Chromosoma (2000) 109:62–71

CHROMOSOMA


# A homologue of the yeast *HOP1* gene is inactivated in the *Arabidopsis* meiotic mutant *asy1*

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Received: 22 September 1999; in revised form: 18 October 1999 / Accepted: 18 October 1999

**Abstract.** Synapsis of homologous chromosomes is a key event in meiosis as it is essential for normal chromosome segregation and is implicated in the regulation of crossover frequency. We have previously reported the identification and cytological characterisation of a T-DNA-tagged asynaptic mutant of *Arabidopsis thaliana*. We have demonstrated that this mutant, *asy1*, is defective in meiosis in both males and females. Cloning and nucleotide sequencing of the *ASY1* gene has revealed that it encodes a polypeptide of 596 amino acids that exhibits similarity to the *HOP1* gene of *Saccharomyces cerevisiae*, which is known to encode a protein essential for synaptonemal complex assembly and normal synapsis. Expression studies indicate that, in common with a number of other *Arabidopsis* meiotic genes, *ASY1* exhibits low-level expression in a range of plant tissues. Southern analysis coupled with database searching has resulted in the identification of an *ASY1* homologue, *ASY2*. Although *asy1* exhibits a strong asynaptic phenotype, a residual low level of synapsis indicates that *ASY1* and *ASY2* may exhibit a low degree of functional redundancy.

## Introduction

Despite a long history of research into meiosis in flowering plants, including the isolation of many meiotic mutants (Rees 1961; Baker et al. 1976; Golubovskaya 1979; Koduru and Rao 1981; Kaul and Murthy 1985), the molecular and functional analysis of plant meiotic genes is relatively undeveloped. *Arabidopsis thaliana* is now regarded as the foremost model organism for plant molecular genetics and opportunities exist to identify, clone, sequence and analyse the expression of many genes of interest in this species, including meiotic genes.

Several sterile or partially fertile mutants of *Arabidopsis* exist that might be defective in meiotic genes, as indicated by reductions in both male and female fertility (Glover et al. 1996) or deviations in the number and size of meiotic products (microspores) at the tetrad stage (Dawson et al. 1993; Chaudhury et al. 1994; He et al. 1996; Peirson et al. 1996). Recent improvements in cytogenetic techniques for *Arabidopsis* (Ross et al. 1996) have enabled detailed analyses of meiotic chromosome behaviour to be conducted and have led to the description of a number of meiotic mutants having specific meiotic defects (Peirson et al. 1997; Ross et al. 1997).

Synaptic mutants represent an important class of meiotic mutants (reviewed by Riley and Law 1965); they have been subdivided into two groups, depending on whether they are defective in homologue synapsis (asynaptic) or in the maintenance of synapsis following a normal synaptic process (desynaptic). Homologous chromosome synapsis, which in the majority of organisms results in the formation of full length synaptonemal complexes (SCs), is an event of crucial significance in the meiotic process since it is implicated in the development and possibly the regulation of crossovers and is essential for the normal progression of homologue disjunction and chromosome reduction. Consequently much effort has been expended in identifying and characterising the meiotic proteins that are involved in synapsis and the genes that encode them or regulate their expression (Roeder 1997).

We have previously reported the identification of four different classes of meiotic mutants of *Arabidopsis* isolated from T-DNA-transformed lines (Ross et al. 1997), one of which is asynaptic. The genetic locus was designated *ASY1* and the mutant allele *asy1-1*. This mutant was initially identified on the basis of reduced fertility, which is about 10% of wild type. Both male and female fertility are affected in this mutant, an observation that might indicate a meiotic defect although this does not necessarily follow.

*Genbank Accession number:* AF157556

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The meiotic phenotype of *asy-1* pollen mother cells (PMCs) was assessed and interpreted in the light of the previously published account of male meiosis in the wild-type WS ecotype of *Arabidopsis* (Ross et al. 1996). The essential feature of male meiosis in this mutant is that synapsis of homologous chromosomes is almost completely lacking, from which other defects follow, culminating in a high proportion of unbalanced microspores and reduced fertility (Ross et al. 1997). Chromosome axis development appears to proceed normally during leptotene, so far as can be judged from 4',6-diamidino-2-phenylindole (DAPI)-stained images, but extensive synapsis of the kind normally observed during zygotene and pachytene in the wild type is never seen in *asy-1*. From diplotene to metaphase I most chromosomes are present as univalents, although a low number of bivalents are also present in some cells (the overall mean bivalent frequency is 1.57 per cell). Chromosome distribution is highly irregular during anaphase I, reflecting the expected erratic behaviour of univalent chromosomes. The resulting inequalities in chromosome number in dyads and metaphase II nuclei are manifested in the unequal chromosome numbers and uneven sizes of meiotic tetrads following the second meiotic division and eventually in the production of microspores of varying size.

In this paper we present further characterisation of *asy1*, including cytological and molecular data. A candidate gene (*ASY1*) has been identified from the *Arabidopsis* genome sequence database. This gene has homology to the *HOP1* gene of *Saccharomyces cerevisiae*, which is known to encode a protein essential for SC assembly and normal synapsis in yeast (Hollingsworth et al. 1990). Our evidence suggests that the *ASY1* gene encodes an important component required for homologous chromosome synapsis in *Arabidopsis*.

## Materials and methods

### *Plant material*

The *A. thaliana* ecotypes Wassilewskija and Columbia were used in this study.

Plants were grown in a soil-based compost in a greenhouse under supplementary light (16 h light, 8 h dark cycle) or in a culture room (16 h light, 8 h dark cycle). Bud material consisted of the top of the inflorescence spike cut above the first open flower, harvested throughout the plant's flowering period. Leaf material was a mixture of rosette and cauline leaves. Stem material consisted of inter-nodal stem sections. Whole plant material was collected 14 days after germination from plants grown on sterile agar plates. Root material was collected from a shaking root culture (hormone free) after 2–3 weeks (Czako and Marton 1991).

### *Genetic analysis*

Genetic segregation of the *asy1* gene was tested by crossing to wild type (WS) and analysing the segregation of fertile versus reduced fertility phenotypes in the F2 generation. Cosegregation of the reduced fertility phenotype with T-DNA was monitored by resistance of seedlings to kanamycin (50 µg/ml) on agar medium (Feldmann and Marks 1987). The chromosomal designation and map position of *ASY1* were determined by making crosses of the mutant line to the multiply marked tester line W100ms$^+$, which carries two visible genetic markers on each chromosome.

### *Cytological analysis – preparation of chromosome spreads*

The method of Zhong et al. (1996) for spreading PMCs was modified for *Arabidopsis* material (Ross et al. 1996) as follows. Entire immature flower buds of up to about 2 mm in size were fixed in Carnoy's fixative (6:3:1 ethanol:glacial acetic acid:chloroform) for 12 h at room temperature and thereafter stored in a freezer at –20°C. Before the spreading procedure was commenced, fixed buds were washed in three changes of 3:1 ethanol:acetic acid to remove all traces of chloroform. They were then washed three times for 5 min in citrate buffer (10 mM, pH 4.5) and digested in an enzyme mixture containing 0.3% (w/v) cellulase, 0.3% (w/v) pectinase and 0.3% (w/v) cytohelicase (Sigma, Poole, UK) in 10 mM citrate buffer, pH 4.5 in a moisture chamber for 1–2 h, until the buds were adequately softened. The buds were then transferred to citrate buffer and maintained at 4°C. These steps were all carried out in a black solid watch-glass, adding and removing solutions with a Pasteur pipette. Single, enzyme-digested buds were transferred to clean glass slides and excess buffer removed. A small drop (10 µl) of 60% acetic acid was added to a bud, which was macerated with a fine needle under a dissecting microscope in a minimum amount of liquid, taking care that the material did not dry out. A further 7 µl of 60% acetic acid was then added to the slide and the droplet stirred on a hotplate at 45°C for up to 1 min. The slide was flooded with freshly made, ice-cold, 3:1 ethanol:acetic acid fixative, initially by adding drops of fixative around the acetic acid droplet. Finally the slide was tilted to remove the fixative and dried using a hairdryer.

This basic spreading procedure was adapted for the analysis of female meiosis in embryo-sac mother cells (EMCs). Fixed flower buds of 0.5–1.0 mm in size were first dissected to isolate intact gynaecia (ovary and style). Gynaecia were washed in three changes of 3:1 ethanol:acetic acid, followed by three changes of 10 mM citrate buffer, pH 4.5. Enzyme digestion was carried out as described above for PMC preparation and the gynaecia were finally transferred to citrate buffer. The spreading procedure was essentially the same as for PMCs from anthers, with minor modifications, and was performed either on intact gynaecia or after carefully dissecting away the carpels to leave the strings of ovules attached to the placenta. Each gynaecium contains about 50 ovules.

For routine meiotic observations in mutant and wild-type PMCs and EMCs the slides were stained with DAPI and viewed by fluorescence microscopy. A small drop (5 µl) of DAPI (1 µg/ml) in Vectashield anti-fade mounting medium (Vector Labs, Peterborough, UK) was placed on each slide and mounted with a 22×22 mm no. 0 coverslip. The mounted slide was squashed between filter paper sheets to remove excess stain and Vectashield.

### *Fluorescence in situ hybridisation*

The DNA clone used for the detection of T-DNA insertion sites by fluorescence in situ hybridisation (FISH) was plasmid pNINJA 1. This plasmid contains the NPT II coding sequence (~1 kb) on a BamHI-KpnI fragment (C. Lichtenstein, Queen Mary Westfield College, University of London), subcloned into pSK+ (pBluescript, Stratagene, Cambridge, UK). The plasmid DNA was purified by the method of Sambrook et al. (1989). The DNA sequence was labelled with digoxygenin-dUTP by nick translation using the manufacturer's protocol (Boehringer Mannheim, Germany).

Fluorescence in situ hybridisation was carried out as described by Fransz et al. (1996) with the following modifications. Before the paraformaldehyde fixation step, slides were incubated in 0.01 g pepsin in 100 ml of 0.01 M HCl for 1 min at 37°C and then washed three times in 2×SSC for 5 min. (1×SSC is 0.15 M NaCl, 0.015 M sodium citrate.) Between 50 and 100 ng of probe was used for each slide, made up to 20 µl in hybridisation fluid con-

taining 50% deionised formamide, 2×SSC and 10% dextran sulphate. The probe mixture was applied to the slides and sealed under 22×22 mm coverslips using vulcanising solution. The slides were placed on a hot block at 75°C for 4 min to denature the target DNA and probe simultaneously, and then hybridised overnight at 37°C in a moisture chamber. Post-hybridisation washes were carried out as described by Fransz et al. (1996). The digoxygenin-labelled T-DNA probe was detected by anti-digoxygenin rhodamine according to the manufacturer's instructions (Boehringer Mannheim, Germany). Slides were counterstained with DAPI (1 µg/ml) in Vectashield anti-fade mounting medium (Vector Labs, Peterborough, UK) and examined using a Nikon Eclipse T300 fluorescence microscope. Image capture and analysis were achieved using an image analysis system (Vysis, Richmond, UK).

*Nucleic acid extraction*

Genomic DNA was isolated using the Nucleon Phytopure Kit (Amersham Life Sciences, UK) according to the manufacturer's instructions. Total RNA was isolated using the RNA isolation kit (Stratagene, Cambridge, UK). As the kit was not designed for plant tissues only 50% of the recommended weight of tissue was used in each extraction. The total RNA was then treated with DNase I (RNase free) (Stratagene, Cambridge, UK) to remove residual genomic DNA.

*Hybridisation*

Five micrograms of digested genomic DNA was transferred from an agarose gel onto Hybond $N^+$ membrane (Amersham Life Sciences, UK) by capillary blotting. The membrane was then baked at 80°C for 2 h to fix the DNA. The blot was hybridised in modified Church and Gilbert (1984) buffer and probed using radiolabelled DNA (Feinberg and Vogelstein 1983, 1984). Blots were washed at low stringency (2×SSC, 0.1% SDS) or high stringency (0.2×SSC, 0.1% SDS). Radioactivity was detected by autoradiography.

*Polymerase chain reaction*

For the expression studies the reverse transcription-polymerase chain reaction (RT-PCR) kit (Stratagene, Cambridge, UK) was used to synthesise cDNA from 2.5 µg total RNA (DNase I treated) from the desired tissue. The cDNA was then subject to PCR using REDTAQ DNA Polymerase (Sigma, Poole, UK) in the manufacturer's buffer with the following primers FP1 5'-CTCCTTCTCTCTGAGAATTCC-3' and RP5 5'-TTGCAAGAATATCTGTGAGC-3' (for *ASY1* expression) or Act-327S 5'-ATGAARATNAARGTNGTNGCNCCNCCNGA-3' and Act8–3'N1 5'-AGCTCCCGGGGTTTTTATCCGAGTTTGAAGAGGCT-3' (An et al. 1996) (for *ACT8* expression).

To generate clones for sequencing, the RT-PCR kit (Stratagene, Cambridge, UK) was used to synthesise cDNA from 10 µg total bud RNA and PCR was performed using Cloned Pfu DNA Polymerase (Stratagene, Cambridge, UK) in the manufacturer's buffer using FP1 and RP6 5'-AGGGAATAATACTCACAGTAATAAC-3'.

To produce the *ASY1* probes used in this study bud cDNA or genomic DNA was amplified with the following primers: FP2 5'-ATGGCTCAGAAGCTGAAGG-3' and RP4 5'-CGTTAACACGAGATGTTTGCTG-3'. The 3' rapid amplification of cDNA ends (RACE) was carried out using the 3' RACE System (GibcoBRL, Paisley, UK) according to the manufacturer's instructions. Two rounds of nested PCR were carried out using the following gene-specific primers: FP3 5'-CTAGTCTAACTGAGAAGAAGC-3' and FP4 5'-GATGTGGATGTTACCGAAAC-3'. The PCR products were cloned into the pCR2.1 vector (Invitrogen, Carlsbad, Calif.) and sequenced. The 5' RACE was carried out as follows: cDNA was synthesised from 10 µg of total bud RNA using the RT-PCR kit (Stratagene, La Jolla, Calif.) with the RP3 5'-GTTGAAGATAGCAATACGAAGC-3' primer. The cDNA was tailed with either dCTP or dATP using Terminal Transferase (Boehringer Mannheim). Nested PCR was then performed using the following gene-specific primers: RP2 5'-ATTTCTAGTCAGAAGAAGCGAG-3' and RP1 5'-TCCTGCTCAGTGATCTCTGC-3', together with either 5'RACE Abridged Anchor Primer 5'-GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG-3' and AUAP 5'-GGCCACGCGTCGACTAGTAC-3' (GibcoBRL, Paisley, UK) for the dCTP tailed cDNA or T7dT 5'-GATCTGCACGCGTAATACGACTCGAGTTTTTTTTTTTTTTT-3' and T7 unique 5'-GATCTGCACGCGTAATACGACTC-3' for the dATP-tailed material (Brady et al. 1995). The PCR products were cloned into the pCR2.1 vector (Invitrogen, Carlsbad, Calif.) and sequenced.

*Nucleic acid sequencing*

Automated nucleotide sequencing was carried out by Alta BioScience, University of Birmingham, UK.

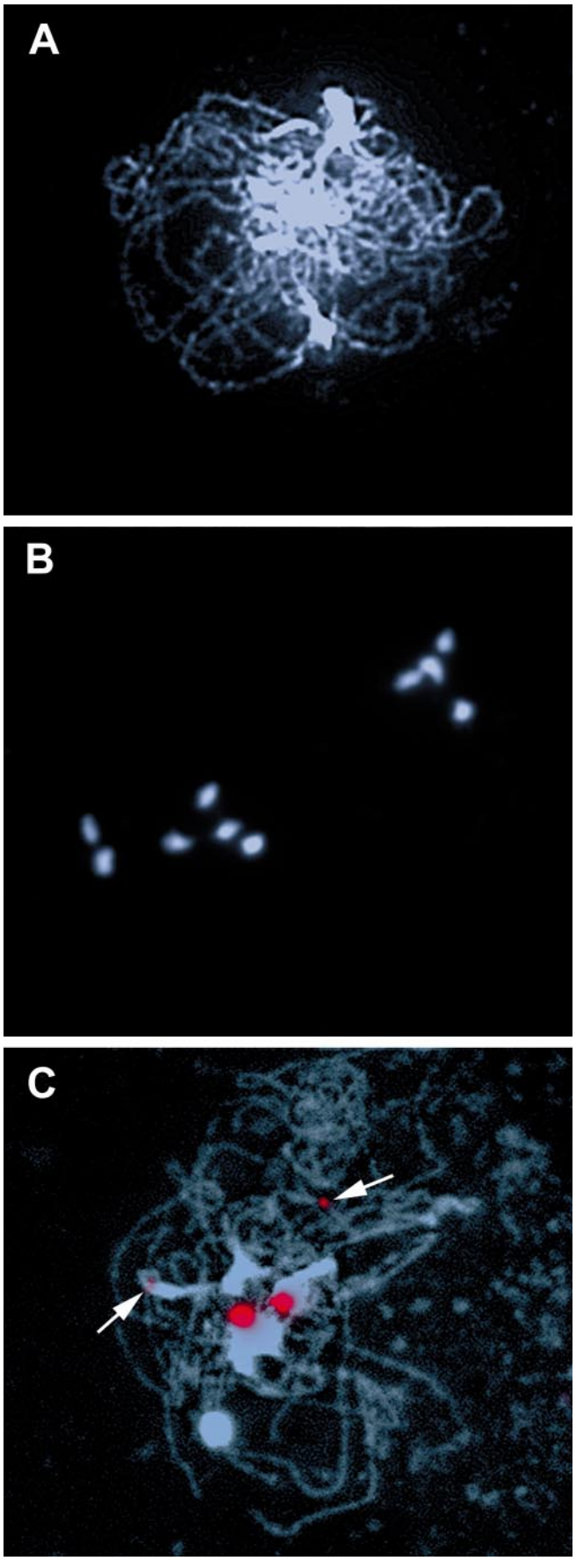
## Results

### *Both male and female meiosis are defective in* asy1

We have previously reported the cytological analysis of male meiosis in *asy1* plants (Ross et al. 1997). Reduced female, as well as male, fertility of mutant plants indicated that the asynaptic phenotype was likely to be expressed in both female and male meiosis. By adapting the cytogenetic technique developed for male meiosis, we succeeded in confirming this by direct observations on female meiosis in mutant and wild-type plants. Spread and DAPI-stained preparations of ovules at the appropriate developmental stages showed that mutant EMCs fail to undergo extensive synapsis during early prophase I, resulting in frequent univalent formation at diakinesis and metaphase I (Fig. 1). By contrast, wild-type EMCs showed normal synapsis during zygotene resulting in full pachytene synapsis and the regular presence of five chiasmate bivalents at diakinesis and metaphase I (Armstrong et al., unpublished observations).

### *Genetic analysis of* asy1 *and cloning of the* ASY1 *gene*

Genetic segregation data indicated that the initial *asy1* material (T3) that we had analysed contained two independent and unlinked T-DNA insertions [90 Km/–; 8 km/km; $\chi^2(15:1)=0.612$; $P>0.3$]. This was confirmed by FISH, which showed the presence of two T-DNA sites on different chromosomes, one being much larger than the other (Fig. 1C). The genetic data also indicated that kanamycin resistance cosegregated with the *asy1* mutant phenotype, since all the 19 *asy1*/*asy1* F2 segregants in the same family of 78 individuals were also found to be homozygous for kanamycin resistance when progeny were tested through the F3 generation. This showed that the *asy1* gene is linked to one of the T-DNA insertions and is potentially tagged. To isolate the single-tagging T-DNA insert, the double-insert line was outcrossed to the wild-type WS ecotype and the F2 and F3




segregants of this cross were screened for the presence of a single-tagging insert by a combination of kanamycin resistance segregation analysis, T-DNA FISH and fertility assay. By these procedures it was established that the smaller of the two inserts is the tagging insert. Both kanamycin resistance and the mutant (reduced fertility) phenotype showed monogenic segregation in selected F3 families (e.g. 233 Km/–, 66 km/km; $\chi^2$=1.37; $P$>0.2; 24 *ASY1*/–, 5 *asy1*/*asy1*; $\chi^2$=0.930; $P$>0.3) and further evidence of their cosegregation was obtained from this segregating material. The single-insert line was then subjected to further genetic analysis. The segregation of the mutant phenotype in 197 F2 progeny indicated that the *asy*1 gene is inherited as a single Mendelian gene (149 *ASY1*/–, 48 *asy1*/*asy1*; $\chi^2$=0.04; $P$>0.8). *ASY1*/*asy1* heterozygotes gave an average seed set of 47.4 seeds per silique, which is not significantly different from the wild type, confirming that *asy1* is fully recessive to the wild-type allele. *ASY1* maps to the bottom arm of chromosome 1, 21.9% towards the centromere from the *AP1* gene. This approximate chromosomal location of the smaller T-DNA insert was confirmed by FISH analysis.

### *Cloning the* ASY1 *gene*

A λ genomic DNA library prepared from heterozygous *ASY1*/*asy1* plant material was screened using a T-DNA probe. Nine positive λ clones were identified and tested for plant DNA content by reverse Southern blotting, using genomic plant DNA as a probe. This revealed the presence of a 9.5 kb SalI fragment containing plant DNA in one of these clones (data not shown). Partial nucleotide sequencing of the fragment and comparison with the *Arabidopsis* genomic sequence databases revealed that it corresponded to one end of a fully sequenced bacterial artificial chromosome (BAC). The BAC F1N21 (Accession number: AC002130) comprises 114,738 bp from the region of chromosome 1 that corresponds to the map position of *asy1*. Eighteen open reading frames (ORFs) are predicted to lie within the segment of chromosome 1 present in F1N21. The T-DNA/plant DNA junction present in this λ clone was sequenced and the T-DNA insertion was shown to lie between two predicted ORFs, referred to as ORF1 and ORF2, at one end of the BAC (Fig. 2).

**Fig. 1A–C.** Meiotic stages in embryo-sac and pollen mother cells of the *asy1* mutant of *Arabidopsis thaliana*. **A** Prophase I of meiosis in *asy1* embryo-sac mother cell, showing absence of chromosome synapsis. This phenotype closely resembles that seen in *asy1* pollen mother cells (**C**; Ross et al. 1997). **B** Metaphase I of meiosis in *asy1* embryo-sac mother cell showing ten univalents and absence of bivalents. **C** Prophase I pollen mother cell from an *asy1* mutant plant containing multiple- and single-copy T-DNA inserts revealed by fluorescence in situ hybridisation. The two smaller red signals (*arrowed*) correspond to the (homozygous) tagging single T-DNA inserts. The unsynapsed condition of the chromosomes is evident from the presence of two large and two small signals

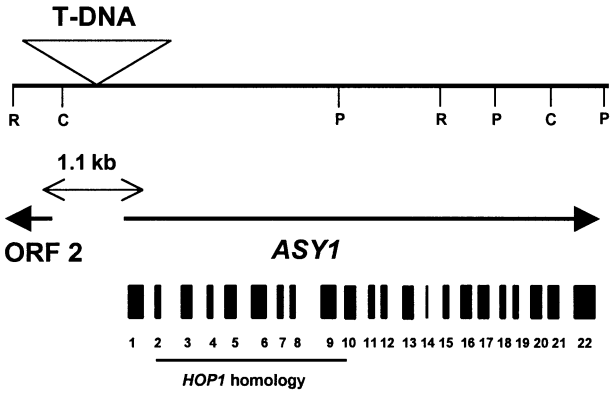



**Fig. 2.** Map of the *ASY1* locus and exon organisation. Map of the 7 kb region of chromosome 1 showing the relative positions of *ASY1* and ORF2. The T-DNA has inserted as a right-border fusion upstream of *ASY1*, and has resulted in a 1.1 kb deletion that extends 74 bp into exon 1 of the *ASY* gene. A partial restriction map is shown (*R* EcoRV, *C* ClaI, *P* PstI). The approximate positions of the 22 exons are shown. The Hop1-homologous region that extends from exon 2 to 10 is *underlined*

Database searches using the BLAST 2.0 program (Altschul et al. 1997) revealed that one of the ORFs (ORF1) possessed significant homology to the yeast meiotic synapsis gene *HOP1*, which therefore appears to be a good candidate gene for *ASY1* (discussed more fully below).

There are two main reasons why OFR2 does not appear to be involved in the *asy1* phenotype. Firstly, RT-PCR analysis failed to detect any evidence of ORF2 expression in any tissue under normal growth conditions (data not shown). Secondly, ORF2 exhibits no similarity to any known meiotic gene but does exhibit similarity to sequences listed as heat shock transcription factor (HSTF) homologues (e.g. Accession number CAB10177), although not to actual HSTFs.

To determine whether the T-DNA in the *asy1* mutant had disrupted the *HOP1* homologue, the T-DNA/plant DNA junction was identified. A 1.8 kb fragment corresponding to the 5' end of ORF 1 was amplified from *Arabidopsis* wild-type DNA. This was then used in conjunction with the T-DNA probe to re-screen the nine λ clones initially isolated. Three were found to hybridise to both probes and therefore contained theT-DNA/ORF1 junction region. DNA from one of these was subcloned and subjected to sequence analysis.

Nucleotide sequencing revealed that the T-DNA insertion was associated with a 1.1 kb deletion immediately upstream of the predicted ORF with homology to *HOP1* (Fig. 2). Based on these observations it seemed likely that the T-DNA insertion had resulted in a deletion of the ORF1 promoter region that possibly extended into the 5'-untranslated region (5' UTR). In order to clarify this situation, a combination of RT-PCR, 5'- and 3'-RACE was used to obtain a full-length cDNA corresponding to ORF1 from wild-type bud mRNA (Fig. 3). The precise 5' end of the gene was delineated using multiple independent 5' RACE experiments. Based on this sequence it was determined that the deletion had removed 74 bp of the 5' UTR of ORF1 and, presumably, the whole of the promoter region.

The predicted protein sequence of ORF1 was determined and compared with that of *HOP1* from the budding yeast *S. cerevisiae*. *HOP1* in yeast encodes a protein having a role in the assembly of SCs, and *hop1* mutants in yeast lack SCs and are asynaptic, producing <1% viable spores (Hollingsworth et al. 1990; Friedman et al. 1994). The predicted protein encoded by ORF1 consists of 596 amino acids, compared with 605 amino acids for yeast Hop1. Alignment of these proteins, shown in Fig. 4, reveals that they overlap each other over the first 250 amino acids, and over this conserved region they exhibit 28% identity and 51% similarity as determined using the BLAST 2.0 program (Altschul et al. 1997). This finding, together with the expression analysis (see below), strongly implicates the *Arabidopsis HOP1* homologue (ORF1) as the *ASY1* gene.

### *The* ASY1 *gene structure*

A comparison of the *ASY1* cDNA sequence and the published genomic sequence of the *ASY1* region on BAC F1N21 allowed the intron/exon structure of the *ASY1* gene (Fig. 2) to be determined. *ASY1* is composed of 22 exons and 21 introns. The sequence comparison revealed some minor discrepancies between the predicted protein sequence (F1N21.3, Accession number AAB95232) and that derived from the *ASY1* cDNA. The prediction did not detect exon 1, which is perhaps unsurprising, considering it encodes only 2 amino acids. It also failed to identify exon 15 (corresponding to residues 11,290–11,388 on the BAC sequence) and exon 14 is slightly different from the prediction, ending at 11,189, rather than 11,191.

### *The Asy1 protein sequence*

Two potential in-frame translational start codons were identified in the *ASY1* ORF, separated by 3 bp; both have the critical purine at position +3 and G at +4 that permit efficient translational initiation (Kozak 1995). While it is likely that most translational initiation occurs at the first ATG, a low level of initiation from the second is also possible (Kozak 1995).

The Asy1 protein is 596 amino acids in length, with a predicted $M_r$ of 67,300 and a pI of 5.04. The protein contains a HORMA domain spanning the first 250 amino acids. This domain is found in proteins that associate in some way with chromatin (Aravind and Koonin 1998). No other recognisable DNA binding motifs are present.

### *The* Arabidopsis *genome contains a second* HOP1 *homologue*

Southern blots of genomic DNA isolated from homozygous and heterozygous mutant plants and wild-type

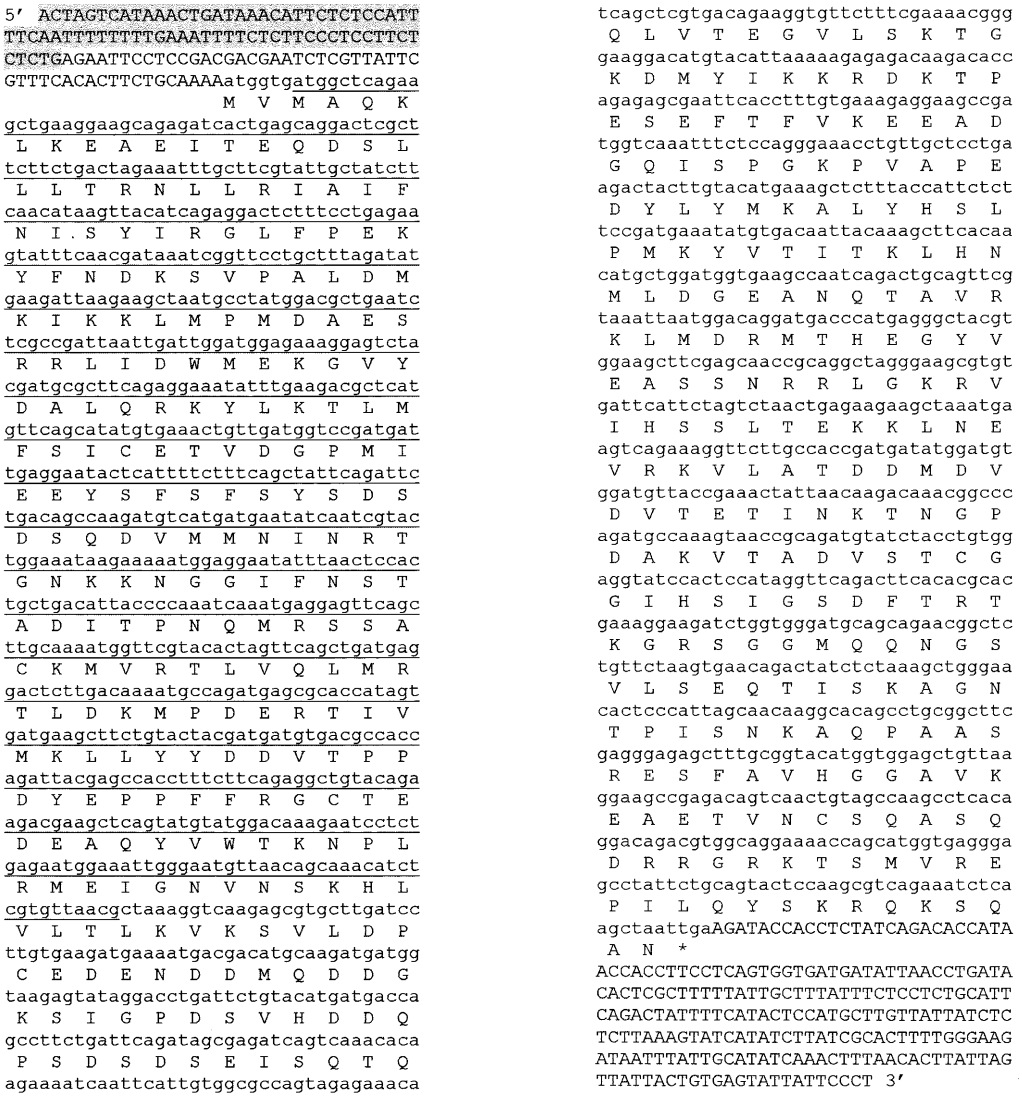



**Fig. 3.** Nucleotide sequence of the *ASY1* cDNA and deduced amino acid sequence. The deduced Asy1 amino acid sequence is presented below the cDNA sequence, which includes both 5' and 3' flanking sequences. The *shaded region* indicates the extent of the T-DNA-induced 74 bp deletion in the 5' untranslated region. The region of Asy1 that exhibits a high level of similarity with Hop1 is *underlined*. The *ASY1* cDNA sequence has the GenBank Accession number AF157556

plants were probed with DNA corresponding to the 5' end of *ASY1* (underlined in Fig. 3). Figure 5A shows that under conditions of high stringency the probe hybridised to a single band in wild-type DNA (lanes 1 and 4). A single signal was also obtained with the homozygous *asy1* mutant sample (lanes 3 and 6), but as expected the position of this band was shifted owing to the presence of the T-DNA insert. As might be anticipated, two bands were obtained with the heterozygous material corresponding to those found in the other two samples (lanes 2 and 5). When an identical blot was probed at lower stringency (Fig. 5B), the same hybridisation signals were obtained together with an additional band of higher molecular weight present in all three samples. As the region of *ASY1* used as the probe in this analysis corresponds to the region of the gene with *HOP1* homology, this result clearly suggested the presence of a second *HOP1* related sequence in *Arabidopsis*.

This was confirmed by further searching of the *Arabidopsis* genome sequencing database, which revealed the existence of a second ORF with significant homology to yeast *HOP1*. The BAC (BAC F10M6, Accession number AL051811) containing this ORF maps on chromosome 4. This ORF (F10M6.160, Accession No. CAA16970), which we provisionally name *ASY2*, encodes a predicted protein of 1552 amino acids. The predicted amino acid sequences of Asy1 and Asy2 overlap each other over the first 300 amino acids and over this

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Asy1 1 -----MVMAQKLKEA EITEQDSLLLTRNLL RIAIFNISYIRGLFP EKYFNDK--------
Asy2 1 -----MVVVSK-N-- --NEQQSLILTTELL RTAIFNISYIRGLFP VRYFKDM--------
Hop1 1 MSNKQLVKPKTETKT EITTEQSQKLLQTML TMSFGCLAFLRGLFP DDIFVDQRFVPEKVE

Asy1   -------SVPALDMK IKKLMP-MDAESRRL IDWMEKGVYDALQRK YLKTLMFSICETVDG
Asy2   -------SVPALDLK MKKLMP-MDAESRRL IGWMEKGVYDALHKK HLKKLIFYICETVDG
Hop1   KNYNKQNTSQNNSIK IKTLIRGKSAQADLL LDWLEKGVFKSIRLK CLKALSLGIFLEDPT

Asy1   PMIEEYSFSFSYSDS DSQDVMMNINRTGNK KNGGIFNSTAD---- -ITPNQMRSSACKMV
Asy2   PLIEEYIFSFSYSDS DSQDVRMNINITGIN TYGGTLNSTADNSTA DMTLNQMSSVDEDFG
Hop1   DLLENYIFSFDYDEE N--NVNINVNLSGNK K--GSKN--ADPENE TISLLDSRRMVQQLM

Asy1   R---------TLVQL MRTLDKMPD------ -----ERTIVMKLLY YDDVTPPDYEPPFFR
Asy2   QNARRSNAFVTYQRF SVYISFHIANYRLCY FFASVQRTILMKLLY YEYV-PPDYQPPFFR
Hop1   R---------RFIII TQSLEPLPQ------ -----KKFLTMRLMF NDNV-DEDYQPELFK

Asy1   GCT-EDEAQYVWTKN PLR--MEIGNVNSKH LVLTLKVKSVLD--- PCEDENDDMQDDGKS
Asy2   GCSEEEEAQYVWPKI PLR--MEIGNVNSQH HVLTVKVKSVLDPYD PCEDENDNMQDDERS
Hop1   DATFDKRATLKVPTN LDNDAIDVGTLNTKH HKVALSV-------- ---------------

Asy1   IGPDSVHDDQPSDSD SEISQTQENQFIVAP VEKQDDDDGEVDED- --------DNTQDPA
Asy2   KGPDSLHDDQP--CK VFTKPSK-----LIL TENKDADHGEVNEEK LLLITPICEILQDVK
Hop1   --------------- --------------- --------------- ---------------

Asy1   EN--EQQLARVKD    310
Asy2   QDQVEHQLAKVKD    337
Hop1   -------------    250
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**Fig. 4.** Alignment of homologous regions of Asy proteins and yeast Hop1. The deduced amino acid sequences of the homologous segments of the *Arabidopsis* Asy1 and Asy2 polypeptides are shown in alignment with the Hop1 protein of *Saccharomyces cerevisiae*. Sequences were aligned with Clustal using the Blosum62 comparison tables (Thompson et al. 1994). Strictly conserved residues are highlighted in *grey blocks*. Gaps are shown as *dashes*

conserved region they show 57% identity and 66% similarity (Fig. 4). During the 5' RACE experiments on *ASY1* the 5' end of *ASY2* was also cloned, confirming that *ASY2* is expressed, although the full extent of this gene was not determined (data not shown).

Several lines of evidence suggest that *ASY1* and *ASY2* are the only *HOP1* homologues in *Arabidopsis*. Firstly, only two bands were detected on low stringency blots of genomic DNA cut with two different restriction enzymes (ClaI and EcoRV) (Fig. 5B). Secondly, when the *ASY1/asy1* genomic λ library was screened only clones corresponding to *ASY1* and *ASY2* were identified (four of each, data not shown). Finally, only *ASY1* and *ASY2* were identified in a BLAST 2.0 tblastn (Altschul et al. 1997) search of the *Arabidopsis* genomic DNA database, which now covers 64.5% of the genome (10 June 1999).

### *Expression of* ASY1

Gel blots of total RNA prepared from a range of *Arabidopsis* tissues were probed with the *ASY1* probe used for the Southern analysis. No hybridisation signal was detectable; moreover, a subsequent experiment in which bud poly(A)+ RNA was probed also failed to produce any signal (data not shown). These results are reminiscent of those obtained with other *Arabidopsis* meiotic genes: *SYN1* (Bai et al. 1999) and *MS5* (Glover et al. 1998); however, in these cases low-level expression has been detected using RT-PCR. A similar approach was therefore employed to investigate *ASY1* expression. *ASY1* specific primers were used to amplify cDNA from reproductive and vegetative tissues isolated from wild-type plants. Figure 6A reveals that *ASY1* is expressed in all tissues (whole plant, roots, stem, bud and leaf) tested. A reduced level of *ASY1* expression was observed in the whole plant tissue sample. This may represent some degree of temporal control of *ASY1* expression as this tissue was harvested 14 days after germination, while the rest of the tissues were harvested after 3–4 weeks. When cDNA prepared from *asy1* bud RNA was analysed no expression of *ASY1* was detected, whereas expression of the actin gene control was detected as normal (Fig. 6C, D). This result clearly establishes a direct link between loss of *ASY1* expression and the asynaptic phenotype.

## Discussion

One proven approach to the identification and isolation of *Arabidopsis* meiotic genes is to exploit PCR technology using primers based on information from sequenced meiotic genes from other eukaryotes (Sato et al. 1995; Klimyuk and Jones 1997). This approach relies on the conservation of significant levels of homology between corresponding genes in different eukaryotes. As the amount of genomic sequence information for *Arabidopsis* increases, we can anticipate a rapid growth in the number of *Arabidopsis* homologues of known meiotic genes identified by genomic database searching. *ASY1* was detected as a T-DNA-tagged mutation of *Arabidopsis* (*asy1*). An homology search using DNA sequences flanking the tagging T-DNA insertion identified an ORF located on a sequenced *Arabidopsis* BAC clone that showed significant homology to the budding yeast meiotic gene *HOP1*, with a key role in meiotic synapsis. Further investigation showed that the T-DNA insert in *asy1* co-localised with a 1.1 kb deletion that removed the

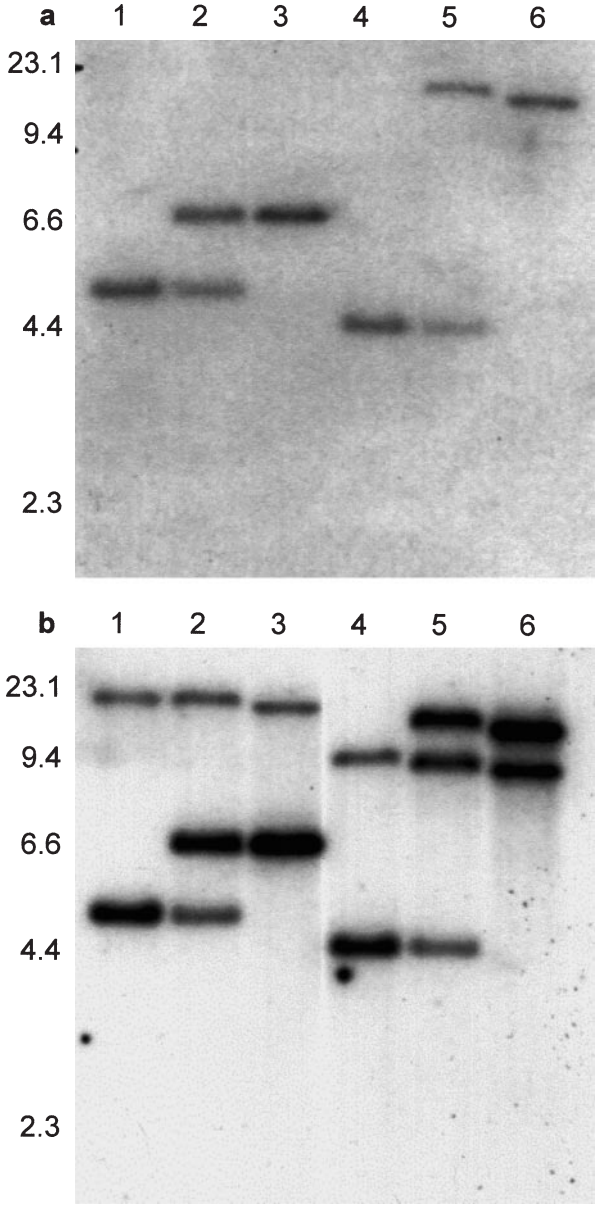


**Fig. 5a, b.** Southern analysis of the *ASY1* gene in *Arabidopsis* genomic DNA. **a** Genomic DNA from wild-type plants (*lanes 1, 4*), *asy1* homozygous mutant plants (*lanes 3, 6*) and *ASY1/asy1* heterozygotes (*lanes 2, 5*) was digested with ClaI (*lanes 1–3*) and EcoRV (*lanes 4–6*) and subjected to Southern analysis using an *ASY1* probe corresponding to residues 6–669 of the coding region. The blot was washed at high stringency. The size of the λ HindIII standard is indicated in kilobases. **b** An identical blot washed at low stringency, revealing the presence of an additional *ASY1*-like gene (*ASY2*) in the *Arabidopsis* genome

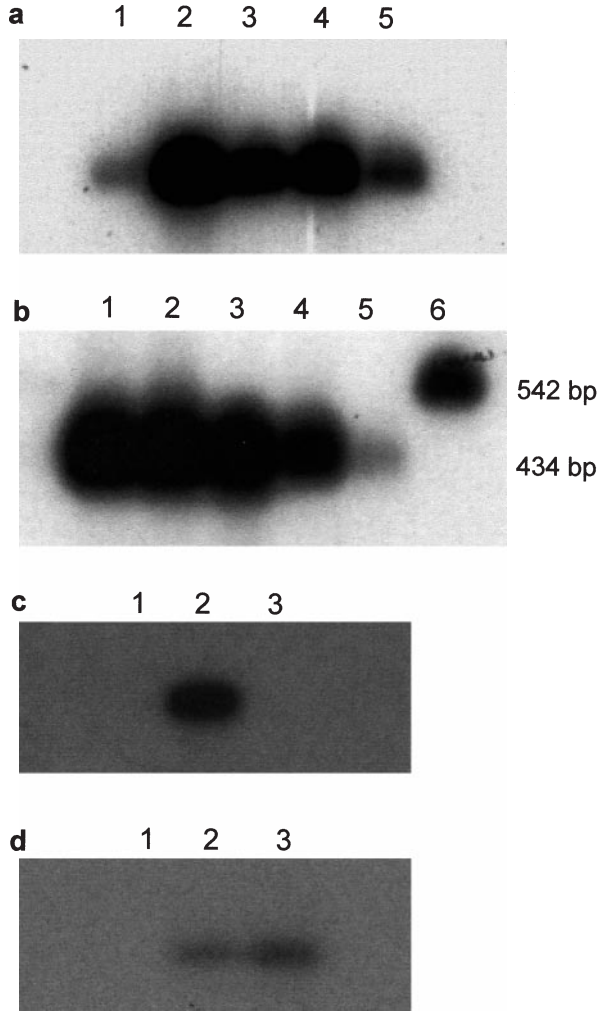


**Fig. 6a–d.** Expression analysis of *ASY1*. The reverse transcription-polymerase chain reaction (RT-PCR) was use to analyse the expression of *ASY1* in various *Arabidopsis* tissues. Reaction products were separated on a 1.2% agarose gel, transferred to nylon and probed with an *ASY1* or *ACT8* probe as appropriate. **a** RT-PCR analysis of *ASY1* expression in wild-type plants: *lane 1* whole plant, *lane 2* root, *lane 3* stem, *lane 4* bud, *lane 5* leaf. **b** Control for **a** using *ACT8* primers designed to flank intron 4: *lane 1* whole plant, *lane 2* root, *lane 3* stem, *lane 4* bud, *lane 5* leaf, *lane 6* genomic DNA. Note that a single product of 434 bp is obtained from the reverse transcribed RNA samples, indicating that these do not contain genomic DNA that produces a product of 542 bp. **c** RT-PCR analysis of *ASY1* expression in *asy1* homozygous mutant plants. *Lane 1* $H_2O$ control, *lane 2 ASY1* wild-type buds, *lane 3 asy1* buds. **d** Control for **c** using *ACT8* primers. *Lane 1* $H_2O$ control, *lane 2 ASY1* wild-type buds, *lane 3 asy1* buds

promoter region and 74 bp of the 5' UTR of *ASY1* and as a result the gene transcript was absent in *asy1* bud tissue. Taken together these observations provide strong evidence that the partial deletion of *ASY1* has resulted in the asynaptic phenotype of *asy1*. We have also provided direct cytological evidence that this lesion affects both male and female meiosis.

Comparison of the the *ASY1* cDNA sequence and the published genomic sequence of the *ASY1* region on BAC F1N21 revealed some differences between the actual and predicted intron-exon junctions. It has been reported that the *Arabidopsis* meiotic gene *MEI1* (He and Mascarenhas 1998) contains introns that do not follow the GU...AG rule for intron splicing (Brown et al. 1996). However, when the splice sites in *ASY1* were examined all 21 introns were found to obey the rule, hence it is likely that the discrepancy is due to the software used to predict potential ORFs.

The *ASY1* gene was found to be expressed in non-meiotic tissues rather than being specifically induced by meiosis as *HOP1* is in yeast (Hollingsworth et al. 1990). Two other meiotic genes from *Arabidopsis* are also expressed in non-meiotic tissues, namely *SYN1* (Bai et al. 1999) and *MS5* (also known as *Jag18* and *Pollenless3)* (Glover et al. 1998; Sanders et al. 1999; Luong, Caryl and Franklin, unpublished observations), although a meiosis-specific splice variant of *SYN1* was reported (Bai et al. 1999). In addition when the expression patterns of the *Arabidopsis* homologues of the yeast meiotic gene *Dmc1* (Doutriaux et al. 1998) and the *Drosophila* meiotic gene *Pelota* (Caryl, Jones and Franklin, unpublished observations) were examined these were also found to be expressed in non-meiotic tissues. Taken together these findings appear to suggest that a substantial amount of meiotic gene expression is regulated at a post-transcriptional level in *Arabidopsis*.

### *Comparison of* ASY1 *and the* HOP1 *gene of S. cerevisiae*

Mutants of the yeast *HOP1* gene show reduced meiotic recombination (10%–25% of wild-type levels), and extremely low levels of spore viability (<1%) (Hollingsworth et al. 1990). Hop1 has been shown to be a component of the budding yeast SC, more specifically of the axial elements (Hollingsworth et al. 1990; Friedman et al. 1994; Smith and Roeder 1997; Kironmai et al. 1998). Antibody staining reveals that Hop1 protein accumulates at numerous discrete foci on meiotic chromosomes during early prophase (Hollingsworth et al. 1990; Smith and Roeder 1997), but by the time the chromosomes have fully synapsed at pachytene, little Hop1 staining remains (Smith and Roeder 1997). Accumulation of Hop1 protein depends on the presence of another yeast SC component Red1 (Smith and Roeder 1997). Yeast two-hybrid experiments (Hollingsworth and Ponte 1997), and genetic studies (Hollingsworth and Johnson 1993; Friedman et al. 1994; Hollingsworth and Ponte 1997) indicate that Hop1 and Red1 interact, and that this interaction may be regulated by Mek1, a putative serine-threonine protein kinase (Hollingsworth and Ponte 1997).

Hop1 is a DNA binding protein of 605 amino acids in length, containing a single putative non-classical zinc finger around amino acid 371 of the protein (Hollingsworth et al. 1990). DNA binding activity, while enhanced in the presence of zinc, is still observed even in the presence of EDTA (Kironmai et al. 1998). The N-terminus is thought to comprise a HORMA domain, which is seen in a variety of proteins that interact with chromatin, in SC formation, DNA repair or DNA synthesis (Aravind and Koonin 1998). It is possible that this domain may be responsible for the DNA binding activity detected with Hop1 in the absence of zinc.

The Asy1 protein (596 amino acids) is almost identical in size to Hop1 (605 amino acids), but homology between the two is restricted to the N-terminus, in the region that corresponds to the proposed HORMA domain (Aravind and Koonin 1998). Notably, Asy1 does not contain the zinc finger region that has been shown to be essential for Hop1 activity in vivo (Hollingsworth et al. 1990). The second *Arabidopsis HOP1* homologue *ASY2* is predicted to encode a longer protein of 1552 amino acids and again homology to Hop1 is restricted to the HORMA domain at the N-terminus. However, homology to Asy1 is slightly more extensive, extending over a further 50 amino acids at the N-terminus, but the remainder of the proteins exhibit no obvious homology. The significance of this variation awaits functional analysis of the *Arabidopsis* proteins and identification of the cellular components with which they interact. While it may suggest substantial functional differences from yeast Hop1, it may be the case that although the proteins are functionally homologous this does not extend to primary amino acid sequence. If so, this would resemble the situation reported for other SC proteins; for example, Scp1 from rat (Meuwissen et al. 1992) and yeast Zip1 (Sym et al. 1993) are both localised to the central region of the SC and are of similar structure, yet they do not exhibit amino acid sequence homology beyond that found when any pair of coiled-coil proteins are compared.

The meiotic phenotype of *asy1* is more accurately described as partially asynaptic, because a low level of synapsis is indicated by the presence of from one to three bivalents in some metaphase I cells, with an overall average of 1.5 bivalents per cell. The scarcity of detectable synapsed regions in early prophase I nuclei suggests that any synapsis that occurs is limited in extent. Given that the *asy1* mutation appears to involve deletion of the promoter region and 5' UTR of the *ASY1* gene, presumably causing an effectively null mutation, the fact that any synapsis occurs at all is indicative of some degree of redundancy between *ASY1* and *ASY2*. It will be of interest to explore the functional relationship of these two genes in future studies.

*Acknowledgements.* We thank John Martin and colleagues for horticultural support. We thank Clare Peterson for her contribution to the analysis of the *ASY1* open reading frame and Trong Luong for helpful discussions. This research was supported by the Biotechnology and Biological Research Council (BBSRC).

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