

Live observation of fission yeast meiosis in recombination-deficient mutants: a study on achiasmate chromosome segregation

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SUMMARY

Regular segregation of homologous chromosomes during meiotic divisions is essential for the generation of viable progeny. In recombination-proficient organisms, chromosome disjunction at meiosis I generally occurs by chiasma formation between the homologs (chiasmate meiosis). We have studied meiotic stages in living *rec8* and *rec7* mutant cells of fission yeast, with special attention to prophase and the first meiotic division. Both *rec8* and *rec7* are early recombination mutants, and in *rec7* mutants, chromosome segregation at meiosis I occurs without any recombination (achiasmate meiosis). Both mutants showed distinct irregularities in nuclear prophase movements. Additionally, *rec7* showed an extended first division of

variable length and with single chromosomes changing back and forth between the cell poles. Two other early recombination deficient mutants (*rec14* and *rec15*) showed very similar phenotypes to *rec7* during the first meiotic division, and the fidelity of achiasmate chromosome segregation slightly exceeded the expected random level. We discuss possible regulatory mechanisms of fission yeast to deal with achiasmate chromosome segregation.

Movies available on-line

Key words: Meiosis, Fission yeast, Chromosome segregation, Achiasmate segregation

INTRODUCTION

In the life cycle of sexually reproducing eukaryotes, meiosis halves the DNA content from diploidy in the germline cells to haploidy in the gametes. This reduction is achieved by two consecutive rounds of chromosome segregation, which follow a single round of DNA replication. The first (reductional) division involves several meiosis-specific events. During meiotic prophase, homologous chromosomes pair and recombine. Meiotic recombination serves not only for the generation of genetic diversity, but the crossing over between homologous chromosomes and the resulting chiasmata is also necessary for faithful segregation of chromosomes at meiosis I (Baker et al., 1976). Proper alignment of a bivalent on the metaphase plate requires at least one functional chiasma, which connects the homologs and balances the pulling forces exerted on the kinetochores attached to opposite poles (Carpenter, 1994; Nicklas, 1997). Furthermore, chiasmata need to be held in place until the signal to start anaphase I occurs. Attachment of sister chromatids along the centromere-distal side of the chiasmata is thought to fulfill this function (Maguire, 1974). Mutants impaired in meiotic recombination are expected to produce frequent nondisjunction of homologs at meiosis I, and those with mutations in genes necessary for sister chromatid cohesion produce precocious separation of sister chromatids. Both missegregation types result in the

generation of aneuploid, and therefore frequently unviable progeny.

The fission yeast *Schizosaccharomyces pombe* is a unicellular eukaryote that undergoes meiotic divisions immediately after the mating of two haploid cells (Egel, 1989). Meiosis in fission yeast has some unusual features. Unlike most of the eukaryotes, *S. pombe* has no synaptonemal complexes (Bähler et al., 1993) and shows no crossover interference (Munz, 1994). Linear elements, which resemble the axial cores of other eukaryotes, have been proposed to play a role in meiotic chromosome organization (Bähler et al., 1993; Kohli, 1994; Kohli and Bähler, 1994). The meiotic prophase in fission yeast is characterized by a strongly elongated nuclear morphology known as the horse-tail nucleus. (Robinow, 1977; Robinow and Hyams, 1989).

Observation of living cells undergoing meiosis has shown that the elongated prophase nuclei perform striking oscillatory movements between the cell poles. These movements are led by the spindle pole body (SPB) and the attached telomere cluster, and this bouquet arrangement of chromosomes is maintained throughout prophase (Chikashige et al., 1994; Chikashige et al., 1997). The oscillatory nuclear movements are mediated by the reorganization of astral microtubules originating from the SPB (Ding et al., 1998; Svoboda et al., 1995), and require cytoplasmic dynein as a microtubule motor protein (Yamamoto et al., 1999). Pairing of homologous

chromosomes and meiotic recombination occur during prophase nuclear movements. It has been proposed that telomere clustering and oscillatory nuclear movements facilitate the encounter of homologous chromosomes, and thus are required for normal homologous pairing and full level of meiotic recombination (Chikashige et al., 1997; Ding et al., 1998; Hiraoka, 1998). Analysis of mutants that show reduced meiotic recombination, and deficiencies in telomere clustering (Shimanuki et al., 1997; Cooper et al., 1998; Nimmo et al., 1998) or in nuclear movement (Yamamoto et al., 1999) support this model.

Considering the evidence for the necessity of prophase nuclear movements for meiotic recombination, we have started live observation of meiosis in meiotic recombination-deficient mutants (Ponticelli and Smith, 1989; DeVeaux et al., 1992). Two of the mutants (*rec8* and *rec7*) that belong to the genetically best characterized recombination-deficient mutants (Molnar et al., 1995; Krawchuk et al., 1999; Parisi et al., 1999; Watanabe and Nurse 1999; Molnar et al., 2001) were selected for detailed study.

Live observation of fission yeast meiosis has so far been concentrated on the examination of prophase nuclear movements. In the present study we demonstrate that live observation of meiosis can also reveal details of the mechanism and accuracy of the meiotic divisions. We have examined the different meiotic stages in living *rec8* and *rec7* mutant cells and have then further analyzed the first meiotic division with the application of a LacO-LacI-GFP construct that visualizes the centromeric region of chromosome I (Nabeshima et al., 1998). We show that the special features of meiotic divisions detected in the *rec7* mutant are also characteristic of other meiotic mutants and represent a common way of achiasmate chromosome segregation.

MATERIALS AND METHODS

Microscope system

Fluorescence images were obtained on a cooled, charge-coupled device (CCD) as an image detector using a computer-controlled, fluorescence microscope system. In this microscope system, a Peltier-cooled CCD camera CH250 (Photometrics, Tucson, AZ) is attached to an Olympus inverted microscope IX70. The microscope lamp shutter, focus movement, filter combinations and CCD data collection are controlled by a Silicon Graphics UNIX workstation. The microscope system is built in a temperature-controlled room; the computer and other controllers are placed outside the room and control the microscope remotely (Haraguchi et al., 1997; Haraguchi et al., 1999).

Strains, media and culture conditions

S. pombe strains used in this study are listed in Table 1. The strains designated *his7⁺::lacI-GFP lys1⁺::lacO* carry tandem repeats of LacO DNA sequences at the centromere-linked *lys1* locus on chromosome I, and the integrated GFP-LacI-NLS fusion construct at the *his7* locus (Nabeshima et al., 1998). Strains were cultivated on yeast extract agar (YEA) complete medium supplemented with 75 µg/ml adenine sulfate, at 30°C. Mating and meiosis were induced by transferring homothallic (*h⁹⁰*) or crossing heterothallic (*h⁺* and *h⁻*) strains on malt extract agar (MEA) sporulation medium (Gutz et al., 1974), and incubating the plates at 26°C. For microscopic observation of meiosis, cells were resuspended in EMM2-N liquid medium (EMM2 minimal medium without nitrogen source). Transformants

Table 1. Strains

Strain	Genotype
CRL 152	<i>h⁹⁰ leu1 ura4-D18 lys1</i>
57-2262	<i>h⁹⁰ leu1-32 rec8::ura4⁺ ura4-D18</i>
88-3485	<i>h⁹⁰ leu1-32 rec7::ura4⁺ ura4-D18</i>
A1	<i>h⁹⁰ ade6-M26 leu1-32 rec14-161::LEU2</i>
A2	<i>h⁹⁰ ade6-M26 ura4-294 leu1-32 rec15-154::LEU2</i>
AY 176-17D	<i>h⁹⁰ ade6-M216 ura4 his7⁺::lacI-GFP lys1⁺::lacO</i>
AY 171-2A	<i>h⁻ leu1 ura4 his7⁺::lacI-GFP lys1⁺::lacO</i>
AY 173-4A	<i>h⁺ ade6-M216 ura4 his5</i>
A3	<i>h⁹⁰ rec8::ura4⁺ ura4-D18 his7⁺::lacI-GFP lys1⁺::lacO</i>
A4	<i>h⁻ rec8::ura4⁺ ura4-D18 leu1 his7⁺::lacI-GFP lys1⁺::lacO</i>
68-2710	<i>h⁺ rec8::ura4⁺ ura4-D18 lys1-131</i>
A5	<i>h⁹⁰ rec7::ura4⁺ ura4-D18 his7⁺::lacI-GFP lys1⁺::lacO</i>
A6	<i>h⁺ rec7::ura4⁺ ura4-D18 ade6-M216 leu1 his7⁺::lacI-GFP lys1⁺::lacO</i>
82-3246	<i>h⁻ rec7::ura4⁺ ura4-D18 lys1-131 ade6-M216</i>

were selected on EMM2 plates supplemented with appropriate nutrients (Moreno et al., 1991).

Preparation of specimens for microscopic observation

For live observation of meiosis, cells of control and *rec* mutant strains were transferred onto MEA plates and incubated overnight (12-16 hours), at 26°C. Fluorescence staining of nuclei was achieved either by staining with Hoechst 33342, a DNA-specific fluorescence dye, or with the application of the jellyfish green fluorescence protein. Hoechst 33342 staining was used to identify and examine the different meiotic stages in living cells. Samples of mating cells were washed in water, and stained with 1 µg/ml Hoechst 33342 in distilled water for 15 minutes at room temperature. Stained cells were resuspended in EMM2-N and mounted either on a coverslip or in a glass-bottom culture-dish (MatTek, Ashland, MA) coated with concanavalin A (1 mg/ml).

To observe the duration of prophase nuclear movements, nuclei were stained with the use of jellyfish GFP protein. Control, *rec7* and *rec8* homothallic strains were transformed with the plasmid pYC551 according to the LiCl method (Moreno et al., 1991). This pREP1 expression vector-based plasmid (Maundrell, 1993) carries the sequences for the NLS-GFP protein and the *Saccharomyces cerevisiae* LEU2 gene as selection marker. Transformants grew on appropriately supplemented EMM2-leu plates containing 2 µM thiamine, and prepared for microscopic observation of meiosis as described above.

To examine the final meiotic phenotypes of *rec* mutants, the MEA plates were incubated for 3 days at 26°C. In each mutant, approx. 200 mature asci were classified in phase-contrast microscope according to their spore numbers. From the same crosses, samples were taken for fluorescence staining of nuclei in the asci. Samples were treated with 70% ethanol for 5 minutes, resuspended in 1 µg/ml DAPI (4',6-diamidino-2-phenylindol) and examined in fluorescence microscope.

Fluorescence imaging of living fission yeast cells

Specimens of living fission yeast cells were observed at 26°C on the CCD microscope system using an Olympus oil immersion objective lens (Plan Apo 60/NA=1.4). Images were obtained on the cooled CCD with an exposure time of 0.2-0.5 seconds under the illumination of a mercury arc lamp. An excitation filter with a narrow peak at 380 nm for Hoechst 33342 and a high-selectivity fluorescein excitation filter for GFP in combination with high-selectivity barrier filters for DAPI and fluorescein (Chroma Technology, Brattleboro, VA), respectively, were used. A single dichroic mirror with quadruple-bandpass properties (Chroma Technology) was used. During the observation of different meiotic stages images were taken in a single optical section. In order to monitor the position and number of GFP signals visualizing the centromere-proximal region of chromosome I, images were taken in ten optical sections covering the whole nucleus at each time-point. In the presented figures, the images are projected into two

dimensions after deconvolution. Image processing, analysis and display were carried out using the DeltaVision program (Applied Precision, Seattle, WA).

RESULTS

Phenotypes of prophase nuclear movements in *rec8* and *rec7* mutants

To gain further insight into the relationship of oscillatory nuclear movements and meiotic recombination, we examined the prophase nuclear movements in *rec8* and *rec7* mutant strains. Cells of homothallic wild-type and *rec* mutant strains were induced to undergo meiosis, stained with Hoechst 33342, a DNA-specific fluorescence dye, and mounted on microscope slides (see Materials and Methods). Fusion of haploid nuclei took place regularly in both mutants (an example for *rec7* mutant shown in Fig. 1C). Immediately after karyogamy, the prophase nuclei started to move. Fig. 1A demonstrates the characteristics of these movements in wild-type meiosis. The nucleus is highly elongated and flexible: it takes U-turns at the cell poles (0 minutes, 6 minutes). A bright spot, representing the clustered telomeres (Chikashige et al., 1994), is situated at the leading edge of movement (indicated by arrows at 6 and 10 minutes in Fig. 1A). Both recombination-deficient mutants showed a deviation from the above-described regular phenotype. In *rec8* mutant cells, the bulk nuclear mass mostly remained at the cell center; only the leading edge protruded to the cell poles (indicated by arrows in Fig. 1B). This rendered a teardrop-like shape to the nucleus. In *rec7* mutant cells, horse-tail nuclear movements were observed, but were somewhat aberrant: the bright spot (indicated by the arrowhead in Fig. 1C) frequently appeared at a specific distance from the leading edge of the nucleus (arrows in Fig. 1C). This phenotype was particularly striking right after karyogamy.

We were interested in whether these aberrations influence the length of prophase nuclear movements. The duration of prophase nuclear movements was determined after fluorescence staining of the nuclei with GFP (see Materials and Methods). In the wild-type strain, in two experiments time lengths of 140 and 160 minutes were measured. These numbers are in good agreement with the previously presented data (146 ± 14 minutes, in Hiraoka et al., 2000). Prophase nuclear movements lasted 147 ± 25 minutes in *rec8*, and 153 ± 6 minutes in *rec7* mutant strains (averages

of three measurements). Because the prophase nuclei did perform oscillatory movements in both mutants, and these movements were of similar duration as those in wild-type nuclei, we conclude that the *rec8* and *rec7* mutations have an effect on the nuclear shape and chromosome organization rather than on the movements per se.

Analysis of meiotic divisions in *rec8* and *rec7* mutants

Live observation of fission yeast meiosis has been centered on the understanding of the most striking meiotic phenomenon, the prophase nuclear movements (see Introduction). Live observation of meiotic divisions in mutants that are strongly impaired in meiotic recombination may contribute to a better understanding of meiotic chromosome segregation. *rec8* and *rec7* are particularly interesting in this respect, as they are the meiotic recombination-deficient mutants, in which the two missegregation types (precocious separation of sister chromatids in *rec8*, Molnar et al., 1995; nondisjunction I in *rec7*, Molnar et al., 2001) have been detected genetically.

To examine the first division, zygotes with a single almost round nucleus were selected after staining the cells with

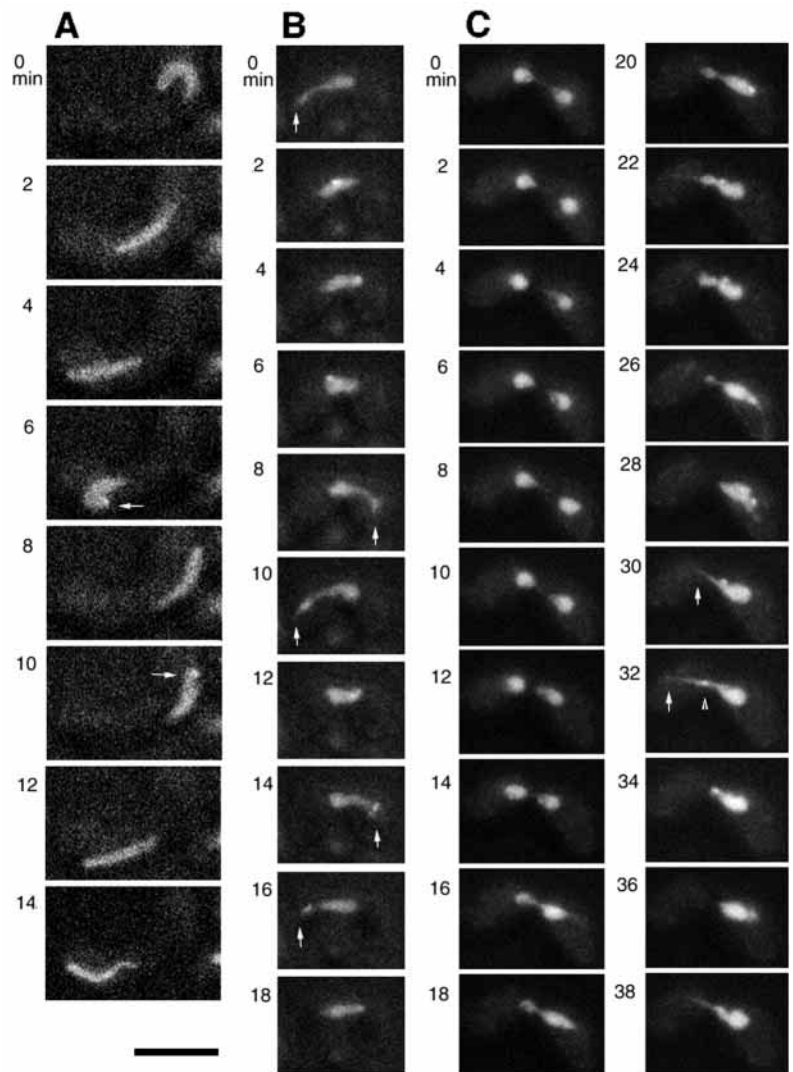


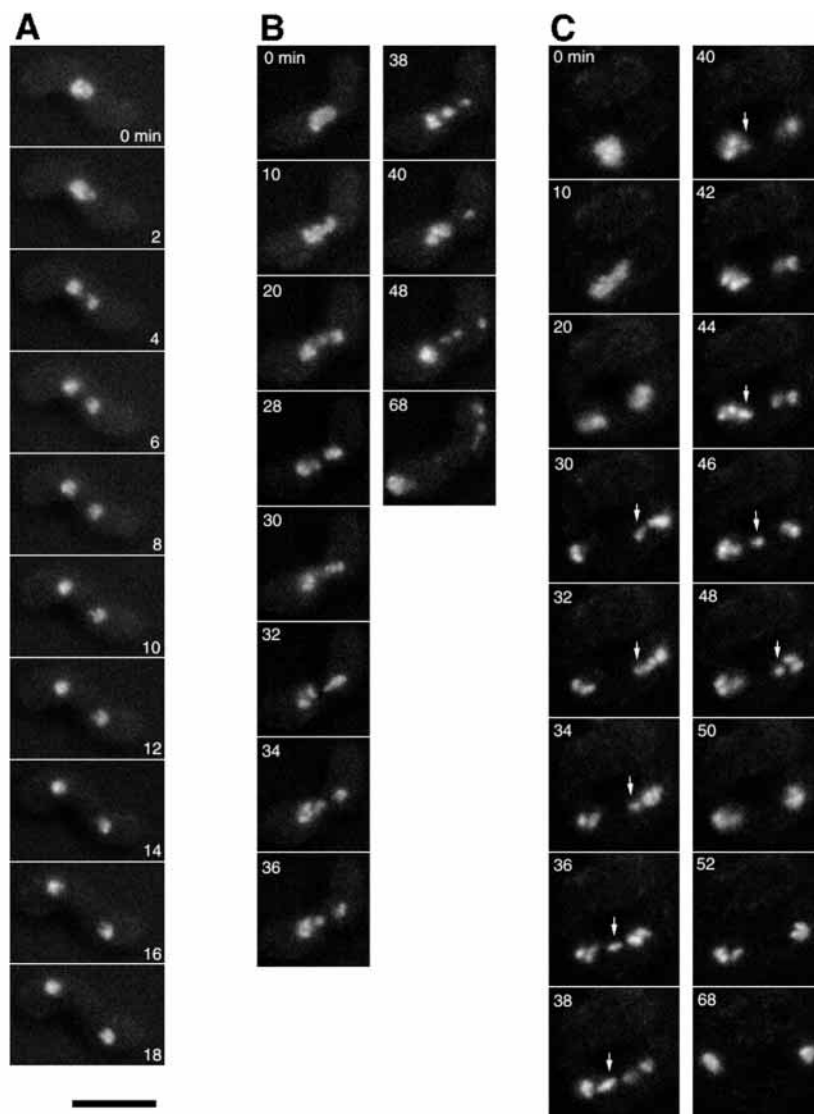
Fig. 1. Prophase nuclear movements in wild-type, *rec8* and *rec7* mutant cells. Nuclei of strains (A) CRL 152 (wild type), (B) 57-2262 (*rec8* mutant) and (C) 88-3485 (*rec7* mutant) were stained with Hoechst 33342. Images were taken in a single optical section with 2 minute intervals. Numbers on the left of each image indicate time in minutes. In A, arrows indicate the position of the presumptive telomere cluster. In B, arrows indicate the position of the leading edge of the nucleus. In C, arrows indicate the position of the leading edge of the nucleus; the arrowhead points at the presumptive telomere cluster. Scale bar: 10 μ m.

Fig. 2. Nuclear dynamics during the first meiotic division in the *rec8* and *rec7* mutants. (A) *rec8* mutant. Nuclei of strain 57-2262 were stained with Hoechst 33342. Numbers on the right of the images indicate time in minutes. (B,C) *rec7* mutant. Nuclei of strain 88-3485 were stained with Hoechst 33342, and two divisions with different outcome were chosen for presentation. Numbers on the left of the images indicate time in minutes. In case of division (B), chromosomes were apparently unevenly distributed between the daughter nuclei. (C) Division resulted in even segregation. Equal numbers of chromosomes moving to opposite poles are clearly discernable at 48 minutes. The arrow indicates a chromosome moving between two poles. Scale bar: 10 μ m.

Hoechst 33342 dye. These nuclei had completed the prophase movements and were in the nuclear condensation stage, which precedes the first division (Chikashige et al., 1994; Hiraoka et al., 2000). To see the detailed dynamics of nuclear divisions, images were taken at 2 minute intervals. Apparently equal amounts of nuclear material moved to the two cell poles in the first division, both in the wild-type and the *rec8* mutant cell. Fig. 2A shows an example of the dividing nucleus of a *rec8* zygote (for wild type, data not shown). In *rec8*, regular distribution of the nuclear material was observed in five cases studied. According to previous studies, which detected precocious separation of sister chromatids on spreads of *rec8* prophase nuclei (Molnar et al., 1995), a more irregular first division was expected in this mutant. However, a complete change of the reductional segregation pattern to equational (Watanabe and Nurse, 1999) could account for the observed regular phenotype, if sister chromatids are held together at least at the centromeric region until anaphase I.

In sharp contrast to the *rec8* mutant, *rec7* exhibited a highly irregular first division. In six divisions out of nine observed in *rec7*, unequal amounts of nuclear masses were distributed to the cell poles. A striking feature of the *rec7* mutant was that chromosomes moved back and forth between the cell poles during the first division. Seven out of the nine divisions exhibited this phenotype. Selected frames of two examples are shown in Fig. 2B,C. Individual chromosomes were clearly discernable in some images. In Fig. 2C, for example, six chromosomes can be seen at 48 minutes and the arrow indicates a chromosome wandering between the cell poles. Chromosome movements ended in an apparently regular segregation in two cases (Fig. 2C), while in five divisions the final chromosome distribution was seemingly irregular (Fig. 2B).

Measurement of duration of the first division further emphasized the difference between the two mutants. The first meiotic division took 22 ± 3 minutes in the wild type and 21 ± 3 minutes in *rec8* mutant cells (averages of three and five measurements, respectively). In *rec7*, an average of 57 ± 26 minutes was measured in five observations. In the *rec7* mutant, the observed divisions were widely different in duration.



Notably, the shortest one (20 minutes) resulted in an apparently equal distribution of the nuclear mass and was followed by a reasonably regular second division (data not shown).

To observe the second meiotic division, zygotes with two nuclei were chosen after Hoechst 33342 staining. Generally, irregular distribution of the nuclear masses was characteristic for the second division in both mutants (Fig. 3B,C; for wild-type, see Fig. 3A). In addition, in *rec7* the second division did not always take place or only one of the daughter nuclei divided. Because the first meiotic division was irregular in both mutants, it was difficult to decide whether the second meiotic divisions themselves were impaired as well, or whether they simply were a consequences of impairment during the first divisions. Therefore, the second meiotic division was not analyzed further.

Tracing individual chromosomes through the first meiotic division

Live observation of the first meiotic division in the *rec7* mutant resulted in a puzzling observation. It gave the impression that chromosomes moved back and forth between the cell poles

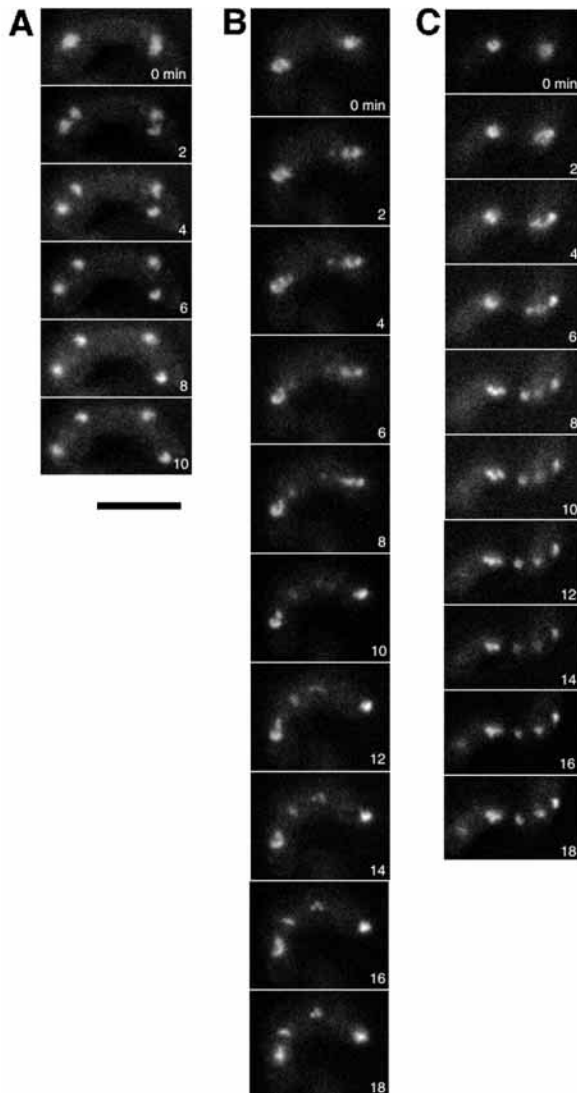


Fig. 3. Selected frames of the second meiotic division. Nuclei of strains CRL 152 (wild type), 57-2262 (*rec8*), and 88-3485 (*rec7*) were stained with Hoechst 33342. Numbers on the right of the images indicate time in minutes. Compare the even distribution of nuclear masses in the wild type strain (A) with the irregular divisions seen in *rec8* (B) and *rec7* (C). Scale bar: 10 μ m.

several times, until finally they stopped at either pole. To understand the chromosome movements better in the *rec7* and *rec8* mutants, we traced individual chromosomes through the first meiotic division.

For visualization of individual chromosomes, a GFP-LacI-NLS fusion protein was used that binds to a LacO array integrated at the centromere-proximal region of chromosome I (Nabeshima et al., 1998). This construct was introduced into homothallic and heterothallic, wild-type and mutant strains (Table 1), and was observed as described in Materials and Methods. Results are summarized in Table 2 (see the next section for details); some examples of images are shown in Figs 4-6 below.

To examine chromosome segregation in *rec7*, a homothallic strain was applied because, according to genetic studies (Molnar et al., 2001), nondisjunction of homologs at the first

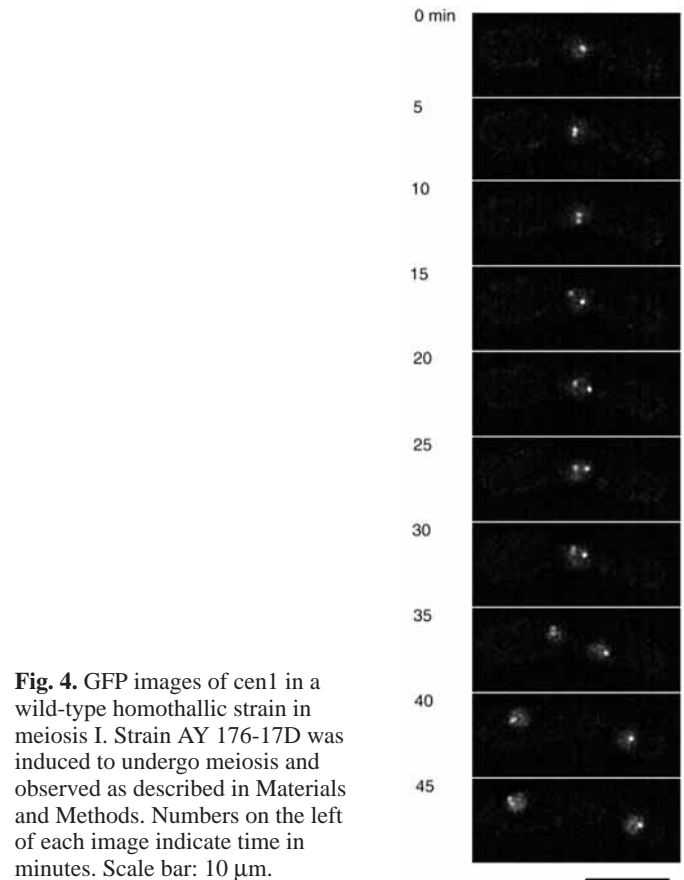
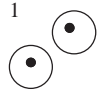

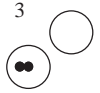



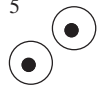






Fig. 4. GFP images of *cen1* in a wild-type homothallic strain in meiosis I. Strain AY 176-17D was induced to undergo meiosis and observed as described in Materials and Methods. Numbers on the left of each image indicate time in minutes. Scale bar: 10 μ m.

division was expected. In homothallic crosses, sister chromatids of the homologs are labeled with GFP fluorescence. In the homothallic wild-type strain (control), segregation of homologous chromosomes occurred in two phases (Fig. 4). After separation the centromere signals maintained their position at a short distance (from 15 to 30 minutes, Fig. 4), then moved to the cell poles. In some images (30, 35 and 45 minutes in Fig. 4), one of the chromosomes showed two very closely spaced signals, suggesting that during this division in one of the chromosomes a loosening of sister chromatid attachment occurred. Fig. 5 shows examples of *rec7* homothallic cross: chromosome segregation occurred in one phase without having a period of short-distance separation. After an initial regular separation (10 minutes to 20 minutes in Fig. 5A; 5 minutes to 15 minutes in Fig. 5B), one of the chromosomes lost orientation, and started to move to the same pole as its homolog (see 35 minutes in Fig. 5A, and 20 minutes in Fig. 5B). This loss-of-orientation resulted in nondisjunction of the homologs in Fig. 5A. In another example, however, a successful re-orientation took place, ending with normal disjunction of the homologs (Fig. 5B).

To observe chromosome segregation in *rec8*, an *h⁻* strain carrying the GFP construct was crossed with an *h⁺* strain lacking the construct. In such a heterothallic cross, sister chromatids of only one of the homologs are labeled; thus, separation of sister chromatids can be distinguished from separation of homologs. In accordance with this, the signals in Fig. 6 are weaker than in the images, where they represent homologous chromosomes (homothallic crosses, Figs 4 and 5).

Table 2. Fidelity of chromosome segregation

Crosses heterozygous for the GFP construct					
					Total number of zygotes observed
<i>rec8</i>	36	4	0	0	40
<i>rec7</i>	1	16	16	7	40
<i>rec⁺</i>	1	21	0	16	38

Crosses homozygous for the GFP construct								
								Total number of zygotes observed
<i>rec8</i>	3	0	10	23	0	1	1	38
<i>rec7</i>	17	3	3	1	11	3	1	39
<i>rec⁺</i>	22	5	0	8	0	0	0	35

To examine the fidelity of chromosome segregation at the first meiotic division, a centromere-proximal region of the chromosome I is labeled with a GFP construct (see Materials and Methods). Heterothallic strains were crossed on sporulation media. The *h⁻ rec8* (A4), *h⁺ rec7* (A6), and *h⁻ rec⁺* (AY171-2A) strains carry the GFP construct; their mating partners, *h⁺ rec8* (68-2710), *h⁻ rec7* (82-3246), and *h⁺ rec⁺* (AY173-4A) strain lack the GFP construct. Homothallic strains carrying the GFP construct, *h⁹⁰ rec8* (A3), *h⁹⁰ rec7* (A5) and *h⁹⁰ rec⁺* (AY176-17D), were transferred on sporulation media. Zygotes with two nuclei were identified by Hoechst 33342 staining, and the number of GFP signals was determined as described in Materials and Methods.

The separating GFP signals in Fig. 6 indicate that sister chromatids moved to opposite poles precociously at the first division. The segregation of sister chromatids also occurred in one phase.

Fidelity of chromosome segregation at meiosis I in *rec8* and *rec7* mutants

Next we tried to answer two questions: how frequently the separation of sister chromatids in *rec8* occurs and how efficiently the chromosome re-orientation works in the *rec7* mutant. To test the accuracy of the first meiotic division, we first stained the cells with Hoechst 33342 dye in order to identify zygotes having two nuclei. Then, the number of GFP signals in the daughter nuclei was determined as described in Materials and Methods.

To examine the separation of sister chromatids, crosses heterozygous for the GFP construct were applied. Crossing a *h⁻ rec8* strain carrying the GFP construct with a *h⁺* strain that lacked the construct revealed that precocious separation of sister chromatids at meiosis I occurred fairly regularly in this mutant (crosses heterozygous for GFP in Table 2). In Table 2, class 1 represents precocious separation of sister chromatids at the first meiotic division. Classes 2 to 4 represent regular segregation; in these divisions the labeled chromosome moved to one pole (classes 2 and 3) and started the second meiotic division (class 4). Precocious separation of sister chromatids was detected in 90% of the divisions observed in *rec8* heterothallic cross (class 1 in Table 2), whereas it was observed infrequently in the control cross (one out of 38 zygotes) and in *rec7* heterothallic cross (one out of 40 zygotes).

To examine the fidelity of chromosome disjunction (regular

segregation versus nondisjunction I), meioses in homothallic (*h⁹⁰*) strains were analyzed (crosses homozygous for GFP in Table 2). In a homothallic strain, segregation of sister chromatids to opposite poles is not distinguishable from segregation of homologs, therefore results obtained from *rec8* are not informative because of the high frequency of separation of sister chromatids. However, precocious separation of sister chromatids is rare in *rec7*, results obtained from *rec7* well reflect disjunction of homologs. In this mutant, classes 5, 6 and 8 are typical for a regular first meiotic division. In class 6, one of the chromosomes was represented by tightly associated signals of sister chromatids. Class 8 represented zygotes, which have already started the second meiotic division. Class 7 indicated that in *rec7* the coordination of second meiotic division between the daughter nuclei was impaired: only one of the daughter nuclei entered the second division. In wild-type strains this class was not observed. The segregation patterns of classes 5 to 8 arose from regular disjunction of homologs, while classes 9 and 10 showed nondisjunction of homologs at the first meiotic division. In *rec7*, nondisjunction I was observed in 36% of divisions analyzed. Precocious separation of sister chromatids occurred in the mutant with low frequency (class 11). This is consistent with results obtained from crossing heterothallic strains, where class 1 gave direct evidence for precocious separation of sister chromatids.

Sister chromatid cohesion was also examined during meiotic prophase nuclear movements. In a *rec8* heterothallic cross (same cross as in Table 2), horse-tail nuclei were selected after Hoechst 33342 staining and the number of signals determined in 40 nuclei. Two separated, but closely spaced signals were observed in three horse-tail nuclei (7.5%). Sister chromatids

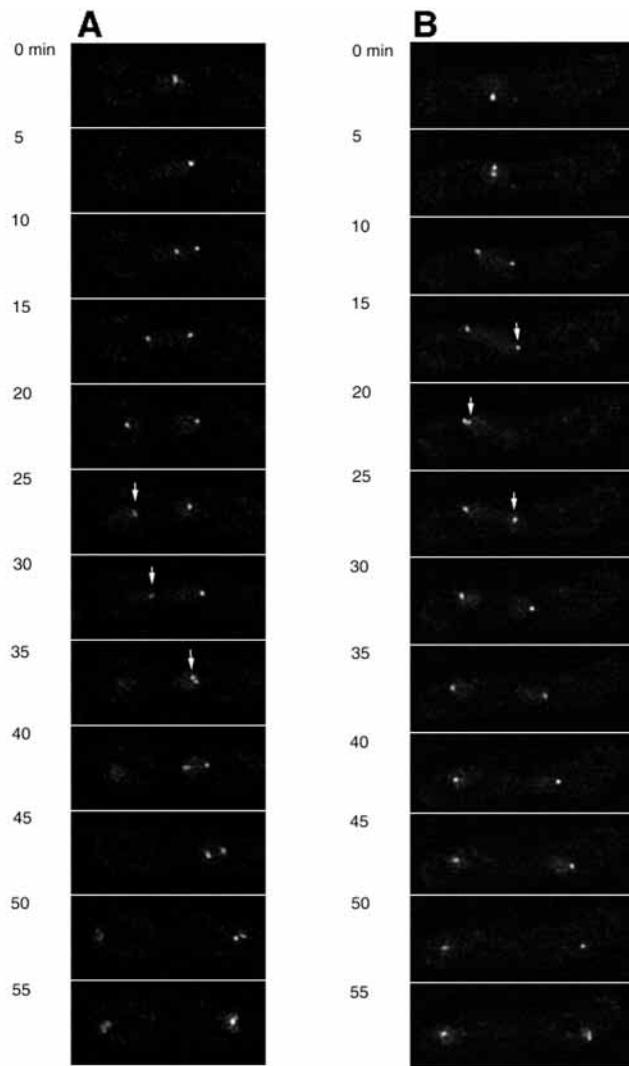


Fig. 5. GFP images of *cen1* in a *rec7* homothallic strain in meiosis I. Meiosis was induced in strain A5, and the divisions were observed as described in Materials and Methods. Numbers on the left of each image represent time in minutes. (A) GFP signals arising from homologous chromosomes which eventually moved to the same pole, resulting in nondisjunction I. The arrow points to the chromosome moving to the same pole as its homolog. (B) Chromosome re-orientation in *rec7*. The arrow points to the chromosome that moved to the same pole as its homolog and subsequently re-oriented to the other pole. Scale bar: 10 μ m.

were represented by tightly associated signals in 11 cases (27.5%). A single signal was observed in the remaining 26 nuclei (65.0%). In the control cross (*rec*⁺ heterothallic cross, same as in Table 2) either a single signal was observed (in 36 out of 40 nuclei) or signals of tightly associated sister chromatids were seen (in four nuclei).

Observation of GFP signals in a *rec7* homothallic strain (same as in Table 2) during horse-tail nuclear movements confirmed that pairing of centromere proximal regions takes place regularly in the mutant, and precocious separation of sister chromatids does not occur more frequently than in the wild-type strain (data not shown; see Molnar et al., 2001).

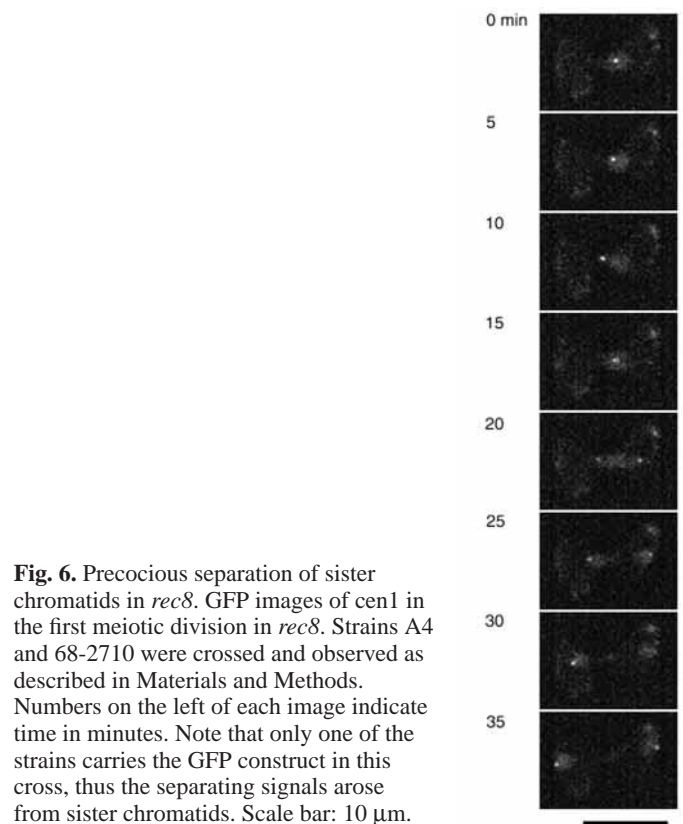


Fig. 6. Precocious separation of sister chromatids in *rec8*. GFP images of *cen1* in the first meiotic division in *rec8*. Strains A4 and 68-2710 were crossed and observed as described in Materials and Methods. Numbers on the left of each image indicate time in minutes. Note that only one of the strains carries the GFP construct in this cross, thus the separating signals arose from sister chromatids. Scale bar: 10 μ m.

Common features of meiosis in *rec7*, *rec14* and *rec15* mutants

Studying additional meiotic recombination deficient mutants revealed that the special features of meiotic divisions observed in the *rec7* mutant are typical not only for this mutant. The phenotype of the first meiotic division in the *rec14* and *rec15* mutants is virtually indistinguishable from that observed in *rec7* (Fig. 2B and data not shown). These mutants were chosen for study because they showed aberrations in meiotic prophase nuclear movements. The observed common features of the three mutants can be described as follows. Irregular distribution of the nuclear mass in the first meiotic division occurred frequently. This was detected in six divisions out of 10 observed in the *rec14* mutant, and in six cases out of 12 observations in the *rec15* mutant. During the first meiotic division the chromosomes moved back and forth between the cell poles. Eight divisions in the *rec14* mutant and 9 divisions in the *rec15* mutant showed this phenotype. Chromosome movements sometimes resulted in seemingly regular segregation (three and four cases in *rec14* and *rec15*, respectively), other times in apparently irregular distribution (five observations in both mutants). The first division lasted significantly longer than in wild-type cells (see Results above and data not shown). Interruption of meiosis after the first division was detected during live observation of all three mutants (data not shown).

In *rec7*, it was demonstrated genetically that interruption of meiosis led to the formation of two-spored asci (Molnar et al., 2001). To analyze sporulation in the mutants, zygotes were allowed to complete meiosis and the number of spores in mature asci was determined (see Materials and Methods). Fig.

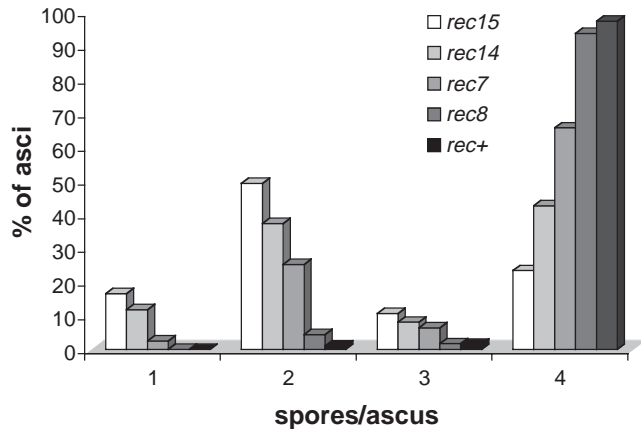


Fig. 7. Spore numbers in *rec+*, *rec8*, *rec7*, *rec14* and *rec15* zygotic asci. Strains CRL 152, 57-2262, 88-3485, A1 and A2 were transferred onto sporulation medium and the spore numbers determined by phase contrast microscopy in approximately 200 mature asci.

7 shows that frequent occurrence of two-spored asci was typical for *rec7*, *rec14* and *rec15*, and that this phenotype was most strongly expressed in *rec15*.

DAPI staining of nuclear material in the asci (Materials and Methods) revealed the final phenotypes of meiotic divisions in the mutants. Our observations on the ascus morphology and chromosome distribution are in agreement with those of Krawchuk et al. (Krawchuk et al., 1999): in *rec8*, regular four-spored asci were the predominant class (Fig. 7) but the spores frequently contained unequal amounts of nuclear material (Fig. 8B). We explain this terminal phenotype by the occurrence of a fairly regular, but equational first division followed by a random second division. Two-spored asci, which contained two large nuclei, were found in all the other mutants (Fig. 8C,D and E), in agreement with the observed interruption of meiosis. Interestingly, *rec7*, *rec14* and *rec15* also formed large spores, in which more than one DAPI-stainable body was enclosed. The distribution of nuclear material was rather unequal in all the three mutants.

DISCUSSION

Accurate distribution of chromosomes during the meiotic divisions is indispensable for the generation of viable progeny. To gain further insight into meiotic chromosome segregation in fission yeast, we studied meiotic recombination-deficient mutants by live observation. This approach has advantages over the classical tetrad analysis of chromosome segregation. Live observation of meiosis is possible, irrespective of whether the progeny are viable or not, and thus provides more reliable unbiased information about the accuracy and ways of divisions. Moreover, monitoring nuclear dynamics in vivo may reveal the mechanisms that govern the chromosome movements during the divisions.

Rec8 is required for proper nuclear movement during meiotic prophase

Live observation of different meiotic stages in *rec8* revealed that prophase nuclear movements are impaired in this mutant.

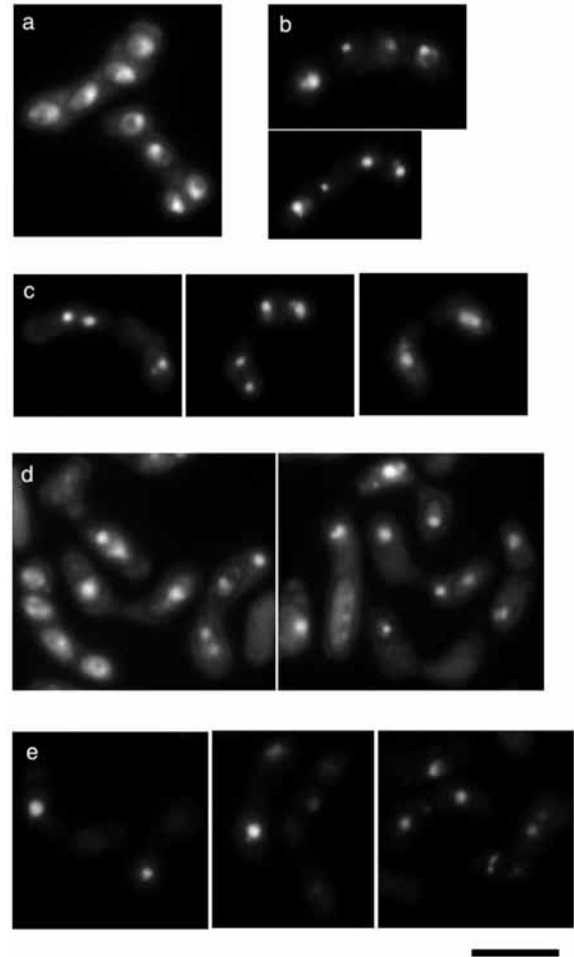


Fig. 8. Ascus formation in recombination-deficient mutants. Morphologies of *rec+* (a), *rec8* (b), *rec7* (c), *rec14* (d) and *rec15* (e) zygotic asci are shown after visualization of the DNA content by DAPI staining (see Materials and Methods). The same strains were used as in Fig. 8. In contrast to the uniform distribution of nuclear masses in the spores of *rec+* asci, each mutant had spores with variable DNA content. Note the large spores in *rec7*, *rec14* and *rec15* asci that enclosed more than one DAPI-stainable body. Scale bar: 10 μ m.

Only the leading end of the horse-tail nucleus moved, whereas the bulk nuclear mass remained in the cell center (Fig. 1B). However, both the dynamics and the duration of these movements were normal, suggesting that the observed phenotype is caused by a deficiency in the nuclear organization. A similar phenotype was observed in *taz1*, a mutant deficient in telomere clustering (Cooper et al., 1998; Hiraoka et al., 2000). Telomere clustering occurs regularly in *rec8* (Molnar et al., 1995), indicating that here another deficiency of the nuclear structure has led to the same phenotype. In *rec8*, the formation of linear elements is impaired (Molnar et al., 1995). An important role for these structures in regular meiotic chromosome organization has been proposed (Bähler et al., 1993; Kohli, 1994; Kohli and Bähler, 1994). We infer that the observed unusual shape of the prophase nucleus is due to the lack of regular linear elements. Linear elements provide scaffolding structures for the chromosomes, which may be necessary for driving the entire chromosomes during prophase nuclear movements.

Meiotic chromosome segregation in *rec8*

The first meiotic division showed basic differences in the *rec8* and *rec7* mutants. In *rec8* the division was regular (Fig. 2A), but mainly equational (Table 2). *rec8* encodes a meiotic cohesin which localizes to chromosomes in premeiotic S phase (Watanabe and Nurse, 1999) and completely dissociates only at anaphase II (Parisi et al., 1999; Watanabe and Nurse, 1999). In *rec8* mutants, impaired linear element formation, reduced meiotic chromosome pairing and, on prophase nuclear spreads, precocious separation of sister chromatids have been detected (Molnar et al., 1995). Watanabe and Nurse (Watanabe and Nurse, 1999) have shown that Rec8p has a role in the establishment of meiosis specific centromere structure; thus, it is required for reductional chromosome segregation at meiosis I. Data obtained from live monitoring of meiosis in the mutant are fully compatible with such a function.

Examination of cen1 GFP signals in horse-tail nuclei showed that the sister centromeres remain together in *rec8* during prophase, although their association is weaker than in wild-type strains (see Results). This may suggest the involvement of additional proteins in sister chromatid cohesion at the centromeres throughout prophase. Alternatively, the association of centromeres might be due to the regular centromere clustering in *rec8* (Molnar et al., 1995). Rec8p fulfills several functions during meiosis: (1) it establishes a meiosis-specific centromere structure, and (2) it contributes to sister chromatid cohesion at the centromeres and centromere-proximal regions of chromosomes. In the *rec8* mutant, the chromosomes are predisposed to undergo an equational division: their sister centromeres face to opposite poles, and they lack the cohesive function of Rec8p in the centromeric region. We detected equational chromosome segregation in 90% of divisions examined.

In *rec8* mutant strains, sister chromatid cohesion at the telomeric regions of chromosomes is likely to be maintained by the Rad21p mitotic cohesin (Watanabe and Nurse, 1999). Meiotic double strand breaks occur in *rec8*, although at strongly reduced levels (Cervantes et al., 2000). Meiotic recombination is practically abolished at the central regions of chromosomes, but a moderate recombination is still detectable at the telomere-proximal regions (Krawchuk et al., 1999; Parisi et al., 1999). These findings suggest that incidental meiotic recombination interfered with a perfect equational division in the *rec8* mutant, and resulted in regular reductional disjunction of chromosomes observed in the minority of divisions (Table 2).

An explanation for the common features of *rec7*, *rec14* and *rec15*: chromosomes segregate in an achiasmate first meiotic division

Live observation of meiosis I detected a lengthened first meiotic division in *rec7*, during which individual chromosomes seemed to change their position several times between the cell poles (Figs 2 and 5). *rec7* is an early meiotic recombination gene (Fox and Smith, 1998; Molnar et al., 2001). In *rec7*, meiotic double strand breaks have not been detected (Cervantes et al., 2000). Rec7p was localized in horse-tail nuclei in live observation, and on meiotic prophase nuclear spreads to about 50 foci per nucleus (Molnar et al., 2001). All these findings are consistent with a role for Rec7p in the initiation of meiotic recombination. A failure of initiation of meiotic recombination

obviously leads to lack of crossover formation, a severe overall reduction in meiotic recombination (DeVeaux and Smith, 1994; Molnar et al., 2001) and the lack of functional chiasmata.

We propose that the observed oscillation of chromosomes between the cell poles is a consequence of the lack of chiasma formation. Studies in higher eukaryotes have shown that mechanical tension stabilizes the proper chromosome configuration on the metaphase plate, and controls the cell cycle checkpoint (Nicklas et al., 1995; Nicklas, 1997). In meiosis, a stable configuration is achieved if chiasmata between the homologous chromosomes balance the pulling forces exerted on the kinetochores that are attached to opposite poles. The short-distance separation of homologous centromeres observed in the wild-type strain probably reflects this stable configuration (Fig. 4). Notably, this phase was not detectable in either *rec8* or in *rec7* (Figs 5 and 6). In the lack of stabilizing tension, chromosomes might detach from the spindle microtubules in *rec7*, and change their position until a new capture by microtubules or final migration to either cell pole occurs.

Studying meiosis I in *rec14* and *rec15* mutants has provided further support for this hypothesis. The phenotypes of first meiotic division in these mutants were virtually indistinguishable from that of *rec7*. *rec14* is a homolog of *REC103*, an early meiotic recombination gene in budding yeast (Evans et al., 1997; Fox and Smith, 1998). Meiotic double strand breaks have not been detected in *rec14* (Cervantes et al., 2000), and a severe reduction of meiotic recombination was measured in all the chromosomal intervals tested (Evans et al., 1997). In *rec15*, occurrence of meiosis initiator breaks have not been tested, and *rec15* shows no sequence homology to any other reported polypeptides (Lin and Smith, 1995). However, its transient induction at early meiotic stages, the observed severe reduction in meiotic recombination in the *rec15* deletion strain (Lin and Smith, 1995) and its additional common phenotypes with early genes (see Results) suggest that *rec15* is also an early meiotic recombination gene. We suggest that the common features of meiosis I in *rec7*, *rec14* and *rec15* can be attributed to the achiasmate segregation of homologs.

All achiasmate mutants showed two additional features: the frequent omission of meiosis II and enclosure of nuclear masses into huge spores. Persistence of Rec7p in the meiotic nuclei after meiosis I has been demonstrated (Molnar et al., 2001); thus, a direct role for Rec7p in the initiation of meiosis II is possible. Surprisingly, interruption of meiosis after the first division was detected in live observation of *rec14* and *rec15* as well; therefore we now propose an alternative explanation for the omission of meiosis II in the early recombination deficient mutants. A failure of initiation of meiotic recombination may trigger a block after meiosis I. This contributes to the improvement of spore viability by the omission of a frequently irregular second meiotic division. The block after meiosis I in recombination-deficient mutants is not complete. Finally, all achiasmate mutants formed two-spored asci, and occasionally enclosed several DAPI-stainable bodies into huge spores. Spindle pole body modification (a differentiation of the SPB into multipole structure which is necessary for the assembly of forespore membranes) usually occurs during meiosis II (Tanaka and Hirata, 1982). The two-spored asci and huge spores in the early *rec* mutants suggest that in these mutants a modification in spore formation might have occurred, in order

to ensure a better enclosure of the irregularly distributed chromosomes.

Fidelity of achiasmate chromosome segregation

In achiasmate meiosis, random segregation of homologous chromosomes at the first division is expected. Random segregation results in regular disjunction of homologs (50%) or in nondisjunction I (50%). For three pairs of chromosomes, a completely random distribution results in proper segregation only in 12.5% of divisions. Some of our observations indicate that the fidelity of achiasmate chromosome segregation in the recombination deficient mutants may exceed randomness. In *rec7*, nondisjunction of chromosome I was detected in only 36% of divisions examined (Table 2). Live observation of the first meiotic division detected apparently regular distribution of the nuclear mass in each mutant more frequently than it would be expected on random basis (see Results).

We consider two possible mechanisms that may contribute to the improvement of achiasmate chromosome segregation. First, occasional crossovers arising from the repair of spontaneously occurring double strand breaks or from meiotic double strand breaks may lead to a regular first division. Although meiosis initiator breaks have not been detected in these mutants (Cervantes et al., 2000) rare occurrence of DNA breaks that escaped the current detection level can not be excluded. Considering that severe reduction in meiotic recombination was measured in each mutant, this mechanism cannot be frequent. Second, a backup system may exist in fission yeast that improves the fidelity of segregation of achiasmate chromosomes. This may be achieved by triggering a checkpoint response that gives time for missegregating chromosomes to correct their error. In Fig. 5B we show that *S. pombe* is able to re-orient a chromosome if it moves to the same pole as its homolog. Chromosome wandering between the cell poles during the first division was common in each mutant. These divisions usually lasted longer than those where segregation happened to be regular. Although they resulted in apparently regular distribution more frequently than 12.5% of the divisions, it has to be noted that chromosome movements did not always lead to an improvement of segregation. Thus, the achiasmate backup system of fission yeast may work with low efficiency.

Meiosis is not an error free process. The presented data (Fig. 4, Table 2) show that both slight irregularities and obvious missegregation occur even in recombination-proficient, wild-type strains. Considering that mutation in a single gene may lead to achiasmate meiosis and that the consequences of aneuploidy is frequently death, to assume the operation of an achiasmate backup system in fission yeast is an appealing hypothesis. Distributive disjunction has been detected also in *S. cerevisiae*, another organism highly proficient in meiotic recombination (Dawson et al., 1986; Guacci and Kaback, 1991; Loidl et al., 1994).

What mechanism re-orient the chromosomes in meiosis I? Does the meiotic spindle checkpoint play as crucial a role in meiotic chromosome segregation in fission yeast as it has been demonstrated in budding yeast (Shonn et al., 2000)? The meiotic spindle checkpoint in fission yeast has not been characterized. Is the second meiotic division really coupled to the initiation of meiotic recombination? Experiments to clarify the answers to these questions are in progress.

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