, and C1 and C2 are Col alleles.

^cRecombination frequency = $(R + 4 \times DR) / (2P + R + DR)$. The observed recombination frequencies in normal plants were lower than the published map distances, possibly due to the inability to score double or multiple crossovers; the chromosome V distance might also reflect a dominant effect of the *sds* mutation on recombination because the plants were *SDS/sds*.

^dThe map distances (cM) are from http://genome.salk.edu/SSLP_info/SSLPsordered.html.

plants that were heterozygous (L1L2/C1C2) for two molecular markers on each of chromosomes II and V (see Materials and methods). Although the *sds* mutant was nearly sterile, it was able to produce 50–100 seeds per plant (a normal plant can easily produce several thousand seeds). When the seeds from *sds* plants were planted, many of them developed to maturity and exhibited the same *sds* defects in fertility and meiosis (data not shown), indicating that the seeds from *sds* plants were not from cross-pollination. The progeny of *sds/sds* plants allowed the analysis of recombination frequency in the *sds* mutant. The *sds*/+ or +/+ siblings were also heterozygous for the same molecular markers and served as positive controls for recombination.

The progeny of both *sds* and normal plants (L1L2/C1C2) were analyzed for genotypes of these markers. As shown in Table I, for the markers nga1126 and AthBIO26 on chromosome II, the control plants had 17.0% recombinants, whereas the *sds* plants had no detectable recombinants. Similarly, for the markers nga76 and nga106 on chromosome V, the control plants showed 16.5% recombinants, in contrast to the 4.5% recombinants among *sds* plants. Therefore, meiotic recombination is greatly reduced in the *sds* mutant. Our results were obtained from viable seedlings, which resulted from viable pollen and embryos.

The progeny of the *sds* mutant are often aneuploid

Because *sds* mutant meiosis appeared to have random chromosome segregation at meiosis I, we suspected that seeds from *sds* plants might carry an abnormal number of chromosomes, which could cause visible developmental defects. When the seeds from *sds* plants were germinated on plates and examined under the dissecting microscope, we found that a considerable fraction of seedlings (53 out of a total of 124) showed abnormal development (Figure 6). Unlike normal seedlings with two similarly sized cotyledons (Figure 6A), seedlings from the seeds of *sds* mutant plants had three cotyledons (two seedlings, see Figure 6B and C), two unequal cotyledons (six, Figure 6D), one cotyledon (four, Figure 6E), one cotyledon and no hypocotyl (one, Figure 6F) or had root defects (40, data not shown). The seedling defects were similar to those of the *Atdmc1* mutant seedlings (Couteau *et al.*, 1999). Because *sds* seedlings from *sds*/+ heterozygous plants never

Fig. 6. Wild-type and *sds* seedlings. (A) A wild-type seedling with two evenly sized cotyledons. (B–F) Abnormal seedlings from seeds of the *sds* mutant, with three cotyledons (B and C), two unequal cotyledons (D), one cotyledon (E), and one cotyledon and defective hypocotyl (F). All panels are at the same magnification; bar = 1 mm.

showed any developmental defects prior to meiosis, the abnormal development of progeny of *sds* plants was unlikely to be caused directly by the *sds* mutation.

To test directly the hypothesis that the abnormal seedlings were aneuploid, we then determined the number of chromosomes in wild-type seedlings and the progeny of *sds* plants. As expected, all 13 wild-type seedlings examined had 10 chromosomes. On the other hand, when 13 normal-appearing progeny of *sds* were examined, one had 11 chromosomes and another had nine chromosomes, whereas the others had 10. Even more strikingly, when 13 *sds* seedlings with abnormal development were tested, only one had 10 chromosomes; the remaining 12 seedlings had the following numbers of chromosomes: nine (four seedlings), 11 (two seedlings), 12 (one seedling), 13 (three seedlings) and 15 (two seedlings). Therefore, progeny of *sds* plants were often aneuploid, presumably due to abnormal chromosome segregation at meiosis in *sds* plants.

The *SDS* gene encodes a cyclin-like protein

The *Ds* element in *sds* carries an *NPT* (neomycin phosphotransferase) gene conferring kanamycin resistance (Sundaresan *et al.*, 1995), which co-segregated with the *sds* mutant phenotype. If the *Ds* element was indeed

Fig. 7. The *SDS* locus and gene structure. (A) The DNA and predicted amino acid sequences of the *SDS* locus near the *Ds* insertional site. The *sds* mutant sequence shows an 8 bp duplication (underlined). The revertants have 9 or 6 bp insertions (bold). (B) The *SDS* gene structure with exons and introns, and the *SDS* cDNA.

inserted into the *SDS* gene, excision of the *Ds* element would produce normal-appearing revertant sectors. We screened among *sds* mutant plants carrying an *Ac* element and found that nine of 13 *sds* plants produced phenotypically normal revertant sectors. The flowers of one large sector (Figure 1C) produced normal pollen (data not shown); this ruled out the possibility that the seeds of the revertant sector were due to contaminating pollen. Seeds of three revertant sectors were planted, and each segregated for mutant plants; therefore, the fertile plant sectors were heterozygous, consistent with an excision of the *Ds* element from one of the two alleles. These results strongly support the idea that the *sds* mutation was caused by a *Ds* insertion.

The genomic sequences flanking the *Ds* element were obtained using thermal asymmetric inter-laced PCR (TAIL-PCR; Liu *et al.*, 1995) and shared the same 8 bp immediately adjacent to the *Ds* sequence, consistent with the 8 bp duplication characteristic of *Ds* insertions (Figure 7A). Using primers matching genomic sequences flanking the *Ds* element, we amplified a PCR fragment of the expected size from wild-type and revertant genomic DNAs, but not from mutant DNAs (data not shown). We analyzed the sequences of the PCR fragment from seven revertants, and found that four had wild-type sequences, while the other three had 6 or 9 bp insertions (*Ds* excision footprints) which did not disrupt the open reading frame (Figure 7A). These results confirm that we have identified the genomic insertion that is responsible for the *sds* mutant phenotype, and that the insertion most probably disrupts a protein-coding region.

Using the *SDS* genomic sequence as a probe, we isolated a 2.1 kb cDNA clone after screening $\sim 1 \times 10^6$ plaques of a floral cDNA library (Fan *et al.*, 1997). The *SDS* sequence also matches a sequenced bacterial artificial chromosome (BAC) clone (F10B6; accession No. AC006917) ~ 20 cM from the distal end of the left arm

of chromosome 1. Comparison between the *SDS* genomic and cDNA sequences indicates that there are seven exons and six introns (Figure 7B). The exons are 1117, 168, 167, 129, 181, 45 and 294 bp, and the introns are 189, 249, 297, 88, 93 and 96 bp. It should be noted that the *SDS* cDNA sequence differs from the exon sequence of the predicted gene (gene ID At1g14750) from the genomic sequence in two places: the actual introns 2 and 6 were longer at the 3' and 5' ends by 51 and 57 nucleotides, respectively. The additional 17 and 19 amino acid residues, respectively, encoded by the additional predicted exon sequences would have interrupted the cyclin core of the SDS protein (see below).

The predicted SDS protein contains 578 amino acids (Figure 8A). The C-terminal one-third of SDS is similar to the cyclin core region of several known *Arabidopsis* cyclins (Figure 8A–C). Like other cyclins, its N-terminal region contains a putative 'destruction box' motif with a conserved element and several lysine residues (Figure 8A; Hunt, 1991). Most of the SDS N-terminal region consists of several putative PEST elements, rich in proline (P), glutamate (E) or aspartate (D), serine (S) and threonine (T) residues (Rogers *et al.*, 1986) (Figure 8A). These PEST elements may play a role in targeting SDS for proteolytic degradation. In addition, the SDS protein contains several potential sites for phosphorylation by CDKs.

Some cyclins, such as the yeast G_1 cyclins Cln1, -2 and -3, have the cyclin core region near their N-termini (Nash *et al.*, 1988; Hadwiger *et al.*, 1989). In plants, previously known cyclins have been classified based on amino acid sequence similarity in the cyclin core into three types, A, B and D, which are grouped further into subtypes (Renaudin *et al.*, 1996). Among these known plant cyclins, D-type cyclins also contain a cyclin core near their N-termini (Figure 9A). In contrast, in plant A- and B-type cyclins, the cyclin core is located near the C-terminus (Figure 9A). Therefore, the location of the cyclin core in the SDS protein is similar to those of plant A- and B-type cyclins, although the SDS N-terminal region is unusually large. Within the cyclin core, members of the same type share $\geq 49\%$ amino acid sequence similarity and those of the same subtype can share 68–97% identity; however, levels of amino acid sequence identity between different types are only 20–39% (Figure 8B and C). Because the putative SDS cyclin core has 23–32% identity to known cyclins, we hypothesize that SDS is a new type of cyclin.

As a first test for the hypothesis that SDS is a new type of cyclin, we investigated whether SDS can interact with known *Arabidopsis* CDKs Cdc2a and Cdc2b (Hirayama *et al.*, 1991), using the yeast two-hybrid assay (Fields and Song, 1989). A portion of the SDS coding region containing the cyclin homology was fused with the coding region for the GAL4 DNA-binding domain to yield the bait construct (Figure 9A). The Cdc2a and Cdc2b coding regions were each fused with that for the GAL4 transcriptional activation domain to produce the prey constructs. The bait and prey constructs were introduced into α or a mating-type yeast strains, respectively, and were brought together by yeast mating. The yeast cells carry a *HIS3* reporter gene that is transcriptionally controlled by GAL4 and that allows cells to grow on media lacking histidine. If a bait and a prey interact to a sufficient level, then the activation of the *HIS3* reporter gene will support yeast

Fig. 8. Sequence and comparison of the SDS protein. (A) The predicted SDS amino acid sequence (accession No. AJ457977). A putative destruction box with additional lysine residues is found near the N-terminus (bold). Eight putative PEST elements (underlined and marked) can be recognized. Most of the remaining portion (underlined region) is similar to cyclin cores. Asterisks are below residues that are potential sites of phosphorylation by CDKs. (B) Alignment of the cyclin-like region of SDS with representative of known *Arabidopsis* cyclins, based on BLAST search results and minor adjustment. The numbers to the left and at the end of the sequences are the amino acid numbers. The regions shown here (~200 amino acids) are smaller than the cyclin cores (~250 amino acids) of cyclins A and B (Renaudin *et al.*, 1996) because of the lack of conservation in SDS at ends of the cyclin core. (C) Percentage identity and similarity (including conservative substitutions; number after slash) between SDS and *Arabidopsis* cyclin Bs, A2.1 and D1, using the regions shown in (B). Accession Nos: A2.1, Z31589; B1.1, X62279; B1.2, L27223; B2.1, Z31400; B2.2, Z31401; and D1, X83369 (Renaudin *et al.*, 1996).

growth without histidine. As shown in Figure 9B, SDS was able to allow yeast cells to grow without histidine in the

Fig. 9. Analysis of the SDS protein. (A) Regions in SDS and several known *Arabidopsis* cyclins. The regions containing putative PEST elements and the cyclin core homologous regions are shaded as indicated. The numbers indicate the beginning and end of each protein, as well as the approximate boundaries of each region. The thick line below the SDS protein represents the region included in the bait construct for the yeast two-hybrid assay. (B) Interaction of SDS with CDKs from the yeast two-hybrid assay. The *Arabidopsis* CycB1.1 protein and the p53 protein serve as positive and negative controls, respectively. The left panel is from a plate lacking leucine and tryptophan; the right panel is from a plate lacking leucine, tryptophan and histidine. The cells with the SDS, CycB1.1 or p53 bait construct alone failed to grow without histidine (data not shown).

presence of Cdc2a or Cdc2b, supporting the hypothesis that SDS can interact with these CDKs.

The SDS gene is expressed specifically in meiotic cells

To obtain additional clues about how SDS functions, we analyzed the expression of the SDS gene. The SDS cDNA clone was very rare; in addition, Northern hybridizations detected only very faint signals in floral bud RNA samples (data not shown). Because the *sds* mutant had only fertility defects, we first examined SDS expression in developing wild-type *Arabidopsis* flowers, particularly at stages near meiosis, by RNA *in situ* hybridization (Figure 10). SDS expression was not detectable in the inflorescence meristem or in very early floral primordia (data not shown). Similarly, no SDS expression was found in a stage 8 developing flower (Figure 10A, as defined previously by Smyth *et al.*, 1990), when anther differentiation had begun but meiosis had not occurred. At about stage 9 (Figure 10B), or the time of male meiosis, we observed a strong SDS signal that was restricted to the male meiocytes (arrows) within the anther locules, but not in other tissues of the anther or in other floral organs. At stage 11 (Figure 10C), when pollen development was progressing, again there was no detectable SDS expression. A sense

Fig. 10. The *SDS* gene expression pattern. (A–C) *In situ* RNA hybridization results from a radioactive probe. The left panel in each is a bright field image showing the tissues, the center panel is a dark field image, and the right panel is a composite image of both. (A) A stage 8 flower. (B) A stage 9 flower. The strong *SDS* expression signal corresponds to male meiotic cells inside the anthers. (C) A stage 11 flower. (D and E) *In situ* results from a digoxigenin-labeled probe. (D) An ovule in a stage 10 floral bud, showing the female meiocyte (arrow) with intense *SDS* signal, whereas the surrounding cells lacked any obvious expression. (E) An ovule from a stage 10 floral bud probed with the control sense probe, without any signal. Scale bars: in (A–C) = 50 μ m; in (D) (for D and E) = 10 μ m. (F) RT-PCR with *SDS*- (top) and *ASK1*- (bottom) specific primers, detected using DNA blot hybridization with gene-specific probes. The source of RNA in each lane (1–6, wild type; 7–9, *sds* mutant): 1, roots; 2, leaves; 3, floral stems; 4 and 7, young floral buds (up to stage 10); 5 and 8, older floral buds (stages 10–12); 6 and 9, open flowers.

probe control produced only background levels of signals (data not shown). Therefore, our results strongly suggest that in the anther, *SDS* is expressed specifically in male meiotic cells.

Because *SDS* was also needed for normal female meiosis, we also tested its expression in female meiocytes. Unlike male meiocytes which are in groups, individual female meiocytes are surrounded by somatic cells. Because RNA *in situ* hybridization using radiolabeled probes does not have high enough resolution to pinpoint accurately expression in a single cell, we switched to using non-radioactive probes for detecting *SDS* expression in the female meiocyte. As shown in Figure 10D, the female meiocyte (arrow) clearly expressed *SDS*, whereas control sense probe (Figure 10E) showed only background. The

somatic cells in the ovule did not have *SDS* signal (Figure 10D); further, other tissues of the female reproductive organ also lacked *SDS* signals (data not shown). Therefore, in the female reproductive organ, *SDS* is expressed specifically in the female meiocyte.

To test whether *SDS* was expressed in other organs at low levels, we performed RT-PCR experiments with RNA samples isolated from roots, leaves, floral stems, young inflorescences, older floral buds, open flowers and young fruits (seedpods). Our results (Figure 10F, top panel) indicate that *SDS* was expressed preferentially in young floral buds, but not in older buds, mature flowers or vegetative organs. As a negative control, we found that *SDS* expression was not detectable in floral buds and flowers of the *sds* mutant (Figure 10F). Moreover, as a positive control for mRNA and RT-PCR, we tested for the presence of mRNA from the non-specific *ASK1* gene (Yang *et al.*, 1999) in the same RNA samples; our results confirmed that *ASK1* mRNA was present in all organs tested (Figure 10F, bottom panel). Therefore, our results strongly suggest that *SDS* is expressed in a highly specific manner, both spatially and temporally.

Discussion

The Arabidopsis SDS gene is required for homolog synapsis and bivalent formation

We have described here the isolation of *solo dancers* (*sds*), a new mutant in *Arabidopsis* that exhibits clear defects in male and female meioses, resulting in greatly reduced male fertility and partial female fertility, respectively. Although the *sds* mutant male meiocytes displayed normal chromosome condensation during prophase I, there were many more zygotene cells and very few pachytene cells. One possibility is that in the *sds* mutant normal synapsis of homologs cannot be achieved for most cells. The observed *sds* zygotene stage cells could include the cells that had partial or no synapsis; further analysis is needed to determine whether there is homolog pairing in *sds* cells. The observations of individual univalents in all *sds* cells at late prophase I and early metaphase I, and of separated arms of sister chromatids for at least some chromosomes at diakinesis (Figure 2J) indicate that *sds* was defective in homolog synapsis and had a severe limitation in the formation and/or maintenance of bivalents.

In female meiosis of the *sds* mutant, many univalents were present at late prophase I and metaphase I, although one or two bivalents were often observed and four or five bivalents occasionally were found. Therefore, female meiosis was partially functional, providing an explanation for a higher female fertility than male fertility. More mild female meiosis and fertility phenotypes than male phenotypes have also been observed in several maize meiotic mutants (I. Golubovskaya and W. Zacheus Cande, personal communication). It is possible that the *SDS* function is not essential for female meiosis because one or more other cyclins (Renaudin *et al.*, 1996) might fulfill the *SDS* function partially. Another explanation for differences in female and male meiotic phenotypes is that female and male meioses may be regulated differently.

We observed that progeny of the *sds* mutant often exhibit abnormal seedling development. Because such abnormal seedlings were not observed among progeny of

SDS/sds plants, the abnormal development was probably a consequence of abnormal chromosome number, resulting from the meiotic defect of the *sds* parent. Indeed, analysis of chromosome number in such abnormal seedlings revealed that their chromosome numbers ranged from nine to 15. It is interesting to note that abnormal embryo and seedling development was also observed when a dominant-negative form of the CDK Cdc2a was expressed in the embryo (Hemerly *et al.*, 2000). The abnormal seedling patterning supports the idea that normal cell division is important for pattern formation during embryo development. Although the molecular basis for the abnormal development of the *sds* mutant progeny is not known, it is possible that an imbalance of gene function resulting from the aberrant chromosome number caused a disruption of normal cell division.

The *SDS* gene encodes a novel meiosis-specific cyclin-like protein

We have isolated the *SDS* gene and found that the predicted SDS protein contains a C-terminal domain that resembles known cyclin proteins from *Arabidopsis* and other plants. Previously identified plant cyclins can be divided into three types: A-, B- and D-type cyclins (Renaudin *et al.*, 1996) (Figure 8). Each type of plant cyclin shares some characteristic sequence motifs with the corresponding type from animals, although the overall levels of amino acid sequence identity in the cyclin core region are not high between corresponding types of animal and plant cyclins (Renaudin *et al.*, 1996). In addition, the levels of amino acid sequence identity are high between members of the same type, ranging from 49 to 84% (Renaudin *et al.*, 1996) (Figure 8C). The SDS cyclin-like domain is most similar to the B2 subtype of *Arabidopsis* B cyclins (31% identity) and slightly less similar to the B1 subtype and A-type cyclins (24–30% identity). The degrees of amino acid identity and similarity between SDS and other plant cyclins is within the range of those between A-, B- and D-type cyclins (20–39%) (Renaudin *et al.*, 1996) (Figure 8C). Therefore, SDS is distinct from all known cyclin types and may represent a novel type of cyclin. Furthermore, the finding that SDS can interact with the *Arabidopsis* CDKs Cdc2a and Cdc2b (Hirayama *et al.*, 1991) in a yeast two-hybrid assay further supports the hypothesis that SDS is a cyclin. It will be interesting to determine whether one or both of the CDKs tested are involved in regulating meiosis.

The SDS protein is unusual in having an extra long N-terminal region. Many cyclins have a non-conserved region in addition to the cyclin core region. In plants, the A- and B-type cyclins have an N-terminal non-conserved region that make up about half of the protein, whereas D-type cyclins have a small C-terminal non-conserved region (Figure 9A). The SDS N-terminal region is rich in hydrophilic residues, particularly in putative PEST elements (Rogers *et al.*, 1986). PEST elements are found in cyclins (Nash *et al.*, 1988; Salama *et al.*, 1994) (Figure 9A) and other unstable proteins (Chevaillier, 1993; Gorl *et al.*, 2001). It was found that the truncated yeast Cln3 proteins with larger deletions in the PEST region had greater stability (Tyers *et al.*, 1992), indicating that the size of the PEST region is correlated with the degree of instability. Therefore, the unusually large PEST

region of SDS suggests that the SDS protein might be very unstable.

Our *in situ* RNA hybridization results indicate that *SDS* is expressed in male and female meiocytes, whereas its expression was not detected in other cells and at other stages. Furthermore, RT-PCR experiments also showed that the only detectable expression was found in flowers containing meiotic cells, and not in vegetative organs or other stages of reproductive structure. These results strongly suggest that *SDS* is a meiosis-specific gene. Molecular genetic studies in yeast indicate that yeast cyclins are all expressed during mitosis. Even the CLB proteins that play important roles in meiosis are not meiosis specific (Grandin and Reed, 1993; Dahmann and Futcher, 1995; Dirick *et al.*, 1998; Stuart and Wittenberg, 1998; Smith *et al.*, 2001). Therefore, our results, including mutant phenotype, protein sequence and expression pattern, argue strongly that SDS may be a novel cyclin that regulates aspects of meiotic chromosome behavior in prophase I.

Meiosis can be considered as a modified cell cycle (Wolgemuth *et al.*, 1995; Zickler and Kleckner, 1999). Similarly to mitosis, meiosis is also preceded by DNA replication and involves chromosome condensation and sister chromatid segregation. Thus, it is reasonable to expect that some cyclin functions would be conserved between mitosis and meiosis. For example, the yeast B-type cyclins CLB5 and CLB6 are required for the meiotic S phase (Smith *et al.*, 2001), as they are for mitotic S phase (Epstein and Cross, 1992; Schwob and Nasmyth, 1993). Similarly, the mouse cyclin A2 is highly expressed in pre-leptotene spermatocytes, suggesting that it may be involved in controlling meiotic DNA replication (Ravnik and Wolgemuth, 1999). In addition, it was shown that the yeast CLB1 and CLB4 proteins, which are redundant with CLB2 and CLB3 for mitosis, are required for meiosis II but not meiosis I (Grandin and Reed, 1993; Dahmann and Futcher, 1995).

However, meiosis differs from the mitotic cell cycle in that it has an additional extended prophase I during which homologs undergo pairing, synapsis and recombination; this is followed by metaphase I through telophase I to allow homologs to segregate in a reductional division. To accommodate two divisions, sister chromatid cohesion removal is biphasic, first along the arms to allow homologs to separate, then at the centromere to allow sister separation (Orr-Weaver, 1999). Therefore, coordination of events in meiosis I would be likely to involve regulatory proteins that are functionally distinct from mitotic cell cycle regulators. Studies with the mouse cyclin A1 protein have provided strong support for meiosis-specific cyclins (Liu *et al.*, 1998, 2000). Mouse cyclin A1 protein is present just before or during meiosis I, but not in meiosis II (Ravnik and Wolgemuth, 1999). Furthermore, in mutant mouse with a disrupted gene for cyclin A1, male meiosis arrests before metaphase I (Liu *et al.*, 1998). Our results indicate that *SDS* also functions as a meiosis-specific regulatory gene, as discussed further in the next section.

Possible roles of *SDS* in regulating synapsis, recombination and/or sister chromatid cohesion

The *sds* mutant is defective in homolog synapsis and bivalent formation. It is possible that during meiosis in the

sds mutant, chromosomes can find their partners and begin pairing, but the synapsis could not be achieved. Such an incomplete or abnormal association might be unstable or indistinguishable from the zygotene stage at the light microscope level. Alternatively, chromosome pairing and SC formation might not occur in the *sds* mutant. Because the SDS protein is similar to cyclins, SDS might activate one or more CDK(s) during early to mid-prophase of meiosis I. The mammalian SCP1 protein, which is a probable component of the SC transverse filaments, has a potential target site for CDK that is thought to play a role in SC assembly and/or disassembly (Meuwissen *et al.*, 1992). In addition, the mammalian Cdk2 protein was found to localize to newly synapsed axes and to disappear by mid-pachytene stage (Ashley *et al.*, 2001). Therefore, it is possible that SDS-dependent CDK(s) might regulate the activity of pairing and/or synapsis proteins.

In animals and yeast, mutants defective in recombination exhibit homolog separation prematurely before metaphase I (Dernburg *et al.*, 1998; Woods *et al.*, 1999). In *Saccharomyces cerevisiae*, meiotic recombination is initiated by DNA DSBs, the formation of which requires several genes, including *SPO11* (Smith and Nicolas, 1998; Paques and Haber, 1999). DSBs are also required for SC formation, so mutations affecting DSBs also cause defects in synapsis. *Arabidopsis* male meiosis undergoes recombination once or twice per chromosome, suggesting that recombination is also needed for normal meiosis in *Arabidopsis* (Copenhaver *et al.*, 1998). In addition, mutations in *Arabidopsis AtSPO11* and *AtDMC1* genes cause defects in meiotic recombination, and SC and bivalent formation (Couteau *et al.*, 1999; Grelon *et al.*, 2001), similar to the *sds* defects. Furthermore, we showed here that the *sds* mutant had greatly reduced frequency of meiotic recombination. Because meioses that lacked any recombinational crossover probably resulted in a greater number of non-viable pollen and embryos, our results were probably an overestimate of the true recombination frequency in the *sds* mutant. It is possible that the *sds* defect in meiotic recombination and that in synapsis may be caused by the same molecular defect. Alternatively, recombination and synapsis may each be regulated by SDS via distinct mechanisms.

The *S. cerevisiae* B-type cyclins CLB5 and CLB6 were found to control recombination initiation and SC formation (Smith *et al.*, 2001). However, the fact that CLB5 and CLB6 are required for meiotic DNA replication suggests that the defects of *Clb5 Clb6* cells in recombination and SC formation might be a consequence of impaired replication. In contrast, meiotic DNA replication in the *sds* mutant appears to be normal. In mouse, cyclin A1 may play a critical role in the activation of the M-phase-promoting factor (CDK) during mouse spermatogenesis prior to metaphase I (Liu *et al.*, 2000). Unlike the *sds* mutant, in the mouse mutant defective in cyclin A1, male meiocytes can form synapsed chromosomes at pachytene (Liu *et al.*, 1998). Therefore, the proposed SDS functions in regulating synapsis and recombination might be distinct from those of the mouse cyclin A1 and the yeast CLB5 and CLB6. The idea that cyclin-CDK may regulate meiotic recombination is also supported by the recent observation that the mammalian Cdk2 protein co-localizes with the mismatch repair protein

MLH1 at sites of reciprocal recombination (Ashley *et al.*, 2001).

The *Atspo11* mutant can form some bivalents (Grelon *et al.*, 2001), suggesting that the *Atspo11* mutant is not totally defective in recombination due to gene redundancy (Grelon *et al.*, 2001). The fact that *sds* male meiosis is more defective than the *Atspo11* mutant in bivalent formation could be explained if the *sds* mutation could possibly affect another aspect of prophase I, such as sister chromatid cohesion, which is also required for bivalent formation. The observations that the meiotic recombination in the *sds* mutant was not zero and that there were a small number of pachytene-like images support the idea that homolog synapsis occurred at a very low frequency in *sds*. Therefore, during *sds* male meiosis, the failure of the infrequent homolog interactions observed at the pachytene stage to be maintained as bivalents at the diakinesis stage could be explained if sister chromatids separated prematurely along the arms, either at or before diakinesis; this idea is supported by the observation that some univalents at diakinesis had 'X' or 'Y' shapes. There are several lines of evidence for the idea that sister chromatid condensation and cohesion are regulated by protein phosphorylation (Wei *et al.*, 1998; Suja *et al.*, 1999; Wei *et al.*, 1999; Kaszas and Cande, 2000). Therefore, if SDS is indeed a meiosis-specific cyclin, one possible mechanism for its function could be to regulate the phosphorylation of proteins involved in sister chromatid cohesion.

Recently, the *Arabidopsis SWITCH* gene was shown to be required for sister chromatid cohesion during meiosis (Mercier *et al.*, 2001). In early male meiosis of the *swi1.2* mutant, homologs fail to synapse and sister chromatids separate during prophase I; consequently, 20 individual chromatids, instead of five bivalents, were observed at metaphase I, followed by abnormal chromosome segregation. In *swi1.2* female meiosis, chromosome segregation resembles that in mitosis, suggesting that sister chromatid cohesion is maintained until anaphase I. Therefore, *SWI1* may play related but distinct roles in male and female meioses; alternatively, female defects in *swi1.2* may be less severe than that in male meiosis. *SWI1* encodes a novel protein, so it is not clear what its biochemical function might be (Mercier *et al.*, 2001). Because chromosomes do condense in the *swi1.2* mutant and cohesins also play a role in chromosome condensation (Bai *et al.*, 1999; Bhatt *et al.*, 1999), the *SWI1* protein may specifically regulate sister cohesion but not condensation. If, as suggested here, SDS plays a role in controlling the timing of the separation of sister chromatids, SDS might regulate the activity of *SWI1*.

Our results presented here strongly support the idea that we have identified a meiosis-specific gene that is required for normal homolog synapsis and recombination in early to mid-prophase I of *Arabidopsis* meiosis. Furthermore, the remaining low frequency of recombination and pachytene-like images, combined with the lack of bivalents at late prophase I and metaphase I, support the idea that SDS may regulate the timing of sister chromatid separation. The SDS sequence and yeast two-hybrid results support the hypothesis that SDS is a new type of cyclin, suggesting that it may regulate recombination and sister chromatid cohesion via phosphorylation of proteins involved in these processes. Further analysis is necessary

to test these possibilities and to identify the target proteins of SDS. SDS provides a new and important entry point into understanding the regulation of homolog synapsis, recombination and bivalent formation in plant meiosis using genetic, cytological, genomic and biochemical approaches.

Materials and methods

Plant materials, mutant isolation and genetic analyses

Both the wild-type and the *sds* mutant plants were of the Landsberg *erecta* ecotype and were grown under long day conditions at 22–25°C unless otherwise indicated. *Ds* insertional lines were generated following crosses between homozygous *Ds*-carrying plants and homozygous *Ac*-carrying plants (Sundaresan *et al.*, 1995; Yang *et al.*, 1999). One *Ds* line was found to segregate for mutant plants that showed a severe reduction in fertility and was chosen for further analysis. F₂ progeny from crosses by pollinating *sds* pistils with normal pollen were grown to maturity and scored for fertility defects. The seeds of *sds* plants were germinated on MS plates and the developmental phenotypes of the seedlings were examined after 8 days. Some of these seedlings were grown to maturity to determine fertility.

To screen for revertant sectors, F₂ progeny of the original *Ds* × *Ac* cross were grown to maturity and *sds* mutant plants were retained. A majority (~3/4) of these *sds* plants should also carry the *Ac* element, and *Ds* excision would produce a revertant sector. Because stable *sds* mutant plants never produced large seedpods with a full seed set, putative revertant sectors were identified as either a normal branch with multiple large seedpods or a single large seedpod. Seeds from these putative revertant sectors were planted and grown to maturity. DNAs were isolated from the phenotypically normal plants for further analysis. For one large sector of a branch, flowers were examined using a dissecting microscope and were found to have normal pollen.

Phenotypic characterization

Fresh plant samples were either photographed and the photographs then scanned to yield digital images or were photographed using a dissecting microscope and an Optronics digital camera. Anthers prior to dehiscence were stained (Alexander, 1969) to distinguish viable and dead pollen grains. Developing microspores and meiotic products from fresh floral buds were stained using an aqueous solution of toluidine blue (0.05%). Chromosome spreads were prepared as described by Ross *et al.* (1996) and stained with 5 µl of DAPI (1 µg/ml). Digital images were obtained by using the Optronics camera mounted on a Nikon fluorescence microscope. Female meiosis was analyzed essentially according to Armstrong and Jones (2001), using floral buds at stages 10–11 (Smyth *et al.*, 1990). Karyotyping was performed on mitotic images essentially as described previously (Maluszynska and Heslop-Harrison, 1991), using root tips from 4-day-old seedlings.

Recombinational analysis

For recombinational studies, plants were generated that had the appropriate genotypes with respect to both the *SDS* gene and molecular markers for detecting recombination. First *sds*+/+ (Ler) was crossed with the Columbia (Col) ecotype to introduce the *sds* mutation into a different ecotype so that we could use molecular markers to detect recombinational events. We identified the desired F₁ plants from such crosses using kanamycin resistance and screened the F₂ generation by PCR for *sds*+/+ plants that were homologous for the Col allele at both of two microsatellite markers (Bell and Ecker, 1994) on either chromosome II or V. The chromosome II markers were nga1126 and AthBIO26, and the chromosome V markers were nga76 and nga106 (http://genome.salk.edu/SSLP_info/SSLPsordered.html). These F₂ plants were then crossed with our original *sds*+/+ (Ler) plants to yield both *sds/sds* mutant plants (experimental) and *sds*+/+ or +/+ normal plants (control); all of these plants were heterozygous (LIL2/C1C2) for two molecular markers on either chromosome II or V. The experimental and control plants were grown to maturity. Their seeds were then germinated on MS plates and DNAs were isolated from the seedlings and used for genotyping by PCR using the primers for the two markers on the relevant chromosome.

DNA and RNA analyses

Genomic DNA was isolated as described previously (Huang *et al.*, 1994). To isolate the *SDS* gene, we obtained ~0.8 and 0.6 kb genomic fragments

(data not shown) from the 5' and 3' ends of the *Ds* element, respectively, using the TAIL-PCR procedure (Liu *et al.*, 1995). TAIL-PCRs were performed using *Ds*-specific primers (Ds3-1, Ds3-2, Ds3-3, D5-1, Ds5-2 and Ds5-3; Grossniklaus *et al.*, 1998) and arbitrary degenerate primers (AD2 and AD4; Liu *et al.*, 1995). The *SDS*-specific primers were designed based on the sequence information of the TAIL-PCR products: oMC257, 5'-CGAAATTTCGAATCACCAAT-3'; oMC258, 5'-TAG-TTTCAGCTTTCGTACG-3'; oMC273, 5'-CGCAGATGCATATAATT-CAAACG-3'; and oMC274, 5'-TGGGAATCGTGGATCTACAG-3'. To isolate the *SDS* cDNA, a probe was generated by PCR using the oMC257 and oMC273 primers and *Arabidopsis* genomic DNA and used to hybridize with a floral cDNA library (Fan *et al.*, 1997). Approximately 1 × 10⁶ plaques were screened and one positive clone was obtained that contained a 2.1 kb insert; it was designated as pMC2311.

RT-PCR was performed using 10 µg of total RNAs from roots, stems, leaves, open flowers, mature buds (larger than ~1.5 mm), young buds (<1 mm), *sds* open flowers, *sds* mature buds and *sds* young buds. The RNAs were treated with RNase-free DNase I (Roche) followed by inactivation of the DNase I. The RNAs were then used for reverse transcription and the resulting cDNAs were used as template for PCRs. The *SDS* gene-specific primers used for the RT-PCRs were oMC299 (5'-GGAGCTTGAGATAGTCGGAT-3') and oMC310 (5'-GTATGAGCG-ACACAAATGGTGGCAC-3'). As a control, the same cDNAs were used to amplify the *ASK1* gene using the oMC221 and oMC222 primers (Yang *et al.*, 1999). The PCR products were hybridized with *SDS*-specific or *ASK1*-specific probes, respectively.

In situ RNA hybridization experiments were performed as described previously (Drews *et al.*, 1991) using probes generated as follows. A 619 bp *EcoRI*-*XhoI* fragment from the *SDS* cDNA in pMC2311 was subcloned into pGEM7Zf(+), and the resultant clone was named as pMC2317. pMC2317 was digested with *XhoI* or *EcoRI* and used for transcription with SP6 or T7 RNA polymerases, respectively, to generate sense or antisense RNA probes. For expression in the female reproductive organ, non-radioactive digoxigenin-labeled probes were synthesized from the same templates as for the radioactive probes, using a Riboprobe transcription system (Promega, Madison, WI) *in vitro* RNA synthesis kit.

Yeast two-hybrid assay

The yeast two-hybrid assay (Fields and Song, 1989) was performed to test for interaction between *SDS* and *Arabidopsis* CDKs Cdc2a and Cdc2b (Hirayama *et al.*, 1991). A plasmid construct expressing a fusion of the *SDS* protein with the GAL4 DNA-binding domain (bait, Figure 9A) and fusions of Cdc2a or Cdc2b proteins with the GAL4 transcriptional activation domain (prey) were created by homologous recombination in yeast (Ma *et al.*, 1987). Briefly, to create the expected construct, yeast cells were transformed with a linearized vector plasmid and a DNA fragment with the desired insert and ends that were homologous to the vector. The linearized plasmid was derived from the pAS1-CYH2 vector (Durfée *et al.*, 1993) by *SmaI* and *NcoI* digestion, and the insert DNA fragments were obtained by PCR using cDNA templates and gene-specific primers. The *SDS* bait construct contains a portion of *SDS* with the cyclin core homologous region, using primers oMC544 (5'-TAC-GCTAGCTTGGGTGGTTCATATGGCCATGGAGCTTGAGATAGTCG-GATGC-3') and oMC545 (5'-ATTAGCTTGGCTGCAGGTCGACGG-ATCCCCCTATTCTTTTAAGAATATGAG-3'). For a positive control, the *Arabidopsis* cyclin cycB1.1 coding region (Hemerly *et al.*, 1992; Renaudin *et al.*, 1996) was amplified using primers oMC546 (5'-TACGTAGCTTGGGTGGTTCATATGGCCATGATGACTTCTC-GTTTCGATT-3') and oMC547 (5'-ATTAGCTTGGCTGCAGGTCGAC-GGATCCCCCTAAGCAGATTCAGTTCCGGT-3'). The bait construct for a fusion between GAL4 and the p53 protein was used as a negative control (Durfée *et al.*, 1993).

Two prey constructs were created similarly using *Cdc2a* and *Cdc2b* PCR fragments and the pAD-GAL4 vector. For *Cdc2a*, the primers oMC323, 5'-GAAGATACCCACCAACCCAAAAAGAGATG-GATCAGTACGAGAAAGTT-3', and oMC324, 5'-TGCGGGGTTT-TTCAGTATCTACGATTCTACTAAGGCATGCCTCCAAGATC-3') were used. For *Cdc2b*, primers oMC548, 5'-CCACCAACCCAAA-AAAAGAGATCGAATTCATGGAGAAGTACGAGAAGCTAG-3' and oMC549, 5'-CTCTGCAGTAATACGACTACTATAGGCTTCAGA-ACTGAGACTTGTCAAG-3' were used. These PCR fragments were co-transformed with the pAD-GAL4 plasmid linearized by digestion with *SmaI* and *EcoRI*.

The bait constructs were introduced individually into the Y187 yeast strain (MATα; Durfee *et al.*, 1993) by selecting for transformants on plates lacking tryptophan. Similarly, the prey constructs were transformed into the PJ69-4A strain (MATα; James *et al.*, 1996) with selection

for growth without leucine. The bait strains and prey strains were mated pair-wise to form diploids that contain one of the baits and one of the preys. Diploid cells were selected on plates lacking both tryptophan and leucine, and then tested for growth on plates without tryptophan, leucine and histidine.

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