

# Meiotic Chromosome Synapsis in Yeast Can Occur Without Spo11-Induced DNA Double-Strand Breaks

Hasanuzzaman Bhuiyan and Karin Schmekel<sup>1</sup>

Department of Molecular Biology and Functional Genomics, Stockholm University, SE-106 91 Stockholm, Sweden

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## ABSTRACT

Proper chromosome segregation and formation of viable gametes depend on synapsis and recombination between homologous chromosomes during meiosis. Previous reports have shown that the synaptonemal complexes (SCs), do not occur in yeast cells with the *SPO11* gene removed. The Spo11 enzyme makes double-strand breaks (DSBs) in the DNA and thereby initiates recombination. The view has thus developed that synapsis in yeast strictly depends on the initiation of recombination. Synapsis in some other species (*Drosophila melanogaster* and *Caenorhabditis elegans*) is independent of recombination events, and SCs are found in *spo11* mutants. This difference between species led us to reexamine *spo11* deletion mutants of yeast. Using antibodies against Zip1, a SC component, we found that a small fraction (1%) of the *spo11* null mutant cells can indeed form wild-type-like SCs. We further looked for synapsis in a *spo11* mutant strain that accumulates pachytene cells (*spo11Δ ndt80Δ*), and found that the frequency of cells with apparently complete SC formation was 10%. Other phenotypic criteria, such as spore viability and homologous chromosome juxtaposition measured by FISH labeling of chromosomal markers, agree with several previous reports of the *spo11* mutant. Our results demonstrate that although the Spo11-induced DSBs obviously promote synapsis in yeast, the presence of Spo11 is not an absolute requirement for synapsis.

**M**EIOTIC chromosome pairing and recombination are two processes that are generally necessary for proper segregation of homologous chromosomes and mixing of parental genomes in the first meiotic division. Both processes are closely linked to the appearance of a proteinaceous structure, the synaptonemal complex (SC), which forms between the homologs along their entire length (synapsis; reviewed in ROEDER 1997). Although valid for a large majority of eukaryotes, the simple and straightforward view that all the meiotic events (pairing, recombination, and synapsis) are needed to assure proper chromosome segregation is not true for all organisms. There are examples of accurate chromosome division without either synapsis or recombination. In fission yeast, *Schizosaccaromyces pombe*, both crossing over and correct chromosome segregation can be achieved without SCs. In *Bombyx mori* females, a modified SC can act as glue between the homologs and thus assure proper chromosome segregation in the absence of crossing over (RASMUSSEN 1976). It is even possible to accomplish proper segregation without either synapsis or recombination: in *Drosophila*, homologous chromosomes that have not recombined can be paired and segregated by a mechanism called distributive segregation, in which

heterochromatic pairing plays an important role (reviewed by WALKER and HAWLEY 2000).

In organisms that require pairing, recombination, and synapsis for normal chromosome segregation there are examples of mutants with obstructed, but not blocked, meiotic progress. In a mutant strain of budding yeast that lacks a major component of the SC, Zip1, some recombination still occurs, showing that the SC is not absolutely required for recombination in yeast (SYM *et al.* 1993; STORLAZZI *et al.* 1996). The spore viability in the *zip1* null mutants is 60%, showing that the chromosome segregation is reasonably good (TUNG and ROEDER 1998). While it seems as if the SC is not required for recombination, it has been generally believed that the initial step of recombination, the formation of double-strand breaks (DSBs) in the DNA, is an absolute prerequisite for successful synapsis in yeast (reviewed in KEENEY 2001). DSBs are generated by a topoisomerase-related meiosis-specific enzyme called Spo11 (SUN *et al.* 1989; CAO *et al.* 1990; BERGERAT *et al.* 1997; KEENEY *et al.* 1997). Together with Spo11, at least 10 other genes are needed for initiation of recombination by DSB formation in yeast (reviewed in KEENEY 2001). Studies show that the sites on the chromosomes where recombination is initiated by DSBs are also the sites where synapsis starts. Several recombination enzymes colocalize with the first synaptic protein, Zip3, which recruits the proteins Zip2 and Zip1 that complete synapsis (AGARWAL and ROEDER 2000). The amount of Spo11-induced DSBs in different

<sup>1</sup>Corresponding author: Department of Molecular Biology and Functional Genomics, Stockholm University, SE-106 91 Stockholm, Sweden.  
E-mail: karin.schmekel@molbio.su.se

*spo11* mutants has been correlated with the level of SC formation, showing that initiation of synapsis is indeed induced by DSBs (HENDERSON and KEENEY 2004). In this study it was also shown that the number of Zip3 sites decreases if the frequency of DSBs decrease.

Both yeast and mouse *spo11* null mutants are defective in DSB formation as well as synapsis (GIROUX *et al.* 1989; WEINER and KLECKNER 1994; MAHADEVIAIAH *et al.* 2001). There are, however, observations of SC-like structures in both mouse and yeast *spo11* mutants. In yeast, traces of SCs have been reported in *spo11* null mutants, and there is an observation of complete SC formation in a yeast strain with a *spo11* point mutation (*spo11-I*; KLAPHOLZ *et al.* 1985; LOIDL *et al.* 1994; MALKOVA *et al.* 2000). In contrast to the yeast and mouse phenotype, neither the Spo11 protein nor DSBs are required for synapsis in *Drosophila melanogaster* and *Caenorhabditis elegans* (DERNBURG *et al.* 1998; MCKIM *et al.* 1998). Thus the formation of DSBs by Spo11 promotes synapsis in, *e.g.*, yeast and mouse, while this system does not seem to be necessary in some other organisms.

The differences in the synapsis and initiation of recombination processes between species, and the uncertainty about the yeast phenotype, led us to reinvestigate the yeast *spo11* mutant. We have examined *spo11* mutants of *Saccharomyces cerevisiae* using immunofluorescence with the aim of gaining a better understanding of the relationship between the initiation of meiotic recombination and synapsis in yeast. We have found complete and homologous synapsis (SC formation) in a small fraction of the cells (1%). In a *spo11* mutant that arrests in pachytene (*spo11Δndt80Δ*), we found a 10-fold increase of cells with full SC complement compared to the single mutant. Other aspects of the *spo11* mutant phenotype fit with data previously published. Spore viability is close to zero. A moderate but notable level of colocalization of both subtelomeric and interstitial parts of homologous chromosomes occurs in the *spo11* mutant cells, which is in agreement with several previously published reports (LOIDL *et al.* 1994; WEINER and KLECKNER 1994; PEOPLES *et al.* 2002). Although initiation of recombination via Spo11 normally precedes synapsis and clearly strongly catalyzes SC formation, the present result demonstrates that chromosome synapsis in yeast can occur in the absence of Spo11.

## MATERIALS AND METHODS

**Strains:** The yeast (*S. cerevisiae*) strains used in this study are as follows: NKY2967 (a/α ho::LYS2/ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG his4X::LEU2/his4B::LEU2 *spo11Δ*::hisG-URA3-hisG/*spo11Δ*::hisG-URA3-hisG), NKY1551 (a/α ho::LYS2/ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG arg4-Nsp/arg4-Bgl his4X::LEU2(Bam)-URA3/his4B::LEU2), NKY2310 (a/α ho::LYS2/ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG *ndt80Δ*::LEU2/*ndt80Δ*::LEU2 his4X::LEU2-MluI-URA3/his4B::LEU2-MluI), NKY 2535 (a/α ho::LYS2/ho::LYS2 lys2/lys2 leu2::hisG/leu2::hisG ura3/ura3 *spo11Δ*::hisG-URA3-hisG/

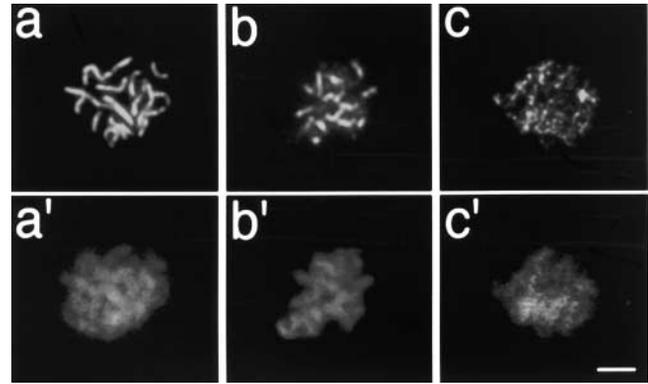


FIGURE 1.—Three classes of Zip1 staining based on the staining pattern illustrated in the NKY1551 wild-type strain. Full complement of SCs (a), SC-like structures (b), and diffuse Zip1 staining (c). DNA is outlined by DAPI (a', b', and c'). Bar, 4  $\mu$ m.

*spo11Δ*::hisG-URA3-hisG *ndt80Δ*::LEU2/*ndt80Δ*::LEU2), and S2888 (a/α his4-280/ his4-260 leu2-27/leu2-3,112 arg4-8/+ thr1-1/thr1-4 cyh10/+ ade2-1/ade2-1 ura3-1/ura3-1 trp1-1/trp1-289 *spo11-ADE2*/*spo11-ADE2*).

**Cell growth:** The cells were cultured as described in SCHMEKEL (2000). Briefly, yeast strains were grown to saturation in YPAD medium and then diluted 1:200 in presporulation medium (2% Bacto-peptone, 1% Bacto-yeast extract, 1% potassium acetate, and 0.003% adenine sulfate) and allowed to grow to a density of  $1.5\text{--}2.0 \times 10^7$  cells/ml. The cells were then transferred to sporulation medium with a density of  $3.0\text{--}4.0 \times 10^7$  cells/ml. Unless otherwise mentioned, the cells were grown at 30° and harvested after 5 or 8 hr.

**Immunofluorescence and fluorescence *in situ* hybridization:** Immunofluorescence experiments were carried out with spread preparations on glass slides (LOIDL *et al.* 1998) as described in BHUIYAN *et al.* (2003). The Red1 antibody, which was a kind gift from Shirleen Roeder's laboratory, was used in dilution 1:1000 (SMITH and ROEDER 1997). For antibody signal strength comparison, the Zip1 antiserum published in SYM and ROEDER (1995) was used. Fluorescence *in situ* hybridization (FISH) was carried out according to WEINER and KLECKNER (1994) with minor modifications (described in BHUIYAN *et al.* 2003), using one probe that binds to an  $\sim 35$ -kb-long subtelomeric region of chromosome III (c9171; American Tissue Culture Collection (ATCC) no. 70884) and another probe that binds to an  $\sim 26$ -kb-long interstitial part of chromosome VIII (c9315; ATCC no. 71216). Images from fluorescence and confocal microscopy were processed using Adobe Photoshop.

**Spore viability:** Both wild-type (NKY1551) and *spo11* mutant (NKY2967) strains were sporulated for 3 days. Tetrads were dissected using a Singer MSM System Series 200 dissecting apparatus and spores were allowed to grow on YPAD plates.

## RESULTS

**A fraction of *spo11* mutant cells exhibit wild-type-like SCs:** To evaluate the level of SC formation, we performed immunofluorescence experiments on spread nuclear preparations of a *spo11* deletion mutant (NKY2967) and on the wild-type strain (NKY1551). SC structures were identified using a polyclonal antibody that recognizes the Zip1 protein (the antiserum is described in BHUIYAN *et al.* 2003). We classified the different appearances of

TABLE 1

Percentage of cells with different Zip1-staining patterns (classes A–D) at pachytene (5 hr in sporulation medium)

Strains	Class A	Class B	Class C	Class D	N
<i>spo11Δ</i>	1	25	47	27	450
Wild type	23	24	35	18	400

Class A, wild-type SC pattern; class B, SC-like structures; class C, diffuse anti-Zip1 staining; D, anti-Zip1 negative nuclei; N, number of cells counted.

SC staining into four classes, A–D. The SC staining pattern that fully resembled the wild-type SC pattern (Figure 1a) is class A. We believe that the SCs in these cells correspond to homologously synapsed chromosomes. Each cell contained  $\sim 16$  SCs, we could usually discern most of the individual bivalents, and we rarely saw structures that could correspond to forked SCs. The class A Zip1-staining pattern was found in 1% of the *spo11* mutants harvested after 5 hr in sporulation medium, which is the time when the highest portion of wild-type cells are in pachytene (see Table 1 and Figure 2, a–c). Many of the mutant nuclei appeared with what we called an “SC-like” staining pattern (class B; Figure 1b). The Zip1-staining structures in these nuclei were short, often faint, and discontinuous. In addition, a large fraction of the *spo11* mutant nuclei showed a more diffuse staining (class C, Figure 1c). Nuclei that lacked anti-Zip1 staining were placed into class D. We saw the same patterns of Zip1 staining in the wild-type nuclei, although in this case the frequencies were different (Table 1).

Yeast cells that lack the *SPO11* gene are not affected by meiotic checkpoint arrest or delay, and they sporulate after incomplete meiosis. Because the cell populations are not in perfect synchrony, only a fraction of the cells are in fact in pachytene at the “pachytene time point.” We realized that the number of SC-forming cells in the *spo11* mutant for this reason may have been underestimated and wanted to investigate how many cells were able to create full synapsis totally. We therefore examined the level of SC formation in the double mutant *spo11Δndt80Δ*, in which pachytene cells accumulate. Ndt80 is a transcription factor needed for meiotic cell-cycle progress, and *ndt80* mutants arrest in pachytene (Xu *et al.* 1995). Cells were collected after 5 and 8 hr in sporulation medium. We found that 10% of the cells in the double-mutant strain formed wild-type SCs (class A) after 5 hr in sporulation medium and 11% after 8 hr (Figure 2, d–f). This is a 10-fold increase compared to the single *spo11* mutant and is one-sixth of the SC content in *ndt80Δ* cells in which  $\sim 66\%$  of the cells in these experiments form class A SCs. With time, class B cells increased more than class A cells in frequency, while the class C cells decreased in relative numbers. A summary of the SC frequency data in *spo11Δ*, *spo11Δndt80Δ*, *ndt80Δ*, and wild type is given in Table 2.

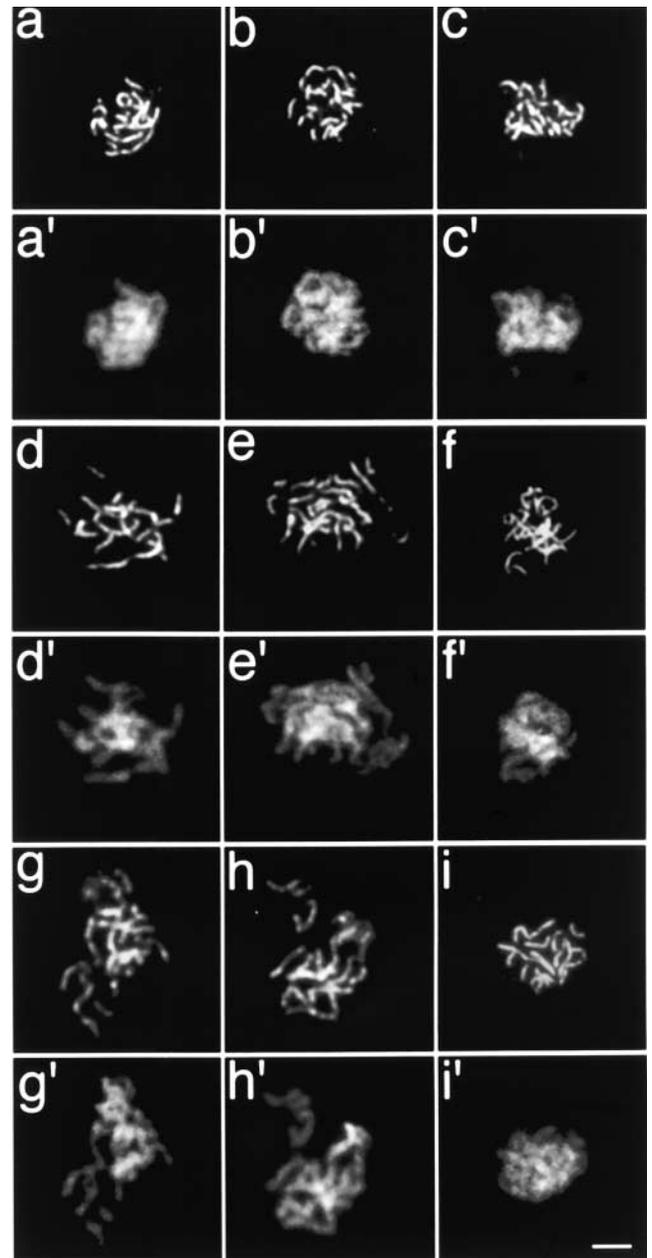


FIGURE 2.—SC structures based on Zip1 staining in spread preparations at pachytene. (a–c) Full complement of SCs in the *spo11* mutant (NKY2967). (d–f) Full complement of SCs in the *spo11ndt80* double mutant (NKY2535). (g–i) Full complement of SCs in wild-type (NKY1551) cells (g and h show average appearance of class A nuclei, while i is an example of an “exceptionally good-looking” nucleus). Corresponding DNA staining is shown in a'–i'. For information on frequency of different classes in the different strains, see Table 2. Bar, 4  $\mu$ m.

The occurrence of SCs in the *spo11* mutant cells was also investigated using an antibody against another SC protein, Red1, which is located in the lateral elements of the SC. The immunolabeling of the SCs in wild-type cells with the Red1 antibody appeared similar to the labeling with the Zip1 antibody, like a group of ribbons. However, since the Red1 antibody stains both axial ele-

TABLE 2

Percentage of cells with different Zip1-staining patterns (classes A–E) after different periods of time in sporulation medium

Strain	Hour:	Class A				Class B				Class C				Class D				Class E:
		3	4	5	8	3	4	5	8	3	4	5	8	3	4	5	8	8
<i>spo11Δ</i>	—	1	1	1	5	14	25	9	80	68	47	19	15	17	27	28	43	
<i>spo11Δndt80Δ</i>	—	7	10	11	23	40	51	58	65	41	28	18	12	12	11	13	—	
<i>ndt80Δ</i>	—	10	35	66	17	32	27	24	70	46	29	3	13	12	9	7	—	
Wild type	—	17	23	8	8	25	24	21	78	38	35	23	14	20	18	26	22	

Cells harvested after growth for different time spans in sporulation medium. Between 400 and 450 nuclei/category were recorded. A, wild-type SC pattern; B, SC-like structures; C, diffuse anti-Zip1 staining; D, anti-Zip1 negative nuclei; E, spores.

ments and synapsed chromosomes, and the Red1 staining often is more discontinuous than the Zip1 staining, we could not perform the classification analysis that was done using the Zip1 antibody. A fraction of the Red1-stained nuclei showed more continuous SCs. Complete synapsis is more reliably confirmed if the SC labeling is continuous, and this class was used for investigation of the presence of Red1 staining SCs in the *spo11* mutant cells. The Red1 antibody stained a fraction of the *spo11ndt80* double-mutant cells in the characteristic wild-type-like SC pattern (Figure 3, a vs. b), a result that supports the finding of SCs in *spo11* mutant cells.

The literature concerning the phenotype of *spo11* mutants in yeast is not consistent as to whether any SCs are formed. We were concerned that our result would not be due to SC formation that had been artificially introduced into the cells. Exogenously induced DSBs may give rise to such artifactual SCs. We have taken extensive

measures to detect any such effect. For example, we have changed all components in the growth media and cell preparation solutions, including the water source, to other brands; we have cultivated the cells on premises other than our own laboratory; we have grown cells in the dark; we have received new cells and restarted the cell strains; and we have measured the radiation in the laboratory. The SCs were present under all of these conditions.

SCs have also been observed in another *spo11Δ* strain (S2888 of BR background; data not shown) using the Zip1 antibody. The yeast strains used in this study are all widely used, well defined, and described in MATERIALS AND METHODS (for source see Acknowledgment).

**Several other aspects of the *spo11* mutant phenotype are in agreement with those previously described:** The phenotype of *spo11Δ* includes aneuploidy and inviable spores, due to missegregation of homologs. The number of viable *spo11Δ* spores would probably increase if DSBs were introduced. We therefore performed spore viability tests to determine the phenotype of the *spo11* mutant cells under the conditions that we use. Tetrads of wild type and *spo11Δ* were dissected and allowed to grow on YPAD. The spore viability of the *spo11Δ* cells was 0.2% ( $n = 440$ ) and that of the wild-type cells was 96.0% ( $n = 360$ ).

We performed FISH experiments using chromosome-specific DNA probes on cells that were surface spread to determine the extent to which homologous chromosomes had been brought into juxtaposition (pairing). One probe, derived from a cosmid clone (ATCC no. 70884), recognizes one end of chromosome III and another probe, ATCC no. 71216, recognizes an interstitial part of chromosome VIII. Wild-type cells were used as positive controls. Unpaired homologs appear as two separate dots, while two homologs that are paired appear very close together and often fuse into one dot. Homologs were classified as paired if the two FISH signals were fused (or separated from each other by  $<0.7 \mu\text{m}$ ; WEINER and KLECKNER 1994). Table 3 shows that the subtelomeric probe was highly paired in both *spo11* mutant strains independent of time of harvesting. The

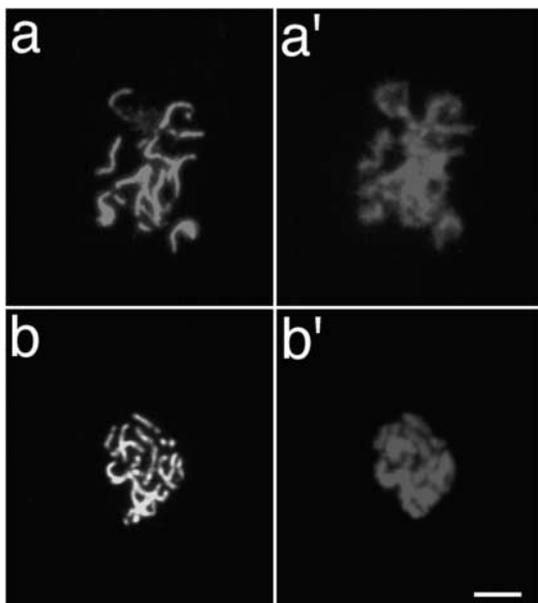


FIGURE 3.—SC in spread preparations at pachytene, stained with the Red1 antibody (a and b) and DAPI (a' and b'). (a and a') Wild-type-like SCs in the *spo11ndt80* double mutant (NKY2535). (b and b') Wild-type (NKY1551) cells. Bar, 6  $\mu\text{m}$ .

TABLE 3

Pairing of chromosomes investigated by hybridization with subtelomeric *vs.* interstitial probes

Strain	Probe chromosome position	Harvesting time (hr)	1 dot (%)	2 dots (%)	Background labeling (%)		% pairing after subtracting background	N
					>2 dots	0 dots		
<i>spo11Δ</i>	Subtelomeric	5	40	46	9	5	47	415
<i>spo11Δndt80Δ</i>	Subtelomeric	5	45	40	8	7	53	224
<i>spo11Δndt80Δ</i>	Subtelomeric	8	46	40	6	8	53	181
Wild type	Subtelomeric	5	47	37	9	7	56	180
<i>spo11Δ</i>	Interstitial	5	23	64	4	9	26	287
<i>spo11Δndt80Δ</i>	Interstitial	5	28	57	7	9	33	172
<i>spo11Δndt80Δ</i>	Interstitial	8	30	59	3	7	34	174
Wild type	Interstitial	5	48	30	12	10	62	129

The percentage of mutant and wild-type cells undergoing homologous chromosome pairing and nonpairing in FISH experiments with subtelomeric (ATCC no. 70884) and interstitial (ATCC no. 71216) probes. Both mutant and wild-type cells were analyzed at pachytene. We judged pairing to have taken place if the two FISH signals were separated by  $<0.7 \mu\text{m}$ .

pairing was almost as high in the mutants as in the wild-type cells. The number of fused signals of the interstitially situated probe was lower than that of the subtelomeric probe (26% in *spo11Δ* and 34% in *spo11Δndt80Δ*), but considerably higher than what can be expected if no homologs are paired. A summary of the chromosome pairing data is given in Table 3.

#### SC formation depends on physical growth conditions:

The difference between our results showing that the SCs form in *spo11* mutant cells and those in previous reports may arise from technical differences in the preparation of the cells. It is possible that slight differences in growth conditions give rise to differences in SC occurrence. We have therefore altered the growth conditions to determine the effect of these conditions on SC occurrence. We cultivated *spo11Δndt80Δ* and wild-type cells under different conditions (26°, 30°, and 34°, in five times higher and five times lower cell concentration than normal, and in two different incubators), harvested the cells at the time for normal pachytene, and recorded differences in SC frequency and sporulation frequency (Table 4). Almost all variations from the standard conditions gave considerably lower SC frequency (in the order of 10–50% of normal SC frequency). Low cell concentration had the greatest effect on the SC count. The low number of cells with SCs in some cases was due to an obvious inability to develop meiotic cells (which resulted in low sporulation frequency) while in other cases it was due to delayed or accelerated meiotic development (resulting in normal sporulation frequency). Surprisingly, two different shaking incubators gave a significantly different SC count. Growth conditions thus clearly influence the occurrence of pachytene cells at a certain time point and sporulation. Notably, these experiments were performed using the double mutant that under optimized conditions has SCs in 10% of the cells. Although the SCs were never totally abolished in these preparations, the same degree of reduction in SC count could

be expected in the single *spo11* mutant, which would result in  $\sim 0.1$ – $0.5\%$  cells with SCs. This level could possibly be considered “nondetectable.”

## DISCUSSION

**SCs can form in *spo11* null mutants:** The Spo11 protein forms DSBs that act as initiation sites for recombination. It seems as if formation of DSBs by Spo11 promotes pairing as well as synapsis (*e.g.*, yeast, KEENEY *et al.* 1997; and mouse, MAHADEVAIAH *et al.* 2001) if homologous chromosomes are not brought together by other mechanisms, like prealignment of chromosomes as in *Drosophila* (*e.g.*, FUNG *et al.* 1998) or meiotic pairing centers as in *C. elegans* (VILLENEUVE 1994). The fact that homologous chromosomes may be synapsed in the absence of Spo11 in some organisms suggests that DSBs do not take active part in the mechanism of synapsis. It is not clear whether any isolated recombination step is directly involved in initiation of synapsis in yeast. Nevertheless, in yeast the chromosomes are synapsed temporally just after the DSB formation (PADMORE *et al.* 1991). Early as well as late recombination enzymes in yeast, like Rad51, Dmc1, and Msh4, may be removed with the chromosomes still capable of synapsis, although the process may be delayed (ROCKMILL *et al.* 1995; NOVAK *et al.* 2001). The removal of yeast Spo11 or any of at least four other proteins that are necessary for DSB formation, on the other hand, has been reported to completely block SC formation (reviewed by BURGESS 2002). Although observations of yeast clearly show that SC formation is strongly promoted by Spo11-induced DSBs, *spo11* null mutants occasionally form SC segments (LOIDL *et al.* 1994) or SC-like structures (0.2–1% of recorded cells; MALKOVA *et al.* 2000). Furthermore, KLAPHOLZ *et al.* (1985) found wild-type-like SC structures in the nuclei of a *spo11* mutant (*spo11-1*) by electron microscopy. However, *spo11-1*

**TABLE 4**  
**Cells with complete SCs (class A) in  $\Delta spo11/\Delta ndt80$  and wild type grown under different physical conditions and sporulation frequency**

Strain	26°:		34°:				N
	Water shaker	Water shaker	Water shaker	Dry shaker:	Water shaker		
	Normal cell concentration	Normal cell concentration	Normal cell concentration	Normal cell concentration	5 × cell concentration	1/5 × cell concentration	
% $\Delta spo11\Delta ndt80$ cells with SCs	2	11	10	5	8	1	98–112
% wild-type cells with SCs	10	7	21	14	9	4	100–138
Sporulation frequency in wild-type cells (%)	70	3	74	74	3	73	124–165

Growth in sporulation medium, at different temperatures, shaking at 160 rpm in water bath or at 250 rpm in dry shaker, at normal cell concentration (see MATERIALS AND METHODS), at 5 times higher cell concentration, or at 1/5 of normal cell concentration. Harvested after 5 hr in sporulation medium. The data are results from one experiment. N, number of cells counted.

is a point mutation and it is unclear how strongly Spo11 function is affected.

Contrary to previously published results, we have found that a small fraction of pachytene *spo11* null mutant cells have fully synapsed chromosomes. Thus, even if the Spo11 DSBs are strong promoters of synapsis, and synapsis does not occur efficiently in the absence of DSBs, our result demonstrates that synapsis in yeast may occur without Spo11 and probably without DSBs. We were concerned with the fact that we reproducibly found small fractions of wild-type-like SCs while other investigators have not detected SCs in *spo11* mutant cells. One possible artifact could be exogenously introduced DSBs. These DSBs would induce recombination and possibly SC formation and thus lead to a higher frequency of cells that undergo proper segregation, resulting in an elevated frequency of spore viability. In a study in which  $\Delta spo11$  cells were moderately X-ray irradiated (15 krad), spore viability increased 40 times, which was 3% of the nonirradiated wild-type level (THORNE and BYERS 1993). We expected the spore viability level to be at least somewhat elevated compared to previously reported levels if we had induced DSBs in our samples. Our spore viability tests, however, showed lower viability than previously reported (0.2% vs. 1%; KLAPHOLZ and ESPOSITO 1982; WEINER and KLECKNER 1994). We thus believe that the SC formation that we have found is not due to exogenously introduced DSBs.

*spo11* yeast mutants go through meiosis but produce aneuploid spores. Because of poor cell synchrony and transient synapsis, only a fraction of the SCs that are totally produced during meiosis are present in a cell preparation at a given time point. To get an estimate of the total number of *spo11* mutant cells that are able

to synapse their homologous chromosomes, we used a *spo11* mutant that arrests in pachytene (*spo11* $\Delta ndt80\Delta$ ). We found a 10-fold increase of SC-forming cells in this strain compared to the single *spo11* mutant strain. Surprisingly, we found that the double mutant showed almost the same number of SC-forming cells after 5 hr as after 8 hr in sporulation medium (10% vs. 11%). Probably this is due to the “pachytene peak” occurring somewhat earlier than at 5 hr in these experiments and to the fact that the double mutant already has accumulated most pachytene cells before 5 hr. Since the *spo11* single mutants only transiently synapse, and thus have only a small number of SC-forming cells at a certain time (1%), it seems reasonable that the total number of cells that have synapsed during meiosis is the same in the single *spo11* mutant and in the double-mutant populations (10%).

***spo11* mutants show some homologous pairing, but results vary:** We, as well as several other investigators, have measured the degree of juxtaposition of homologous chromosome loci in yeast *spo11* mutants by FISH (here called pairing; LOIDL *et al.* 1994; WEINER and KLECKNER 1994; CHA *et al.* 2000; NEALE *et al.* 2002; PEOPLES *et al.* 2002). The absolute level of pairing varies between the investigations. CHA *et al.* (2000) and NEALE *et al.* (2002) did not find any meiotic pairing in *spo11* deletion mutants, whereas the other studies show moderate capacity of *spo11* mutant cells to bring homologs together. Subtelomeric markers seem to show significantly higher pairing level [using the same probe, WEINER and KLECKNER (1994) find 42% pairing while the present study shows 47% pairing in *spo11* mutants]. Using interstitial probes, different reports show different levels of pairing in *spo11* yeast mutants, probably

depending on the location of the probe: 4, 12, and 14% (WEINER and KLECKNER 1994); 9% (LOIDL *et al.* 1994); 26% (this report); and 41–46% in a double-mutant *spo11Δndt80Δ* (PEOPLES *et al.* 2002). Interestingly, Loidl and coworkers found that the level of pairing rose from 9% in all *spo11* mutant cells to 26% if only pachytene cells were counted. Together these reports indicate that meiotic *spo11* mutant cells to some extent are able to bring homologs into juxtaposition. We do not believe that the pairing that we see is remnant Spo11 independent premeiotic pairing because in the strain background that we use (SKI) disruption of premeiotic pairing occurs after 2.5 hr in sporulation medium (CHA *et al.* 2000), and we harvest our cells well after this time, after 5 hr in sporulation medium. The premeiotic pairing explanation seems especially unlikely since we do not see any difference in pairing level in the double-mutant cells harvested after 5 hr and those harvested after 8 hr in sporulation medium. The bottom line is that the pairing phenotype of the *spo11* mutant shown in this report does not significantly differ from previously published reports.

It is also interesting that PEOPLES *et al.* (2002) find pairing levels that are 4 to 10 times higher than what has been reported for the single-mutant *spo11Δ* (LOIDL *et al.* 1994; WEINER and KLECKNER 1994; this report) when they perform FISH pairing measurements on the double-mutant *spo11Δndt80Δ*. Thus, the pairing pattern of the *spo11Δndt80Δ* vs. the *spo11Δ* cells are in accordance with the SC-forming cell pattern that we see in our experiments; prolonged pachytene, which is achieved by cell-cycle arrest in the *ndt80* mutant cells, leads to an elevated incidence of both pairing and synapsis in *spo11* mutants in the absence of Spo11. It should, however, be noted that in the study of NEALE *et al.* (2002), where a *spo11ndt80* double-mutant strain was used, no SCs were found.

**Variations in preparation may lead to different results:** The difference between our results that show SC formation in *spo11* mutant cells and other reports where SCs were not detected may arise from technical differences in the preparation of the cells. Although the different meiosis laboratories use similar methods, it is possible that slight differences in growth conditions could give rise to differences in SC occurrence or detection level. BÖRNER *et al.* (2004) show that the ability of several recombination mutants to carry through both synapsis and recombination steps is temperature dependent. In our experiments, altered temperatures, cell concentrations, and change of incubator resulted in lower SC counts. In these experiments the double-mutant *spo11Δndt80Δ*, which under normal conditions contains a relatively large amount of SC-containing cells (10%), was used. So, although SCs were present to some extent in preparations from all conditions, the interesting observation is that almost all alterations resulted in only 10–50% of normal SC count. The same level of reduction can be expected in *spo11* single-mutant cells. In these

cells we saw 1% SC-containing cells under normal growth conditions, and reduction in the same order may be considered undetected. Another reason for the contradictory result may be the quality of the antibody. Different Zip1 antisera give signals of different strength. In a comparison with another Zip1 serum, we found our antibody to give a particularly strong signal (data not shown). The differences in SC result in the *spo11* mutant may also be due to differences in nuclear spreading and detection techniques. We always screened at least five slides, all with cells spread using different quantities of the paraformaldehyde and the Lipsol solutions (see MATERIALS AND METHODS), and all samples were screened using the Zip1 antibody, which we have found more reliable than silver staining.

**DSB-independent pairing may aid synapsis:** Several studies show involvement of telomeres in the pairing of homologs during meiosis. Prior to pairing of the entire chromosomes, telomeres often cluster in one area on the nuclear envelope (bouquet), a process that in yeast is mediated by the telomere-binding protein Ndj1 (TRELLES-STICKEN *et al.* 2000). ROCKMILL and ROEDER (1998) show in a genetic study that telomeres as well as ends of chromosomes interact to promote homolog pairing. Further, it has recently been shown that telomerase-deficient mice fail in synapsis and recombination (LIU *et al.* 2004). It is interesting to note that *spo11* mutants show a considerably higher level of homologous pairing in telomeres than in interstitial sites of chromosomes monitored by FISH (WEINER and KLECKNER 1994; this study). It thus seems as if homologous pairing of telomeres is much less dependent on DSB formation than are the interstitial parts of the chromosomes. It is possible that the telomere pairing facilitates the SC formation that we detect in our *spo11* preparations.

WEINER and KLECKNER (1994) show that homologous chromosomes pair before meiotic S-phase, but disassociate during replication and then reassociate as a first step in the meiotic pairing process. PEOPLES *et al.* (2002) could distinguish stable meiotic from unstable premeiotic pairing by the detection of “close, stable homolog juxtaposition” in a Cre/loxP-based recombination assay, which requires close chromosome contact. Several recombination mutant strains that were investigated for the Cre/loxP interaction showed much lower than wild-type levels of contact between the homologs during meiosis. Not surprisingly, the *spo11* null mutant strain showed the lowest recombination frequency in the assay. Interestingly, the Cre/loxP interaction, however, was not abolished, but was found to be at a significantly higher level than the ectopic recombination. Peoples *et al.* suggest that the early, DSB-independent homolog interactions are not sufficient to establish connections that are stable enough to perform recombination, while meiotic pairing that involves DSBs bring the homologs close together. They further suggest that both DSB-

dependent and DSB-independent pairing mechanisms may be involved in meiotic pairing. If DSB-independent pairing also takes part in linking the homologous chromosomes together in normal pachytene, these forces should also be present if the DSB-dependent pairing is absent, as in *spo11* mutants. The low but significant FISH pairing level seen in *spo11* mutants, and the notable level of close contact shown by PEOPLES *et al.* (2002), may be remnants of the second round of DSB-independent pairing that has not been disrupted by chromatin rearrangement or spreading force. The remnant DSB-independent pairing could contribute to the formation of the SCs that we found in this study.

Similar to the distributive segregation described in *Drosophila*, GUACCI and KABACK (1991) found evidence for an alternative segregation pathway in yeast. In their experiments nonhomologous chromosomes or chromosome fragments segregate from each other in 90% of the cells. It would be tempting to regard the incidents described above as parts in a salvage pathway to rescue a cell that has been unsuccessful in recombination. Telomeric pairing that normally occurs, DSB-independent pairing that obviously allows some close connection also in *spo11* mutants, and DSB-independent synapsis that we show in this study, as well as distributive segregation, could all possibly contribute to an alternative way of proper chromosome segregation. However, although we find fully formed SCs in a fraction of the *spo11* mutant cells, we also find that the meiotic products are inviable. Thus the hypothetical salvage pathway is unable to rescue all 16 nonrecombinant chromosome pairs from nondisjunction.

Our results show that even if the majority of meiotic yeast cells fail in chromosome synapsis in the absence of Spo11, a fraction of the cells manage to synapse in a wild-type-like manner. So, even if the Spo11-induced DSBs are strong promoters of synapsis in yeast and the absence of Spo11 strongly reduces synapsis, it seems as if the presence of Spo11 is not an absolute requirement for synapsis.

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#### LITERATURE CITED

- AGARWAL, S., and G. S. ROEDER, 2000 Zip3 provides a link between recombination enzymes and synaptonemal complex proteins. *Cell* **102**: 245–255.
- BERGERAT, A., B. DE MASSY, D. GADELLE, P. C. VAROUTAS, A. NICOLAS *et al.*, 1997 An atypical topoisomerase II from Archaea with implications for meiotic recombination. *Nature* **386**: 414–417.
- BHUIYAN, H., G. DAHLFORS and K. SCHMEKEL, 2003 Lateral elements inside synaptonemal complex-like polycomplexes in *ndt80* mutants of yeast bind DNA. *Genetics* **163**: 539–544.
- BÖRNER, G. V., N. KLECKNER and N. HUNTER, 2004 Crossover/non-crossover differentiation, synaptonemal complex formation and regulatory surveillance at the leptotene/zygotene transition of meiosis. *Cell* **117**: 29–45.
- BURGESS, S. M., 2002 Homologous chromosome associations and nuclear order in meiotic and mitotically dividing cells of budding yeast. *Adv. Genet.* **46**: 49–90.
- CAO, L., E. ALANI and N. KLECKNER, 1990 A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. *Cell* **61**: 1089–1101.
- CHA, R. S., B. M. WEINER, S. KEENEY, J. DEKKER and N. KLECKNER, 2000 Progression of meiotic DNA replication is modulated by interchromosomal interaction proteins, negatively by Spo11p and positively by Rec8p. *Genes Dev.* **14**: 493–503.
- DERNBURG, A. F., K. McDONALD, G. MOULDER, R. BARSTEAD, M. DRESSER *et al.*, 1998 Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. *Cell* **94**: 387–398.
- FUNG, J. C., W. F. MARSHALL, A. DERNBURG, D. A. AGARD and J. W. SEDAT, 1998 Homologous chromosome pairing in *Drosophila melanogaster* proceeds through multiple independent initiations. *J. Cell Biol.* **141**: 5–20.
- GIROUX, C. N., M. E. DRESSER and H. F. TIANO, 1989 Genetic control of chromosome synapsis in yeast meiosis. *Genome* **31**: 88–94.
- GUACCI, V., and D. B. KABACK, 1991 Distributive disjunction of authentic chromosomes in *Saccharomyces cerevisiae*. *Genetics* **127**: 475–488.
- HENDERSON, K. A., and S. KEENEY, 2004 Tying synaptonemal complex initiation to the formation and programmed repair of DNA double-strand breaks. *Proc. Natl. Acad. Sci. USA* **101**: 4519–4524.
- KEENEY, S., 2001 Mechanism and control of meiotic recombination initiation. *Curr. Top. Dev. Biol.* **52**: 1–53.
- KEENEY, S., C. N. GIROUX and N. KLECKNER, 1997 Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell* **88**: 375–384.
- KLAPHOLZ, S., and R. E. ESPOSITO, 1982 A new mapping method employing a meiotic rec-mutant of yeast. *Genetics* **100**: 387–412.
- KLAPHOLZ, S., C. S. WADDELL and R. E. ESPOSITO, 1985 The role of the SPO11 gene in meiotic recombination in yeast. *Genetics* **110**: 187–216.
- LIU, L., S. FRANCO, B. SPYROPOULOS, P. B. MOENS, M. A. BLASCO *et al.*, 2004 Irregular telomeres impair meiotic synapsis and recombination in mice. *Proc. Natl. Acad. Sci. USA* **101**: 6496–6501.
- LOIDL, J., F. KLEIN and H. SCHERTHAN, 1994 Homologous pairing is reduced but not abolished in asynaptic mutants of yeast. *J. Cell Biol.* **125**: 1191–1200.
- LOIDL, J., F. KLEIN and J. ENGBRECHT, 1998 Genetic and morphological approaches for the analysis of meiotic chromosomes in yeast. *Methods Cell Biol.* **53**: 257–285.
- MAHADEVAIAH, S. K., J. M. TURNER, F. BAUDAT, E. P. ROGAKOU, P. DE BOER *et al.*, 2001 Recombinational DNA double-strand breaks in mice precede synapsis. *Nat. Genet.* **27**: 271–276.
- MALKOVA, A., F. KLEIN, W.-Y. LEUNG and J. HABER, 2000 HO endonuclease-induced recombination in yeast meiosis resembles Spo11-induced events. *Proc. Natl. Acad. Sci. USA* **97**: 14500–14505.
- MCKIM, K. S., B. L. GREEN-MARROQUIN, J. J. SEKELSKY, G. CHIN, C. STEINBERG *et al.*, 1998 Meiotic synapsis in the absence of recombination. *Science* **279**: 876–878.
- NEALE, M. J., M. RAMACHANDRAN, E. TRELLES-STICKEN, H. SCHERTHAN and A. S. GOLDMAN, 2002 Wild-type levels of Spo11-induced DSBs are required for normal single-strand resection during meiosis. *Mol. Cell* **9**: 835–846.
- NOVAK, J. E., P. B. ROSS-MACDONALD and G. S. ROEDER, 2001 The budding yeast *msh4* protein functions in chromosome synapsis and the regulation of crossover distribution. *Genetics* **158**: 1013–1025.
- PADMORE, R., L. CAO and N. KLECKNER, 1991 Temporal comparison of recombination and synaptonemal complex formation during meiosis in *S. cerevisiae*. *Cell* **66**: 1239–1256.
- PEOPLES, T. L., E. DEAN, O. GONZALEZ, L. LAMBOURNE and S. M. BURGESS, 2002 Close, stable homolog juxtaposition during meiosis in budding yeast is dependent on meiotic recombination, occurs independently of synapsis, and is distinct from DSB-independent pairing contacts. *Genes Dev.* **16**: 1682–1695.
- RASMUSSEN, S. W., 1976 The meiotic prophase in *Bombyx mori* females analyzed by three-dimensional reconstructions of synaptonemal complexes. *Chromosoma* **54**: 245–293.
- ROCKMILL, B., and G. S. ROEDER, 1998 Telomere-mediated chromo-

- some pairing during meiosis in budding yeast. *Genes Dev.* **12**: 2574–2586.
- ROCKMILL, B., M. SYM, H. SCHERTHAN and G. S. ROEDER, 1995 Roles for two RecA homologs in promoting meiotic chromosome synapsis. *Genes Dev.* **9**: 2684–2695.
- ROEDER, G. S., 1997 Meiotic chromosomes: it takes two to tango. *Genes Dev.* **11**: 2600–2621.
- SCHMEKEL, K., 2000 Methods for immunoelectron microscopic and fine analysis of synaptonemal complexes and nodules in yeast. *Chromosoma* **109**: 110–116.
- SMITH, A. V., and G. S. ROEDER, 1997 The yeast Red1 protein localizes to the cores of meiotic chromosomes. *J. Cell Biol.* **136**: 957–967.
- STORLAZZI, A., L. XU, A. SCHWACHA and N. KLECKNER, 1996 Synaptonemal complex (SC) component Zip1 plays a role in meiotic recombination independent of SC polymerization along the chromosomes. *Proc. Natl. Acad. Sci. USA* **93**: 9043–9048.
- SUN, H., D. TRECO, N. P. SCHULTES and J. W. SZOSTAK, 1989 Double-strand breaks at an initiation site for meiotic gene conversion. *Nature* **338**: 87–90.
- SYM, M., and G. S. ROEDER, 1995 Zip1-induced changes in synaptonemal complex structure and polycomplex assembly. *J. Cell Biol.* **128**: 455–466.
- SYM, M., J. A. ENGBRECHT and G. S. ROEDER, 1993 ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. *Cell* **72**: 365–378.
- THORNE, L. W., and B. BYERS, 1993 Stage-specific effects of X-irradiation on yeast meiosis. *Genetics* **134**: 29–42.
- TRELLES-STICKEN, E., M. E. DRESSER and H. SCHERTHAN, 2000 Meiotic telomere protein Ndj1p is required for meiosis-distribution, bouquet formation and efficient homologue pairing. *J. Cell Biol.* **151**: 95–106.
- TUNG, K.-S., and G. S. ROEDER, 1998 Meiotic chromosome morphology and behavior in *zip1* mutants of *Saccharomyces cerevisiae*. *Genetics* **149**: 817–832.
- VILLENEUVE, A. M., 1994 A *cis*-acting locus that promotes crossing over between X chromosomes in *Caenorhabditis elegans*. *Genetics* **136**: 887–902.
- WALKER, M. Y., and R. S. HAWLEY, 2000 Hanging on to your homolog: the roles of pairing, synapsis and recombination in the maintenance of homolog adhesion. *Chromosoma* **109**: 3–9.
- WEINER, B. M., and N. KLECKNER, 1994 Chromosome pairing via multiple interstitial interactions before and during meiosis in yeast. *Cell* **77**: 977–991.
- XU, L., M. AJIMURA, R. PADMORE, C. KLEIN and N. KLECKNER, 1995 NDT80, a meiosis-specific gene required for exit from pachytene in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **15**: 6572–6581.

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