An Analysis of Univalent Segregation in Meiotic Mutants of Arabidopsis thaliana:

A Possible Role for Synaptonemal Complex

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Running head: Synapsis and Univalent Segregation

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ABSTRACT

During first meiotic prophase homologous chromosomes are normally kept together by both crossovers and synaptonemal complexes (SC). In most eukaryotes the SC disassembles at diplotene, leaving chromosomes joined by chiasmata. The correct co-orientation of bivalents at metaphase I and the reductional segregation at anaphase I are facilitated by chiasmata and sister chromatid cohesion. In the absence of meiotic reciprocal recombination, homologues are expected to segregate randomly at anaphase I. Here, we have analyzed the segregation of homologous chromosomes at anaphase I in four meiotic mutants of Arabidopsis thaliana, spo11-1-3, dsyl, mpa1 and asyl, which show a high frequency of univalents at diplotene. The segregation pattern of chromosomes 2, 4 and 5 was different in each mutant. Homologous univalents segregated randomly in spo11-1-3, whereas they did not in dsyl and mpa1. An intermediate situation was observed in asyl. Also, we have found a parallelism between this behavior and the synaptic pattern displayed by each mutant. Thus, whereas spo11-1-3 and asyl showed low amounts of SC stretches, dsyl and mpa1 showed full synapsis. These findings suggest that in Arabidopsis there is a system, depending on the SC formation, that would facilitate regular disjunction of homologous univalents to opposite poles at anaphase I.
INTRODUCTION

Meiosis is a specialized form of cell division that makes haploid gametes from diploid cells. Chromosome number is reduced because there is a single round of DNA replication followed by two consecutive rounds of chromosome segregation. In most organisms, alignment of homologs, genetic recombination and synaptonemal complex (SC) assembly are the processes that link homologous chromosomes together during the first meiotic prophase (ROEDER 1997). SC usually disassembles at diplotene and homologous chromosomes remain joined by chiasmata, stable connections between homologs formed at the sites of crossovers, that in conjunction with sister chromatid cohesion promote the correct co-orientation of bivalents at metaphase I and the reductional segregation of homologues at anaphase I. In absence of meiotic reciprocal recombination homologues are expected to segregate randomly at anaphase I. However, in certain organisms non-exchange chromosomes segregate properly in most meioses (WOLF 1994). Among them, those systems displayed by Drosophila melanogaster males and females, Saccharomyces cerevisiae and Schizosaccharomyces pombe are well documented (DAWSON et al. 1986; GUACCI and KABACK 1991; HAWLEY and THEURKAUF 1993; MOLNAR et al. 2001a, 2001b; SHARIF et al. 2002; MCKIM et al. 2002; DAVIS and SMITH 2003).

In Drosophila females two different achiasmate segregation systems are involved in the accurate segregation of chromosomes (HAWLEY 1989; HAWLEY and THEURKAUF 1993; HAWLEY et al. 1993a, 1993b). In the first, pairing and segregation of non-exchange chromosomes require both homology between centromere adjacent heterochromatin (HAWLEY et al. 1993a; DERNBURG et al. 1996; KARPEN et al. 1996) and the presence of the Nod protein (CARPENTER 1973; ZHANG et al. 1990; THEURKAUF and HAWLEY 1992; CUI and HAWLEY 2005). In the second one, two achiasmate
heterologous chromosomes also segregate from each other with high fidelity (DERNBURG et al. 1996). The existence of these systems is relevant because during Drosophila female meiosis the small fourth chromosomes never recombine and the X chromosomes fail to exchange in 5% of meioses (THEURKAUF and HAWLEY 1992). In Drosophila males all chromosomes are achiasmate and the stable pairing requires a specific region of heterochromatin on the sex chromosomes and euchromatin on the autosomes (see for review MCKEE 1998).

In S. cerevisiae, two non-exchange artificial chromosomes (DAWSON et al. 1986) or two monosomic non-homologous chromosomes (GUACCI and KABACK 1991) segregate properly in about 90% of meioses. On the other hand, in rec7, rec14 and rec15 mutants of Schizosaccharomyces pombe, which are devoid of meiotic recombination, each chromosome segregates properly more than 75% of the time (MOLNAR et al. 2001a, 2001b). Furthermore, DAVIS and SMITH (2003) verified that in rec12 mutants, in which DNA double-strand breaks are essentially eliminated, homologous achiasmate chromosomes segregate to opposite poles at anaphase I in 63% of cells. In these mutants, Meu13, a homolog of the S. cerevisiae Hop2 protein, contributes to the recombination-independent chromosome pairing observed (NABESHIMA et al. 2001). Recently, DAVIS and SMITH (2005) have demonstrated that Dlc1, a member of dynein light-chain family, is involved in the correct segregation of achiasmate chromosomes in S. pombe.

To address whether the plant model species Arabidopsis thaliana (2n=10) possesses an achiasmate segregation system similar to those described above, we have analyzed the segregation of chromosomes 2, 4 and 5 in four different meiotic mutants. All of them characterized by showing a high frequency of univalents at diplotene-metaphase I, namely: mpa1 (meiotic prophase aminopeptidase), dsy1 (desynaptic), asy1 (asynaptic)
and spo11-1-3 (sporulation defective). The MPA1 gene encodes a puromycin-sensitive aminopeptidase that is essential for meiosis in Arabidopsis (SÁNCHEZ-MORÁN et al. 2004). The DSY1 gene has not been identified yet but synopsis appears normal in the mutant (ROSS et al. 1997). On the contrary, ASY1, homologous to the HOP1 gene of S. cerevisiae, is known to be essential for normal synopsis (CARYL et al. 2000). Finally, SPO11 encodes a type II topoisomerase which is likely required for initiation of meiotic recombination in all species (KEENEY et al. 1997; GRELON et al. 2001).

MATERIALS AND METHODS

**Plant material:** Four different meiotic mutants of Arabidopsis thaliana (L.) (2n=10), produced by T-DNA insertion mutagenesis, were used in this study: dsy1, mpa1, asy1 and spo11-1-3. The spo11-1-3 mutant, line Salk_009440, was obtained from the collection of T-DNA mutants of the Salk Institute Genome Analysis Laboratory (Col-0 accession) (SiGnAL, http://signal.salk.edu/cgi-bin/tdnaexpress) (ALONSO et al. 2003) and provided by NASC (http://nasc.nott.ac.uk/). The dsy1 and asy1 mutants (Ws accession), previously described by ROSS et al. (1997), were kindly provided by Dr. Gareth Jones (School of Biological Sciences, University of Birmingham, UK). The mpa1 mutant was isolated in our laboratory from Spanish T-DNA transformed lines. Plants were cultivated in greenhouses under a 16-hour day/8-hour night photoperiod, at 20° and 70% humidity.

**Cytology**

**Fixation:** Flower buds in stages 9/10 and 10/11 of floral development (SMYTH et al. 1990) were used to analyze metaphase I and second division stages in pollen mother cells (PMCs) and metaphase I in embryo-sac mother cells (EMCs). They were fixed in Carnoy’s fixative (absolute ethanol-chloroform-glacial acetic acid; 6:3:1) overnight at
room temperature. The fixative was replenished and buds were stored at -20º until required.

**Slide preparations:** Air-dried spreads of PMCs and EMCs were carried out according to FRANSZ et al. (1998) and ARMSTRONG et al. (1998), with minor modifications as described by SÁNCHEZ-MORÁN et al. (2001). Fixed buds were transferred to a watch-glass containing absolute ethanol-glacial acetic acid fixative (3:1) that was changed twice to remove the original Carnoy’s fixative. Buds were then washed twice in 10 mM citrate buffer pH 4.5 at room temperature. Buffer was removed before incubation with an enzyme mixture, 0.3% w/v pectolyase, 0.3% w/v cytohelicase and 0.3% w/v cellulose (Sigma), in citrate buffer, for 2 h at 37º. The enzyme mixture was replaced by ice-cold citrate buffer to stop the reaction. Single buds, immersed in a small volume of buffer, were transferred to slides with a Pasteur pipette and macerated with a needle. Afterwards, 10 µl of 60% acetic acid were added to each slide that was placed on a hot plate at 45º for 1 min and another 10 µl when the slide was out the hot plate. Preparations were refixed with 200 µl of cold 3:1 fixative. Once the fixative was removed, the slides were dried and stained with the fluorochrome 4´,6-diamidino-2-phenylindole (DAPI, 10 µg/ml) in Vectashield antifade mounting medium (Vector Laboratories), to choose those containing metaphase II cells.

**Fluorescence in situ hybridisation:** The FISH technique was performed according to SÁNCHEZ-MORÁN et al. (2001). The following DNA probes were used:

*Clone pTa71* (GERLACH and BEDBROOK 1979): Containing a 9 kb EcoRI fragment of *Triticum aestivum* consisting of the 18S-5.8S-25S rRNA genes and the spacer regions. Digoxigenin dUTP was incorporated by nick translation following the manufacturer’s instructions (Roche) and it was detected by FITC-antibodies.
Plasmid pCT4.2: Containing the 5S rRNA gene from *A. thaliana* as a 500 bp insert cloned in pBlu. Biotin dUTP was also incorporated by nick translation and detected by Avidin-Cy3 antibodies.

Preparations were counterstained with DAPI in Vectashield antifade mounting medium. Chromosomes were examined by using epifluorescence microscopy (Olympus BX60) and recorded with an Olympus DP70 camera. Figures were composed using PHOTOSHOP 7.0 software (Adobe Systems, San Jose CA).

**Synaptonemal complex preparations:** Surface spreads of meiocytes were performed according to ALBINI (1994) with minor modifications. Briefly, fresh unfixed anthers in stage 9 of floral development (SMYTH *et al.* 1990) were squashed onto a cavity slide with a drop of digestion medium (0.01 g snail gut enzyme cytohelicase, Sigma, 0.125 g polyvinyl pyrrolidone and 0.19 g sucrose in 12.5 ml sterile distilled water). After about 5 min of enzymatic treatment, a single drop of meiocyte suspension was transferred into 0.05% Triton X-100 detergent on slides. The meiocytes were allowed to spread for about 5 min and then about five drops of the fixative solution (4% paraformaldehyde and 1.7% sucrose in distilled water, adjusted to pH 8.9 with borate buffer) were added. Slides were dried overnight on a warm plate at 30º, rinsed in distilled water for 1 min at room temperature and air dried. For silver impregnation, a few drops of aqueous AgNO₃ (30%) were placed on the preparations which were then covered with a patch of nylon cloth at 30-40º until they turned into a yellow color (about 45 min). Once the mesh was removed, the slides were rinsed in distilled water and dried. Prophase I nuclei were examined under an Electron Microscope (EM) (Jeol 1010).
RESULTS

**FISH identification of Arabidopsis meiotic chromosomes:** In the present work we have applied FISH, using 5S and 45S rDNA probes, to distinguish all of the five chromosome pairs of the Arabidopsis complement (Fransz et al. 1998; Sánchez-Morán et al. 2001, 2002). The acrocentric chromosomes 2 and 4 bear 45S sequences on their short arms (corresponding to the location of the nucleolus organizing regions, NORs), whereas 5S sequences are located proximally on the short arms of chromosomes 4 and 5. Furthermore, in the Col-0 accession there is a 5S locus in the short arm of chromosome 3 (Figure 1, A and B). Although chromosomes 1 and 3 differ in size, this difference is not appreciable at metaphase II cells because chromosomes are extremely condensed. On these grounds, the present research will be focused to study the meiotic segregation patterns of chromosomes 2, 4 and 5 in four meiotic mutants. It must be noted that this analysis has been carried out, except for the mutant spo-11-1-3, in meiocytes from flower buds of the same *dsy1*, *asy1* and *mpa1* plants employed in previous studies (Sánchez-Morán et al. 2001, 2004).

**Segregation analyses**

**The spo11-1-3 mutant:** This meiotic mutant, denominated *spo11-1-3*, arose as consequence of a T-DNA insertion in the *SPO11* gene and corresponds to the line Salk_009440. Figure 2 shows the location of the T-DNA insertions in *spo11-1-1* (Grelón et al. 2001) and *spo11-1-3* mutants, and the location of the point mutation produced by treatment with ethyl-metane sulfonate (EMS) in *spo11-1-2* (Grelón et al. 2001).

Synaptonemal complex (SC) formation was very limited in the 20 zygotene-pachytene nuclei analyzed. Indeed, the overall amount of synapsis never exceeded 10% of the total axial element length (Figure 3A). Consequently, a total absence of bivalents
at metaphase I (MI) was the main feature of both male and female meiosis (200 and 20 meiocytes analyzed, respectively). Univalents always moved entirely to one of the anaphase I poles (reductional segregation). However, some chromosome segregation abnormalities must occur at the first division because abnormal metaphase II cells with three (13.87%; 19/137) and four (3.65%; 5/137) separate groups of chromosomes were observed (Supplementary Figure 1S). Canonical meiosis II pollen mother cells, mostly metaphase II pairs (MII PMCs), were classified in two groups depending whether homologous chromosomes migrated to different poles at anaphase I (1:1) or not (2:0), and this was performed separately for each pair of the chromosomes analyzed (see Figure 4, A and B, as examples of regular disjunction and non-disjunction, respectively). Assuming random chromosome segregation, we would expect 50% MII cell pairs of each type. Observed and expected values are shown in Table 1. Chi-square analyses did not reveal significant differences for any of the chromosomes studied.

The *dsy1* mutant: Synapsis appeared to proceed normally until completion at pachytene (25 nuclei analyzed, Figure 3B), but at diplotene most homologues were not associated by chiasmata. The mean cell bivalent frequency at metaphase I was 0.51 with an overall mean cell chiasma frequency of 0.57.

Those MII PMCs containing chromatids (0%, 1.45% and 1.18% for chromosomes 2, 4 and 5, respectively) were excluded from the segregation analysis. This finding, in addition to the fact that some chromosome pair/s could not be accurately identified in some cells, explains the different number of MII cells analyzed for each chromosome (Table 1). In the segregation analysis, we have taken into account the mean cell bivalent frequency of each chromosome at MI since bivalent formation implies that homologues will migrate to opposite poles at anaphase I (AI) with a very high probability. This is the reason because the expected values under the random homologous segregation
hypothesis do not correspond with 50% of MII cells analyzed. Chi-square analyses revealed that univalents for chromosomes 2, 4 and 5 segregate to opposite poles at AI with a frequency significantly higher than that expected by random (Table 1; Figure 4, C and D).

**The mpa1 mutant:** Although five apparent normal SCs were always observed in each of the 20 pachytene nuclei analyzed (Figure 3C), univalents were predominant at diplotene. The mean cell bivalent frequency at MI was 0.75, with an overall mean cell chiasma frequency of 0.9.

The segregation analysis revealed similar results to those obtained in the *dsyl* mutant, i.e., chromosomes 2, 4 and 5 displayed significantly higher frequencies of regular disjunction than those expected by random (Table 1; Figure 4, E and F).

**The asyl mutant:** At zygotene-pachytene, axial/lateral elements showed gaps indicating a certain fragmentation throughout their length. A small amount of SC stretches, never exceeding the 30% of the total axial element length, was observed in some of the 20 nuclei analyzed (Figure 3D). The mean cell bivalent frequency was 1.27, with an overall mean cell chiasma frequency of 1.39.

Those MII PMCs containing chromatids, as result of univalent equational segregation at AI (10.48%, 12.04% and 11.00% for chromosomes 2, 4 and 5, respectively) were excluded from the segregation analysis. The results of such analysis, bearing in mind the mean cell bivalent frequency for each chromosome, are shown in Table 1. Examples of regular homologous disjunction and non-disjunction are showed in Figure 4, G and H. Whereas univalents for chromosome pair 2 segregated to opposite poles with a frequency significantly higher than that expected by random, those univalents for chromosomes 4 and 5 did not.
DISCUSSION

Although reciprocal recombination is generally required for proper homologous segregation at AI, some organisms possess systems capable of partitioning one or more pairs of homologous when they appear as univalents at MI. This is the case of Drosophila males and females in which different mechanisms called upon to segregate achiasmate chromosomes in every meioses (Hawley and Theurkauf 1993; McKee 1998). Similar systems, although not so efficient, have been described in *S. cerevisiae* and *S. pombe* (Dawson et al. 1986; Guacci and Kaback 1991, Davis and Smith 2003). It must be noted, however, that mutations that abolish meiotic recombination in Drosophila females display significantly increased levels of AI non-disjunction, perhaps because the system cannot accommodate the whole chromosome complement (Hawley 1989). Likewise, *S. cerevisiae* is unable to promote non-random segregation when meiotic recombination is abolished throughout the genome. It is known that chromosome V segregates at random in *spo11* mutant meioses (Klein et al. 1999). On the contrary, in recombination defective mutants of *S. pombe*, reductional segregation of chromosomes at AI is non-random (Molnar et al. 2001a, 2001b; Davis and Smith 2003).

The results obtained in the univalent segregation analysis carried out in the Arabidopsis mutants are intriguing: (i) There is not an apparent relationship between the segregation univalent behavior and their synaptic history. Thus, reductional segregation is the rule in asynaptic univalents of *spo11-1-3* and desynaptic univalents of *dsy1*. On the other hand, in *assy1* each pair of asynaptic univalents displayed about 11% of equational segregation. The fragmentation of the axial elements observed in zygotene-pachytene nuclei of *assy1* (Figure 3D) concurs with the hypothesis that Asy1 is required for morphogenesis of the SC, possibly by defining regions of chromatin that associate
with the developing SC structure (ARMSTRONG et al. 2002). Perhaps this protein is also involved in some aspect/s of chromosome reorganization that predispose chromosomes to unipolar orientation. (ii) Whereas chromosome pairs 2, 4 and 5 segregated randomly at AI in spo11-l-3, they did not in dsyl and mpa1. An intermediate situation being observed in asyl, in which chromosome pairs 4 and 5 segregated at random and those of chromosome 2 migrated preferentially to opposite poles (Table 1, Figure 4). These differences in chromosome behavior among mutants are also accompanied with differences in SC formation (Figure 3), in such a way that a relationship between presence of complete SC at pachytene and regular disjunction of homologues at AI can be established. It is perhaps surprising the existence of a low amount of SC formation in spo11-l-3 because in Arabidopsis recombination is a prerequisite for synapsis. Therefore, one would expect that total absence of recombination led to complete absence of synapsis (GRELON et al. 2001). Whether this synapsis is between non-homologous chromosomes or whether it is independent of DNA double strand break (DSB) formation remains to be established. Likewise, nuclei with full synapsis have been observed in spo11 mutants of budding yeast (BHUIYAN and SCHMEKEL 2004). Random chromosome segregation has also been claimed to exist in the almost sterile dmc1 mutant of A. thaliana (COUTEAU et al. 1999), however the authors stated that they did not detect proper metaphase II cells in the DAPI stained slides and also they did not identify any of the univalent chromosome pairs. Unfortunately, no data about SC formation were provided. The different behavior of univalents in asyl might be explained assuming that chromosomes 2 are more actively involved in SC formation than chromosomes 4 and 5 (Figure 3D) and this would facilitate their significant non-random regular disjunction at AI. Indeed, in this mutant, chromosome 2 exhibits the
highest bivalent and chiasma frequencies of the set, the reverse situation of that found in 
_dsyl_ and in wild type (SÁNCHEZ-MORÁN et al. 2001).

It is feasible to think that spindle checkpoint genes (CHESLOCK et al. 2005) and SC 
components may play a role in the segregation of the univalents at the first meiotic 
division. From pachytene onwards, some SC components might secure homologous 
centromeres with the active faces of their kinetochores facing away from one another in 
the meiotic spindle. In fact, in the desynaptic mutants, _mpa1_ and _dsyl_, homologous 
univalents maintain a conspicuous parallel disposition throughout diplotene-diakinesis 
that could facilitate the adequate interactions between kinetochores and microtubules 
(Supplementary Figure 2S). In budding yeast SC does not seem critical for non-
exchange segregation because the segregation of achiasmate homologues was not 
randomized by the absence of SC in _zip1_ mutants (KEMP et al. 2004). However, the SC 
protein Zip1 may play a role in coupling yeast centromeres that would facilitate 
homologous pairing and subsequent synapsis initiation (TSUBOUCHI and ROEDER 2005). 
Meiotic centromere pairing of non-exchange chromosomes, really of the 
heterochromatic adjacent regions, seems to be sufficient to establish an initial bipolar 
attachment of microtubules following by segregation of chromosomes to opposite poles 
(STEWART and DAWSON 2004; KEMP et al. 2005). In Arabidopsis, initial localization of 
Zyp1 is dependent upon DSB formation and occurs when recombination is at an early 
stage, prior to extensive strand invasion. However, subsequent Zyp1 polymerization to 
bring about homologous chromosome synapsis cannot occur if recombination is 
blocked at an early step (HIGGINS et al. 2005). On the other hand, in some meiotic 
systems the segregation of achiasmate chromosomes is mediated by modified SC 
structures that persist after SC breakdown (RASMUSSEN 1977; WOLF 1994). In 
Drosophila females the Mtrm protein (matrimony) might act by modifying some
component of the SC that originally connected the heterochromatic regions of chromosomes during meiotic prophase. These modifications could play an important role in the achiasmate chromosome segregation by holding homologues together long enough to ensure their orientation to opposite poles (HARRIS et al. 2003). In mouse a meiosis-specific structural protein, Sycp3, which appears in axial and lateral elements of the SC is involved in the monopolar attachment of sister kinetochores during the first meiotic division (PARRA et al. 2004). Recently, PAGE et al. (2003, 2005) have reported that in marsupial males the pairing of the achiasmatic X and Y chromosomes during meiotic prophase is maintained by a dense plate developed from their axial elements.

At present, the real role of the SC remains enigmatic. However, its ubiquitous presence and its structural conservation throughout different organisms reflect the importance of this proteinaceous structure in the meiotic process. This study suggests that in Arabidopsis thaliana there is a SC dependent system that promotes the accurate segregation of univalents to opposite poles at AI. It is less efficient than those described in Drosophila males and females and does not seem to specifically ensure the segregation of non-exchange chromosomes, since the chromosomes of A. thaliana do not generally fail to recombine and form five bivalents at meiosis. The further characterization of SC proteins and spindle checkpoint genes in plants may lead to a better understanding of this segregation system.

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Birmingham, Birmingham, UK) for providing *asyl* and *dsyl* mutants and for useful discussions during the course of this work. Finally, we thank the editor and reviewers for their insight, which has enabled us to significantly improve the manuscript.
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TABLE 1

Mean cell bivalent frequencies and segregation patterns displayed by chromosomes 2, 4 and 5 in the four meiotic mutants analyzed

Observed and expected values of regular disjunction (1:1) and non-disjunction (2:0) for each chromosome pair in MII cells. Expected values were calculated assuming random chromosome segregation at anaphase I.
<table>
<thead>
<tr>
<th>Mutant</th>
<th>Chromosome</th>
<th>Mean cell bivalent frequency</th>
<th>Regular disjunction 1:1</th>
<th>Non-disjunction 2:0</th>
<th>No. MII cell pairs</th>
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<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>Expected</td>
<td>Observed</td>
<td>Expected</td>
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</tr>
<tr>
<td><em>spo11-1-3</em></td>
<td>Chr. 2</td>
<td>~0</td>
<td>50</td>
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<td>56</td>
<td>57.00</td>
<td>58</td>
<td>57.00</td>
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<tr>
<td></td>
<td>Chr. 2</td>
<td>0.06</td>
<td>59</td>
<td>42.93</td>
<td>22</td>
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<tr>
<td><em>dsyl</em></td>
<td>Chr. 4</td>
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<td>55</td>
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<td><em>asyl</em></td>
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*Significant differences at 5% level
FIGURE LEGENDS

FIGURE 1.- FISH images of metaphase I cells from wild type Arabidopsis plants, accessions Col-0 (A) and Ws (B). 45S rDNA loci and 5S rDNA loci are identified by green and red signals, respectively. Bars represent 10 µm.

FIGURE 2.- Genomic organization of the $AtSPO11-1$ locus and localization of $spo11-1$ mutations. Black boxes represent exons and gray lines represent 5´ and 3´ untranslated regions. The locations of the T-DNA insertions in $spo11-1-1$ (GRELON et al. 2001) and $spo11-1-3$ (Salk_009440), and the point mutation produced by ethyl-metane sulfonate EMS in $spo11-1-2$ (GRELON et al. 2001) are indicated by vertical arrows.

FIGURE 3.- Electron micrographs of silver stained zygotene-pachytene nuclei. (A) $spo11-1-3$. (B) $dsy1$. (C) $mpa1$. (D) $asy1$. Arrows in (A) and (D) indicate short SC stretches that are enlarged. Bars represent 5 µm.

FIGURE 4.- Examples of regular disjunction (A,C,E,G) and non-disjunction (B,D,F,H) of chromosome pairs 2, 4 and 5 in meiosis II cells. (A, B) $spo11-1-3$. (C, D) $dsy1$. (E, F) $mpa1$. (G, H) $asy1$. Individual chromosomes bearing 45S and 5S rDNA sequences are identified. (D) Telophase I. (A) Prophase II. (B,C,E,F,H) Metaphase II. (G) Anaphase II. Bars represent 10 µm.