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Isolation of *COM1*, a New Gene Required to Complete Meiotic Double-Strand Break-Induced Recombination in *Saccharomyces cerevisiae*

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ABSTRACT

We have designed a screen to isolate mutants defective during a specific part of meiotic prophase I of the yeast *Saccharomyces cerevisiae*. Genes required for the repair of meiotic double-strand breaks or for the separation of recombined chromosomes are targets of this mutant hunt. The specificity is achieved by selecting for mutants that produce viable spores when recombination and reductional segregation are prevented by mutations in *SPO11* and *SPO13* genes, but fail to yield viable spores during a normal Rec$^+$ meiosis. We have identified and characterized a mutation *com1-1*, which blocks processing of meiotic double-strand breaks and which interferes with synaptonemal complex formation, homologous pairing and, as a consequence, spore viability after induction of meiotic recombination. The *COM1/SAE2* gene was cloned by complementation, and the deletion mutant has a phenotype similar to *com1-1*. *com1/sae2* mutants closely resemble the phenotype of *rad50S*, as assayed by phase-contrast microscopy for spore formation, physical and genetic analysis of recombination, fluorescence *in situ* hybridization to quantify homologous pairing and immunofluorescence and electron microscopy to determine the capability to synapse axial elements.

IN order to allow repeated conjugation and thus exchange of genetic material between different individuals, an organism needs to be able to separate again the two complete genomes that contributed to the diploid. Eukaryotes generally use a process called meiosis, which consists of two subsequent cell divisions preceded by only a single round of DNA replication to generate haploid cells for sexual reproduction. Both divisions resemble mitosis in many aspects, but during the first (reductional) division typically homologous parental chromosomes are recognized, paired and recombined and parental centromeres are segregated to opposite poles, whereas the sister centromeres do not come apart before the second (equational) meiotic division.

Thus the reductional division seems to encompass the newly acquired meiosis-specific functions for chromosome sorting and genetic recombination and accordingly has been the focus of many investigations. The yeast *Saccharomyces cerevisiae* has served as a remarkable model organism, helping to dissect meiotic mechanisms through the use of genetic screens combined with biochemical and cytological methods. Genetic screens have become more and more specific, concentrating on defined aspects of the meiotic process, *e.g.*, recombination or homologue interaction (Esposito and Esposito 1974; Rockmill and Roeder 1988; Hollingsworth and Byers 1989; Ajimura *et al.* 1993; Hollingsworth *et al.* 1995).

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In yeast meiotic recombination and synapsis are initiated by meiosis-specific DNA double-strand breaks (DSBs) (Nicolas *et al.* 1989; Sun *et al.* 1989; Cao *et al.* 1990). These breaks are central to meiosis and all genes acting before DSB formation can be regarded as early prophase I genes. A class of mutations that eliminate DSBs have been characterized by a genetic screen, selecting for suppressors of spore lethality caused by unrepaired DSBs in sporulating haploid *rad52, spo13* double mutants (Malone *et al.* 1991). Such mutants are generally deficient for the initiation of meiotic recombination and thus can be rescued by relieving the requirement for reductional segregation in *spo13*. Other mutations, like *hop1* (Hollingsworth and Byers 1989) and *red1* (Rockmill and Roeder 1990), can also be rescued by *spo13*, although some DSBs are formed (Rockmill and Roeder 1990; Schwacha and Kleckner 1994). This suggests that DSBs can be fully repaired in these mutants without ensuring correct reductional segregation.

A number of mutations that cannot be rescued by *spo13* have been characterized and some of these interfere with processing of the DSBs. A class of nonnull alleles of *rad50* designated *rad50S* are defective at the earliest stage of DSB repair, namely in the processing of 5′ ends at the initiation site to form the 3′ single-stranded ends required for strand invasion (Alani *et al.* 1990). This is different from the phenotype of the *rad50*-null mutant, which does not initiate meiotic recombination and therefore is rescued by *spo13*, demonstrating the important role of *RAD50* both for the initiation and processing of meiotic DSBs. Recently, covalent

attachment of an unidentified protein to the unresected 5′ ends in *rad50S* mutants has been demonstrated (DE MASSY *et al.* 1995; KEENEY and KLECKNER 1995; LIU *et al.* 1995). These attachments are very likely to prevent efficient repair of DSBs during meiosis, causing a block or at least a delay before the first meiotic division. The unrepaired lesions, rather than absence of recombination, may be the reason for lethality of the diploid spores produced by *spo13, rad50S* double mutants.

Dmc1p, Rad51p, Rad55p and Rad57p all show homology to the bacterial recombination protein recA. Consequently their function is implicated in the strand invasion step of DSB repair, which is supported by their phenotypes. *dmc1, rad51, rad55, rad57, rad52* and *sep1*-null mutants accumulate DSBs at least transiently, but, in contrast to *rad50S,* produce 3′ single-stranded tails even longer than wild type (reviewed in SHINOHARA and OGAWA 1995; TISHKOFF *et al.* 1995). According to results obtained by SUGAWARA *et al.* (1995) the role of *RAD51, RAD55* and *RAD57* is rather indirect, namely to provide access for other recombination enzymes to the homologous donor copy. A recently characterized member of the group of genes required for DSB repair is *RAD58 (XRS4)* (CHEPURNAYA *et al.* 1995).

Some of these mutants (*e.g., dmc1, rad51* and *sep1*) have been shown to cause an arrest or delay in pachytene in addition to a strong ascospore formation defect. Inactivation of *SPO13* can neither alleviate this block nor restore spore viability, at least in the SK1 strain background (TISHKOFF *et al.* 1991; BISHOP *et al.* 1992). In contrast, by blocking meiotic recombination (*e.g.,* in a *spo11* mutant), the arrest or delay of *dmc1* or *rad51* cells can be abolished. However in a strain background where *dmc1* does not arrest, restoration of wild-type levels of spore viability after inactivation of *SPO13* alone has been reported (ROCKMILL *et al.* 1995). The role of the *SEP1* gene is less clear. *In vitro* DNA strand exchange activity (KOLODNER *et al.* 1987), as well as *in vitro* microtubule polymerizing activity (INTERTHAL *et al.* 1995), has been reported for the Sep1 protein, stimulating diverse interpretations for the role of this important factor. However since the defects of *sep1* mutants are not suppressed by *rad50, spo13* (TISHKOFF *et al.* 1991), it can be concluded that a pathway not related to DSB-induced recombination is affected. The list of genes known to date to be involved in repair of meiotic DSBs is certainly not complete.

Mutations in *ZIP1, TOP2* and *MEI5* are not believed to directly impede processing of meiotic DSBs, yet they cause the cells to arrest or delay in pachytene independently of the *SPO13* function. Zip1p (SYM *et al.* 1993; SYM and ROEDER 1994) is a component of the synaptonemal complex (SC) required for complete synapsis. Separation of recombined homologues during meiosis I was shown to be dependent on topoisomerase II with the use of a cold-sensitive allele of *TOP2* (ROSE *et al.* 1990a). *MEI5*, a recently isolated meiosis-specific gene, is probably required for disassembly of the SC since *mei5* mutant strains arrest at pachytene with complete SCs (M. MODESTI and C. GIROUX, personal communication). However, the meiotic arrest of a *mei5* mutant strain is relieved in the presence of a *spo11* mutation, which eliminates DSB formation and synapsis (GIROUX *et al.* 1993). These mutants affect processes subsequent to but dependent on meiotic recombination, as in each case the elimination of recombination eliminates also the mutant phenotype. It is likely that this interesting class of mutations will expand as more genes involved in SC formation and chromosome separation are identified.

Mutations in genes exerting meiotic cell cycle control also are not expected to be influenced by *spo13. cdc28, cdc36* and *cdc39* cells arrest (or delay) in the pachytene stage of meiotic prophase, although they block at Start during the vegetative cell cycle (SHUSTER and BYERS 1989). *NDT80* (XU *et al.* 1995) is a meiosis-specific gene required for exit from meiotic pachytene, but in contrast to *mei5, ndt80* cells arrest even in a *spo11, spo13* background. Thus *NDT80* is required for processes independent of meiotic recombination, possibly being involved in meiotic cell cycle control.

In this article we introduce a genetic screen that permits the identification of mutations that prevent the formation of viable spores in a *spo13* background, but that permit successful sporulation in *spo11, spo13* strains. We describe the identification and characterization of a new gene *COM1/SAE2* that is required for the processing and the repair of meiotic DSBs.

MATERIALS AND METHODS

Media, growth and sporulation conditions: Cells were grown in YPD or synthetic complete medium supplemented with appropriate amino acids (ROSE *et al.* 1990b). Selection of G418 resistant transformants was performed on YPD plates containing 200 mg/liter of G418 (geneticin; GIBCO BRL, Gaithersburg, MD). Sporulation conditions as well as spheroplasting were as described previously (LOIDL *et al.* 1991). Sporulation of strains Y164 and Y219 on solid medium was performed on SPM+ (0.25% yeast extract, 0.1% glucose, 1.5% potassium acetate plus 1/5 of amino acid or base supplement of a regular synthetic complete medium).

Plasmid constructions: To construct plasmid p21 the *SPO13* gene was cloned as a 2.2-kilobase (kb) *Bam*HI-*Nru*I fragment derived from pTW15 (a gift from R. E. ESPOSITO) into pRS316 (SIKORSKY and HIETER 1989) cut with *Hin*dIII, blunted and cut with *Bam*HI. For the construction of p155 the *Sal*I-*Sma*I fragment of YCp50, containing *URA3*, was deleted. Into the *Bam*HI site of the resulting vector the *ADE2* gene was cloned as a *Bam*HI cassette. *SPO11* was introduced as a *Cla*I fragment derived from pGB429 (a gift from C. N. GIROUX). Plasmid p259, which can be easily lost due to an unstable centromere *CEN3*, was obtained by first subcloning *SPO13* as a 2.2-kb *Bam*HI-*Nru*I fragment into pIC19H from which it was inserted into pNC161. The original plasmid complementing *com1-1* containing an ~6-kb insert, was isolated from a yeast genomic library (the shuttle vector M111 carrying the *TRP1* gene and *CEN3*, containing partial *Sau*3AI-digested genomic yeast DNA inserted into its *Bam*HI site; a gift from G. AMMERER) as described below and was designated p260.

TABLE 1

***Saccharomyces cerevisiae* strains used in this study**

Strain	Genotype	Source
SK1	*MAT***a***/α HO*	KANE AND ROTH (1974)
Y47	NKY1238 × NKY1240	
NKY1238	*MAT***a** *ho::LYS2 lys2 leu2::hisG his4X::LEU2-URA3 ura3 arg4-Nsp*	N. KLECKNER[a]
NKY1240	*MATα ho::LYS2 lys2 leu2::hisG his4B::LEU2 ura3 arg4-Bgl*	N. KLECKNER[a]
Y164	*MAT***a***/α HO spo11::URA3 spo13::hisG leu2 ade2::hisG trp1 ura3 com1-1* +p155 (YCp*SPO11-ADE2*)	This study
Y219	*MAT***a***/α HO spo11::URA3 spo13::hisG leu2 ade2::hisG trp1 ura3* +p155 (YCp*SPO11-ADE2*) p259 (YCp*SPO13-TRP1*)	This study
Y235	*MAT***a** *ho::LYS2 lys2 leu2::hisG his4X ura3 com1::tn3/10-LEU2* *MATα ho::LYS2 lys2 leu2::hisG his4B ura3 com1::tn3/10-LEU2*	This study
Y236	*MAT***a** *ho::LYS2 lys2 leu2::hisG his4X ura3 com1::tn3/20-LEU2* *MATα ho::LYS2 lys2 leu2::hisG his4B ura3 com1::tn3/20-LEU2*	This study
Y248	*MAT***a***/α HO leu2 com1-1*	This study
Y278	*MATα ho::LYS2 arg4-Nsp leu2::hisG ura3 ade2::hisG his4X-LEU2-MluI::BamHI-URA3*	This study
Y319	*MAT***a** *ho::LYS2 leu2R TRP1 his4X rad50S::URA3 com1-1* *MATα ho::LYS2 leu2R trp1 HIS4 rad50S::URA3 com1-1*	This study
Y322	*MAT***a** *ho::LYS2 leu2 trp1 ade2 HIS4 spo11::URA3 spo13::hisG com1-1* *MATα ho::LYS2 leu2 trp1 ADE2 his4 spo11::URA3 spo13::hisG com1-1*	This study
Y329	*MAT***a** *ho::LYS2 lys2 his4B-LEU2-MluI leu2 ura3 arg4-Bgl or arg4Δ10 rad50S::URA3* *MATα ho::LYS2 lys2 hs4X-LEU2-MluI::BamHI-URA3 leu2 ura3 arg4-Nsp or arg4Δ10 rad50S::URA3*	This laboratory[b]
Y340	*MAT***a** *ho::LYS2 LEU2 trp1::hisG ura3 ade2 spo13::hisG com1-1* *MATα ho::LYS2 leu2 TRP1 URA3 ADE2 spo13::hisG com1-1*	This study
Y355	*MAT***a** *ho::LYS2 lys2 leu2 ura3 com1-1 arg4-Bgl his4BLEU2-MluI TRP1 ade2* *MATα ho::LYS2 lys2 leu2 ura3 com1-1 arg4-Nsp his4X-LEU2-MluI::BamHI-URA3 trp1 ADE2*	This study
Y356	*MAT***a** *ho::LYS2 lys2 leu2::hisG his4X ura3 com1::KanMX4*	This study
Y359	*MAT***a***/α HO leu2::hisG ura3 his4X com1::KanMX4*	This study
Y361	NKY1303 × 278	
NKY1303	*MAT***a** *ho::LYS2 lys2 arg4-Bgl leu2::hisG ura3 ADE2 his4-BLEU2-MluI*	N. KLECKNER[c]

[a] This strain was provided by D. BISHOP.
[b] This strain was provided K. NAIRZ.
[c] This strain was provided by L. XU.

Tn3-LEU2 transposon mutagenesis was performed on p190 according to SEIFERT *et al.* (1986). p190 was obtained by subcloning a 4-kb *Eco*RI-*Bgl*II fragment containing *COM1* from p260 into the *Eco*RI and *Bam*HI sites of pHSS6 (SEIFERT *et al.* 1986). Plasmids carrying insertions were cut with *Not*I to release the insert, which was used to replace one of the mutated *com1-1* copies in strain Y164, to test for complementation. The insertions were mapped by restriction analysis with a cluster of six noncomplementing insertions outlining the position of the open reading frame (ORF) of *COM1*. *com1* ::tn3/10 maps ~600 base pairs (bp) downstream of the ATG, whereas *com1* ::tn3/20 was localized to the very 3′ end. Plasmid p188 was obtained by cloning the *COM1* gene on a 1545 bp *Apa*I-*Sal*I fragment into pRS315 (SIKORSKY and HIETER 1989).

Strains: All strains used in this study are isogenic derivatives of SK1 (KANE and ROTH 1974) (Table 1). All markers were introduced into this background by genetic transformation only. Transformation followed the protocol of GIETZ *et al.* (1992). Disruptions were confirmed by Southern analysis. One-step gene replacement (ROTHSTEIN 1983) was used for integration. *SPO11* was disrupted in a haploid parent strain of Y164 and Y219, using a *Hin*dIII-*Bgl*II fragment of p(spo11)35 from R. E. ESPOSITO carrying the *URA3* gene inserted at the *Eco*RI site of *SPO11*. To construct strains Y235 and Y236 haploid parents were transformed separately with transposon tagged inserts as described above, mated and zygotes were isolated by micromanipulation. The *com1*-null allele was constructed by PCR targeting using *KanMX*4 as a dominant resistance marker (WACH *et al.* 1994). We designed oligonucleotides COM L5 (5′-cctgcatttccatccatgctgtagccatta-ggtgtttgtatgtgagatgGTACGCTGCAGGTCG ACGGATCC CC-3′) and COM L6 (5′-gtatttgaagtaatgaataaagaatgatgatcgctggc-gtttaacatcTATCATCGATGAATTCG AGCTCG-3′) containing ~50 bp of homology to sequences at the 5′ or 3′ end of *COM1* (lowercase letters) as well as ~25 bp of *KanMX*4 sequence at the 3′ end (capital letters) to amplify the resistance marker. The resulting disruption cassette was used to construct Y356 by one-step gene replacement, thereby deleting all bases from position +6 to +1029 representing 99% of the ORF of *COM1*. Y359 is a G418-resistant derivative of a cross of Y356 to wild-type homothallic SK1.

Genetic methods: mutagenesis: Strain Y219 was sporulated on SPM+, digested with zymolyase [1 mg/ml in 10 mM dithiothreitol (DTT) for 1 hr at 30°], sonicated on ice to obtain single spores and incubated at 30° with MNNG (*N*-methyl-*N′*-nitro-*N*-nitroso-guanidine; Sigma) at a final concentration of 20 ng/ml. After 30 min the assay was diluted 100-fold in 15% glycerol and stored at −80°. After determination of spore

viability (25%) appropriate dilutions were plated out on SC-ade and the screening was carried out as described in the RESULTS section.

Return to growth experiments: The experiments followed methods described previously (SHERMAN and ROMAN 1963; ESPOSITO and ESPOSITO 1974). Cultures were grown from a single colony on YPD, diluted in YPA, grown overnight to ~2 × 10^7 cells/ml and transferred to sporulation medium (4 × 10^7 cells/ml). To reduce cell aggregation, cells were sonicated immediately after transfer to SPM. Onset of meiosis was followed by staining of ethanol-fixed samples with 4′6-diamidino-2-phenylindole (DAPI). At the specified time after transfer to SPM aliquots were taken, diluted appropriately and plated on YPD, SC-his and SC-arg, where colonies were counted after 3–5 days.

Patch assay for spore viability: Cells were grown in single colonies or patched out on vegetative medium. After 2–3 days cells were replica plated to SPM or SPM+. After 3 days of sporulation a replica on vegetative medium was produced and subjected to ether treatment to kill unsporulated cells selectively. The following variation of the protocol of ROCKMILL and ROEDER (1988) was used for ether killing. A large plastic box was filled with diethyl ether (in the hood) up to 1 cm. Plates containing spores were placed for 30 min on a metal grid as close as possible to the ether in an inverted position and the box was closed with a lid. The plates then were removed and left open for the ether to evaporate for 1 hr before incubation at 30°. The diethyl ether can be reused. If melting of the plastic petri dishes is a problem, glass petri dishes can be used. We found vegetative cells 2000-fold more sensitive to this treatment than spores.

γ-Irradiation assay: Single colonies were grown overnight in YPD to stationary phase. Cultures were diluted in 1 mM KPi to a densitiy of ~1 × 10^4 cells/ml. One milliliter aliquots were irradiated at a dose of 50, 100 and 200 Gy using a ^{60}Co-source (γ-cell 220; Nordion International Inc., Canada). Irradiated and nonirradiated control samples were diluted 1:10 in water and plated out in triplicate on YPD. Colonies were counted after 3–4 days of incubation at 30°.

Cytological methods: Chromosome spreads were prepared as described previously (LOIDL *et al.* 1991). In brief, cells were pelleted and digested in 1:10 volume of SPM with 140 μg/ml zymolyase 100T (Seikagaku Kogio), 10 mM DTT at 37° until spheroplasts lysed to ~90% upon addition of an equal volume of 2% sodium lauryl sarcosyl on a test slide. The digest was stopped by adding ice-cold solution II [0.1 M morpholino ethane sulfonate (MES), 1 M sorbitol, 1 mM EDTA, 0.5 mM $MgCl_2$] and pelleting the spheroplasts at low speed (4 min, 2000 rpm). Spheroplasts were resuspended in 1:10 of the original volume and spread by pipetting 20 μl spheroplasts, 40 μl fixative (4% w/v paraformaldehyde, 3.6% sucrose), 80 μl 1% lipsol and 80 μl of fixative on a clean glass slide. The suspension was evenly distributed by moving a glass rod carefully back and forth and the slide was air dried. For immunostaining, after 2 hr of drying slides were immersed into PBS for 10 min, incubated in blocking buffer (PBS, 0.5% gelatine, 2% BSA) for 10 min and incubated overnight at 4° with the primary antibody diluted 1:50 in blocking buffer in a humid box. Before adding the appropriate secondary antibody, slides were washed again for 10 min in PBS. Before analysis preparations were mounted in antibleach medium (Vectashield; Vector Laboratories, Burlingame, CA) supplemented with 0.5 μg/ml DAPI for staining the chromatin. To demonstrate synapsis mouse-anti-Zip1p antibody (a generous gift from P. B. MOENS) was used in combination with a CY3-conjugated goat-anti-mouse antibody (Dianova) or with FITC-conjugated goat-anti-mouse antibody (Sigma). For electron microscopy, cells were spread and stained with silver nitrate (1 g $AgNO_3$ plus 2 ml distilled water) following the protocol of LOIDL *et al.* (1991).

Fluorescence *in situ* hybridization (FISH): One to two micrograms of chromosome I probe (cosmid 9218, ATCC 70893, 32.9 kb, left arm) were labeled with Cy3-dUTP (Amersham Life Science) and 1–2 μg of chromosome IV (cosmid 9665, ATCC 71003, 31.4 kb, left arm) were labeled with Cy5-dUTP (Amersham Life Science) by nick translation. The labeled probe was separated from free nucleotides using a sephadex G-50 column, and 1/10 of the labeled probe was used for FISH, 20 μg carrier DNA was added and DNA was precipitated with 1:10 volume 3 M LiCl and 2.5 volume ethanol for at least 30 min at −80°. After centrifugation the pellet was resuspended in 5 μl 100% formamide, an equal volume of hybridization buffer (20% dextran sulfate, 4× SSC) was added and the probe was denatured for 5 min at 95°, put on ice for 2 min and then added to the denatured slide. Slides with spread yeast nuclei were incubated with 50 μl RNase A (100 μg/ml) under a coverslip for 30 min at 37° in a moist chamber, and then put for 2 hr in 4× SSC/0.1% Tween at 37°. Slides were denatured for 2 min in formamide at 60° and put directly into cold 70, 80 and 96% ethanol (5 min each) and briefly air dried. Denatured probe was added to the slide, the slide was covered with a coverslip and the coverslip was sealed with rubber cement. The slide and the probe were then co-denatured for 10 min at 95° in a thermocycler capable of holding slides (HYBAID; OmniGene) and hybridized for at least 12 hr at 37°. After hybridization the rubber cement was removed and the slide was placed into the first wash solution (50% formamide in 2× SSC) at 37° for 5 min. Slides were then transferred to 2× SSC at 37° for 5 min and finally to 1× SSC at room temperature again for 5 min. Finally slides were mounted in antibleach medium as described above. For the experiment reported the products of meiotic divisions arising at the late time points were excluded from distance analysis by virtue of their smaller DNA content.

Detection and quantification of DSBs: DNA extraction and Southern blot hybridization were done as described (DE MASSY and NICOLAS 1993). To study the *THR4* hotspot (GOLDWAY *et al.* 1993) *Bgl*II digested DNA was probed with a ^{32}P-labeled 888-bp *Hin*dIII fragment derived from pMJ338 (kindly provided by M. LICHTEN). Direct quantification was done using a Phosphoimager and evaluated using ImageQuant (both Molecular Dynamics). For each lane an intensity profile was generated. Peaks corresponding to the bands were identified, separated from the background and integrated.

RESULTS

A mutant screen to identify new genes required during late events in prophase I: To identify new genes required after initiation of meiotic recombination we designed a mutant screen that took advantage of the fact that *spo13, spo11* double mutants undergo a truncated meiosis, consisting of only a single cell division in which chromosomes do not recombine and segregate in an equational manner, as has been shown for *rec104* (GALBRAITH and MALONE 1992) and *hop1* (HOLLINGSWORTH and BYERS 1989). As a result two viable, diploid spores are formed. Because cells do not initiate meiotic recombination and probably as a consequence do not undergo reductional division, genes required specifically for these processes may be dispensable during the *spo11, spo13* meiosis. In other words we predicted a class of meiotic lethal mutations to be bypassed by the *spo11, spo13* double mutation. Such mutations

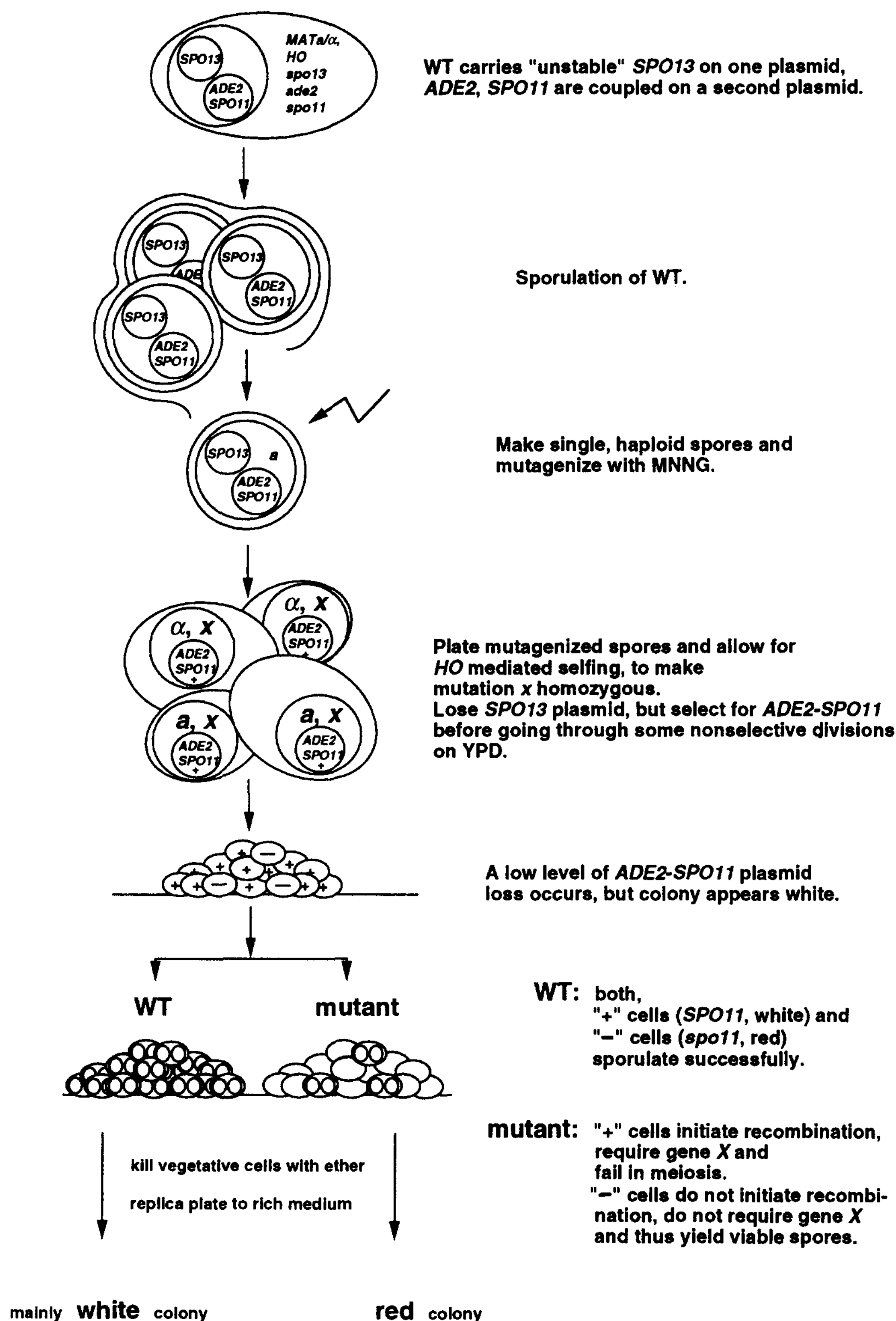

FIGURE 1.—Schematic representation of the mutant screen. The mutant screen is presented, illustrating the crucial steps in mutagenesis. Vegetative cells containing two *CEN* plasmids (p259, p155) are sporulated and single spores are mutagenized. On vegetative medium they germinate, switch mating type and form diploid colonies that carry homozygous mutations. +, cells carry *ADE2* and *SPO11* on the same plasmid p155; −, loss of the plasmid. A mutation *x* is desired, which will derail meiosis only if meiotic recombination is initiated (in + cells). The − cells carrying mutation *x* will produce viable spores because recombination is not initiated and *spo13* rescues viability in a Rec⁻. Due to the loss of the *ADE2* gene, these surviving cells will ultimately form red colonies in contrast to the predominantly white colonies produced from wild-type cells.

could affect completion of meiotic recombination, SC degradation or chiasma resolution.

To identify such mutations, a screen based on a plasmid loss assay (Figure 1) was used. The wild-type strain (Y219) is *HO ade2 spo11 spo13* and carries the *SPO11* gene linked to a color marker (*ADE2*) on one plasmid (p155) and the *SPO13* gene on another unstable plasmid (p259). It is thus able to produce haploid, viable spores that can be mutagenized. A colony originating from such a spore will be a homozygous diploid due to *HO* and will harbor some cells that have lost both plasmids, thereby potentially forming red sectors. For a colony comprised of cells carrying the desired mutation only cells lacking *SPO11* as well as *SPO13* can survive

meiosis. After meiosis these colonies will give rise to patches strongly enriched for such red sectors or will be completely red.

Identification of recombination-dependent meiosis-defective mutants: The haploid spores of strain Y219 were mutagenized with MNNG and plated out on medium lacking adenine (SC-ade). During growth haploid cells switched mating type, resulting in the formation of homozygous diploid cells. Colonies were then transferred to complete medium (YPD) for 12 hr and then to sporulation inducing medium (SPM+). After 3 days spore patches were replica plated onto YPD plates and treated with ether to eliminate nonsporulated diploid cells. These cells would otherwise interfere with the mutant hunt by forming white or weakly sectored colonies even if carrying the desired mutation. Spore patches giving rise to red colonies were selected as possible mutant candidates.

After mutagenizing 75,000 colonies, 56 mutant candidates were isolated and rescreened to confirm their phenotype. An obvious class of false positives was mutants defective in mitotic plasmid maintenance, which we could sort out easily by virtue of their mitotically enhanced red sectoring. Meiosis-specific plasmid loss mutants were rare and could be distinguished from the desired mutants by their high spore viability after meiosis. Ten mutants passed the rescreening procedure and were analyzed further. One of these mutants, strain Y164, fulfilled the criteria of the screen most strikingly and thus was chosen to be investigated further.

Molecular cloning and transposon mutagenesis: We used the fact that Y164 could not produce viable spores in a recombination proficient *spo13*, as well as in a *SPO13* background, to clone the wild-type copy of the gene by complementation. A potential problem for selection were Rec^- mutations, which could arise during transformation and would suppress spore lethality in some rare *spo13* colonies, thereby mimicking successful complementation of the mutation. Therefore the selection was performed using *SPO13* cells that require both Rec^+ functions and the desired gene. Y164 was transformed with a stable *SPO13* plasmid (p21) and then with a CEN -based genomic library (see MATERIALS AND METHODS). The resulting transformants were subjected to a procedure similar to the mutant screen. The colonies were sporulated and subsequently replica plated to SC-leu-trp plates to select for p21 as well as the library plasmid and ether treated. Spore patches giving rise to colonies were picked as possible candidates and reexamined for sporulation in the presence of *SPO13*. The library plasmid was isolated from each candidate, amplified in *Escherichia coli* and reintroduced into strain Y164. Plasmid p260 containing an ~6-kb insert was obtained that reproducibly complemented both the spore formation and the spore viability defect (Figure 2). The complementing fragment was localized to the left arm of chromosome VII using the p260 insert to probe a blot of *Sfi*I and *Not*I-digested yeast chromosomes separated

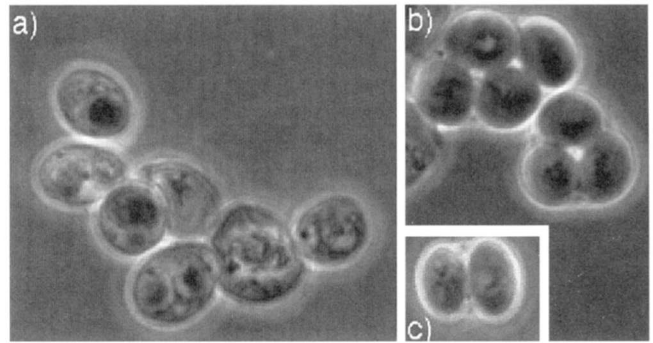

FIGURE 2.—The spore formation defect of the *com1-1* mutant can be complemented by *COM1/SAE2* on a plasmid. Cells are shown after 48 hr in SPM: (a) strain Y164 (*com1-1*) transformed with p21 (*SPO13*); (b) strain Y164 carrying p21 and the wild-type *COM1/SAE2* on p260; (c) same as in b but a cell that had lost p21 before sporulation.

by pulsed-field gel electrophoresis (PFGE; data not shown). Transposon mutagenesis according to SEIFERT *et al.* (1986) was used to localize the complementing DNA sequence within a subcloned 4-kb fragment derived from p260 (see MATERIALS AND METHODS). Deleting a 456-bp *Sac*I-*Sac*I restriction fragment from this insert destroyed its ability to complement the mutation. Sequence analysis of this fragment provided the information to identify the corresponding ORF in the database GenBank using BLAST (ALTSCHUL *et al.* 1990). It represented an ORF of unknown function, designated G1639, that had been sequenced by COGLIEVINA *et al.* (1995) in the course of the yeast sequencing project.

Because the phenotype suggested that G1639 mutants are unable to complete essential meiotic processes after initiation of recombination we refer to this gene as *COM1* for completion of meiotic recombination, an interpretation that proved compatible with all subsequent results obtained. The same gene was identified simultaneously by others (MCKEE and KLECKNER 1997) by complementing their *sae2* mutation. *com1* and *sae2* are allelic, because the DNA sequences that complement each mutation are identical. We will therefore use the double acronym *COM1/SAE2* to indicate this fact. *COM1/SAE2* encodes a 345- amino-acid protein with a predicted molecular weight of 40 kD. It exhibits no significant homology to other known proteins. The protein is rich in charged amino acids, including both positively (6.4% arginine and 9% lysine) and negatively (6.7% aspartic acid and 9.6% glutamic acid) charged amino acids. In a Northern analysis of total yeast RNA the *COM1/SAE2* message was below the level of detection (data not shown). Therefore we assume that the protein is required in small quantities. No regulatory sequences common to many early meiotic genes, such as a URS1 site and UAS_H or T_4C sites, were found in the upstream region of *COM1/SAE2* (MITCHELL 1994; PRINZ *et al.* 1995; GAILUS-DURNER *et al.* 1996). A plasmid (p188) containing *COM1/SAE2* with only 256 bp of the upstream region is still able to complement the spore

lethality phenotype of the *com1-1* mutant, suggesting that all regulatory elements for meiotic expression are contained within this region.

As spore viability of strain Y164 is restored by transformation with p260, we were able to backcross *com1-1* to wild type. These crosses showed that the *com1-1* mutation is recessive and that the sporulation defect segregates 2:2. When homozygous *com1-1, spo13* diploids were constructed, they were unable to produce viable spores as expected. In contrast the homozygous *com1-1, spo11, spo13* triple mutant is meiosis proficient according to our patch assay for spore viability, thus confirming the premise of our screen.

Several disruptions of *COM1/SAE2* were obtained by transposon mutagenesis (see above). Insertions of *Tn3-LEU2* into *COM1/SAE2* were generated in *E. coli* and introduced into SK1 strains. The resulting diploids were sporulated and showed reduced spore formation and a spore viability defect similar to *com1-1.* We constructed a *com1/sae2*-null allele by PCR targeting using *KanMX*4 as a dominant resistance marker (WACH *et al.* 1994), removing 99% of the coding sequence from *COM1/SAE2.* Neither the homozygous *com1::KanMX4*-null mutant (Y359) nor the heterozygous *com1::KanMX4/com1-1* construct produced viable spores, thus confirming that we had indeed cloned *COM1/SAE2.*

In the following investigations the phenotypes of the *com1-1* allele, the *com1-Tn3* disruptions and the *com1::KanMX4*-null mutant were indistinguishable when compared, except that the level of accumulated DSBs in the null mutant and in the disruption of *COM1/SAE2* was noticeably higher than in *com1-1.*

Both meiotic divisions are delayed and spore formation is reduced in com1/sae2 mutants: *com1-1* mutations confer a strong reduction in spore formation when present in an otherwise wild-type SK1 background (Figure 2a). However, on close examination using phase-contrast microscopy spores aberrant in shape and number can be detected in many cells. Their frequency varied from 12 to 42% and depended on culture conditions. The frequency of tetrads with wild-type morphology was invariably smaller than 0.5%. The same results were obtained with the *com1/sae2Δ* mutant. The defect in spore formation was alleviated only slightly in the *spo13, com1-1* double mutant. Whereas the level of unsporulated cells remained unaffected by *spo13* the frequency of morphologically normal asci (dyads) climbed to 7% in the *com1/sae2, spo13* cells. However, when 10 dyads of these 7% were dissected, none gave rise to a visible colony.

Meiotic progression in a liquid culture was followed by DAPI staining of aliquots of ethanol-fixed cells. *com1-1* (Y248, Y355) as well as *com1/sae2Δ* strains (Y359) reached the 50% level of mononucleate cells 2–8 hr later than wild-type SK1 (Figures 3 and 5a). In addition to this retardation of the first meiotic division a minimum delay of 2 hr was found for the second division, separating both divisions by at least 2.5–3 hr instead of

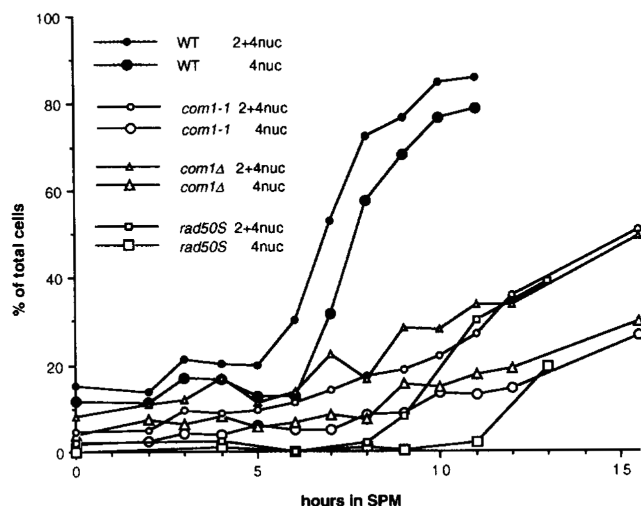

FIGURE 3.—*com1-1, com1/sae2Δ* and *rad50S* mutations cause a similar retardation of meiotic divisions. Percentages of binucleate plus tetranucleate (completed meiosis I) and tetranucleate cells (completed meiosis II) are shown for each strain [wild type (SK1), *com1-1* (Y248), *com1/sae2Δ* (Y359), *rad50S* (Y329)], documenting the rates at which these strains progressed through both meiotic divisions. Except for Y329, every data point represents the average of three independent cultures of the same strain. Whereas for wild type 50% of the cells had completed meiosis I at the 7-hr time point and had after one additional hour also passed meiosis II, the three mutants reached 50% meiosis I completion only after 15 hr (8 hr later than wild type). Meiosis II occurred 3 (rarely up to 4.5) hr later than meiosis I, which represents a meiosis II delay of at least 2 hr relative to wild type.

<1 hr as in wild type. The same holds true for *rad50S* (Y329) in which a delay in the first as well as the second meiotic division similar to *com1/sae2* mutants was observed. In other strain backgrounds (s288c) *rad50S* causes a tight block before the first meiotic division (B. DE MASSY, personal communication).

***com1/sae2* mutants accumulate unresected DSBs during meiotic prophase:** DSBs in the *com1/sae2* mutants were examined at a hotspot near *THR4* because genes involved in the repair of meiotic DSBs are potential targets of our screen. Figure 4 demonstrates that *com1/sae2* mutants accumulate a single species of DNA fragments, indicative of largely unresected breaks similar to *rad50S.* In addition to the prominent DSB-I band two additional bands corresponding to weaker hotspots were also detected by our probe. Only for the *com1::KanMX4*-null mutant did the labeling intensity of the DSB-I fragment reached a level of ~15% of the parental band (18% 6 hr, 15% 10 hr, 14.8% 14 hr). The other mutants arrived at a plateau at the 6-hr time point with ~10% of the parental signal (*com1-1*: 7.5% 6 hr, 9% 10 hr; *rad50S*: 10.4% 6 hr, 11.1% 10 hr; *com1-1, rad50S* double mutant: 7.9% 6 hr, 8.8% 10 hr). We also used the *THR4* probe to hybridize a blot from chromosome-sized DNA separated by PFGE. Because several bands accumulated just as in the *rad50S* mutant (ARBEL *et al.* 1992; GAME 1992), we conclude that DSBs accumulate

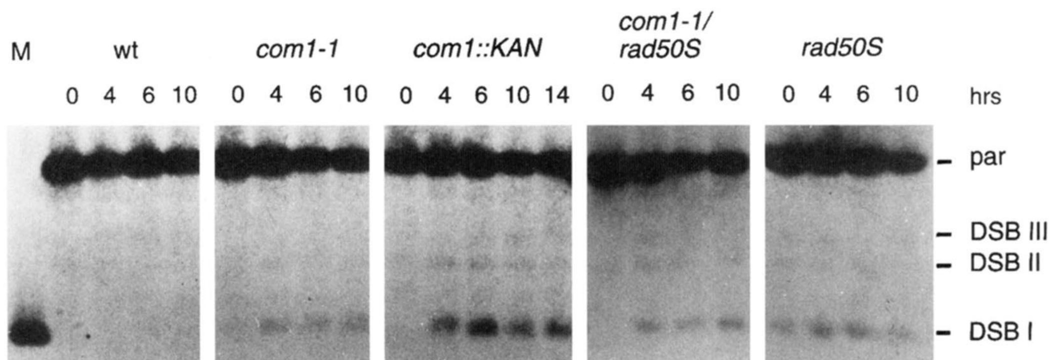

FIGURE 4.—*com1/sae2* mutants accumulate DSBs. DNA was isolated from cells at the indicated times after transfer to SPM and digested with *Bgl*II. par, parental 10.5-kb *Bgl*II restriction fragment. The appearance of sharp bands corresponding to unprocessed DSBs at three sites (5 to 7.5 kb upstream of *THR4*) designated DSB I, II and III was observed. M, size marker obtained by digesting genomic DNA with *Pst*I, resulting in a 4.9-kb fragment highlighted by the probe. The faint band (DSBI) expected for the 4-hr lane of wild type (SK1) was below the level of detection in this experiment. For all mutant strains however (Y248, Y359, Y319 and Y329), DSB I is visible as a prominent ~5-kb band even after 10 or 14 hr in SPM. Two other, weaker double-strand breaks (DSB II and III) share the same characteristics as DSB I. The weak signal at the 0-hr time point of *rad50S* (seen only in this experiment) most likely reflects premature entry of some cells into meiosis during presporulation, a phenomenon known to occur sometimes for SK1 strains.

in *com1/sae2* mutants at many, if not all meiotic hotspots (data not shown).

***com1-1* cells are able to induce meiotic gene conversion 40-fold in return to growth experiments:** *S. cerevisiae* cells that have entered meiotic prophase are nevertheless able to resume vegetative growth when returned to rich medium such as YPD (SHERMAN and ROMAN 1963; ESPOSITO and ESPOSITO 1974). Since *com1-1* cells cannot repair DSBs during meiosis, we asked whether the recombination intermediates would also constitute a problem for vegetative cells. Furthermore, would these intermediates be suited to commit cells to meiotic recombination? Figure 5a demonstrates that *com1-1* cells lose viability at least 5 hr before they undergo the first meiotic division. A drop in colony-forming ability down to 30% at only 4 hr in SPM suggests that the lethal events take place during zygotene or very early pachytene. According to our own observations (Figure 4) and in agreement with the published timing of meiotic events in SK1 strains, the lethal events correspond well with the formation of meiotic DSBs (PADMORE and KLECKNER 1991; GOYON and LICHTEN 1993). We postulate that loss of colony-forming ability is due to the failure of cells to cope with the recombination intermediates accumulating in the absence of Com1/Sae2p in meiotic as well as in mitotic cells and not to failure of chromosome pairing or malsegregation.

Among the surviving cells gene conversion was induced ~40-fold at two different sites (*HIS4, ARG4*), indicating a pathway capable of processing the recombination intermediates in the absence of Com1/Sae2p (Figure 5b). The true meiotic induction of recombination may actually be higher than this for the following reasons. First, because of the low viability at the later time points, the surviving population will be strongly enriched for nonmeiotic cells, reducing the possible fraction of recombinants. Also, cells will lose viability due to a secondary lethal event (when entering meiosis I division), so that accumulation of recombinants as in wild type is not possible. The early time points are least affected by these obscuring processes. We found a steep increase at the first time point analyzed, most strongly pronounced for the *HIS4* heteroallele, where induction was almost similar to wild type. We interpret this to mean that the meiotic lesions of a *com1-1* strain commit the cells to meiotic recombination during return to growth, but that many cells die prior to repairing all the damage. Figure 5c shows that *com1-1* does not interfere with mitotic levels of recombination within the limits of this method and that there is only transient, if any net increase of Arg^+ and His^+ prototrophs. *rad50S* was previously reported not to induce recombination in a return to growth experiment (ALANI *et al.* 1990). To clarify whether the residual recombination competence of *com1-1* cells during return to growth represented a true difference from *rad50S*, we compared both strains in the same experiment. In our hands there is no difference; *rad50S* as well as *com1-1* cells show a similar 30- to 40-fold induction of gene conversion per viable cells (data not shown; K. NAIRZ and F. KLEIN, unpublished results). In addition, we observed a significant loss of viability in *rad50S* cells with progression into meiosis just as observed in *com1/sae2* cells (data not shown).

***com1/sae2* mutants are not hypersensitive to ionizing radiation:** To test whether *COM1/SAE2* is required for repair of DNA damage caused by γ-rays, stationary cultures of *com1-1* (Y248) and *com1::KanMX4*-null mutant (Y359) were exposed to various doses delivered by a ^{60}Co-source and their survival rate was compared to that of wild type. Results shown in Figure 6 demonstrate that radiation resistance in *com1/sae2* mutants was similar to wild type at all doses tested (up to 200 Gy, 70% viability). We conclude that Com1/Sae2p is not required for recombinational repair of radiation-induced DSBs.

However a role of *COM1/SAE2* during vegetative

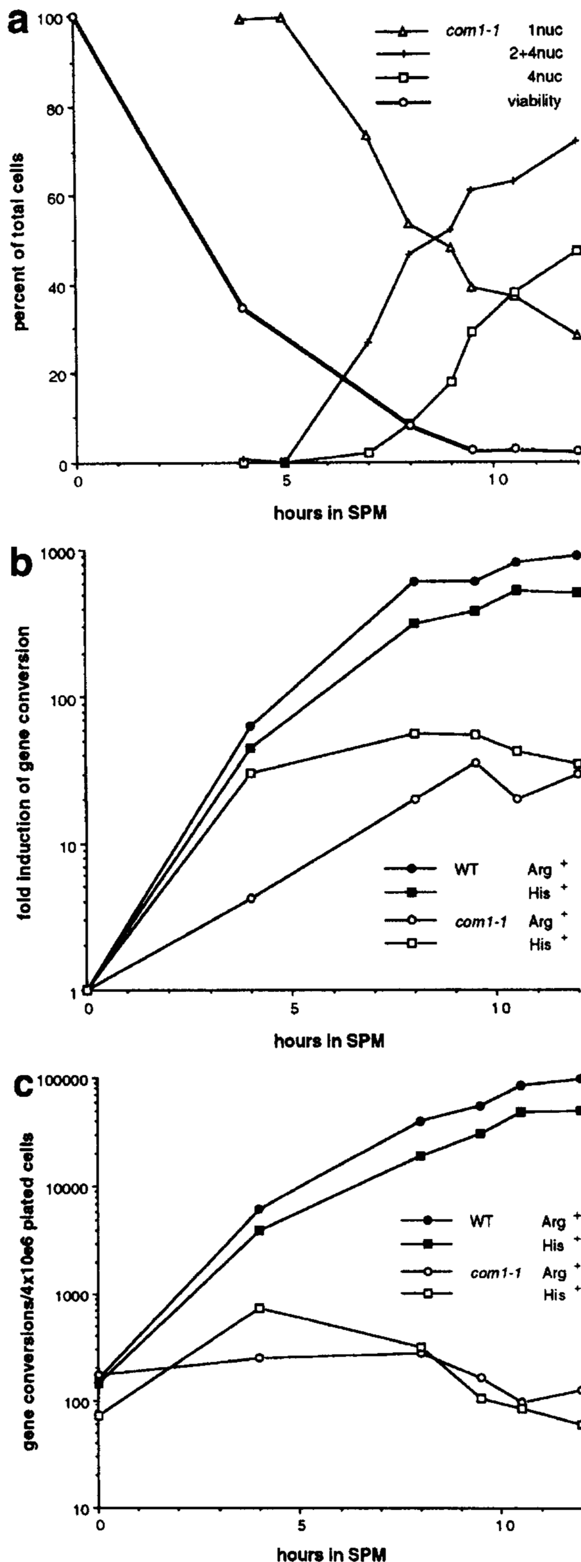

FIGURE 5.—Time course analysis of cell viability and commitment to meiotic recombination. Cells of strain Y355 (*com1-1*) were analyzed for meiotic progression using DAPI staining of ethanol fixed cells, for colony-forming ability on YPD and for induction of gene conversion on SC-arg and SC-his selective plates. (a) Loss of viability precedes meiosis I by at least 5 hr. The decreasing fraction of colony-forming cells (in percent) is compared with the decrease of mononucleate cells (in percent) as a result of cells entering meiosis I division. Kinetics of tetranucleate and binucleate plus tetranucleate cells are also shown. (b) Induction of meiotic gene conversion is reduced to ~40-fold in the *com1-1* mutant compared to 800-fold in wild type. Levels of Arg^+ and His^+ cells per colony-forming cells resulting from gene conversion between heteroalleles are represented relative to their levels at $t = 0$. (c) Yield of Arg^+ and His^+ cells defined as prototrophs per 4×10^6 plated cells. There is no strong net increase of Arg^+ or His^+ prototrophs, because induction seems to be compensated by early loss of colony-forming ability. Mitotic ($t = 0$) prototroph levels in *com1-1* are equal or even slightly lower than in wild type, suggesting that the mitotic gene conversion rate is not increased in *com1-1*.

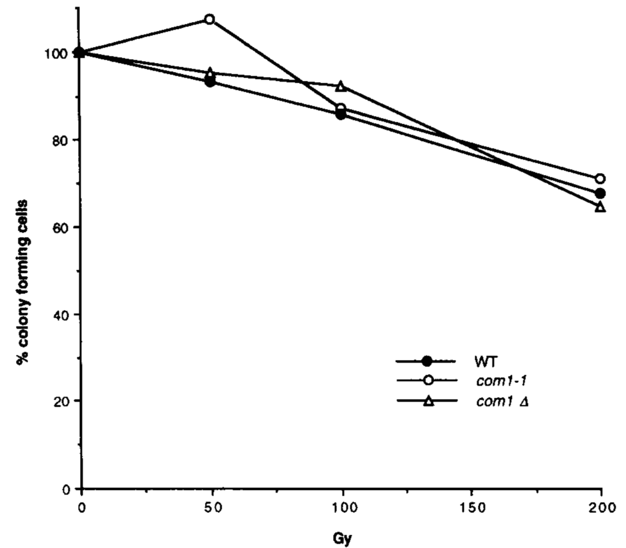

FIGURE 6.—*com1-1* and *com1/sae2Δ* mutants can repair γ-ray-induced DSBs. Wild type (SK1), *com1-1* (Y248) and *com1/sae2Δ* (Y359) were subjected to ionizing radiation and tested for colony-forming ability as described in MATERIALS AND METHODS. Every data point represents the average number of colonies obtained on three plates.

growth cannot be completely excluded, although we did not observe any alteration of the growth rate of *com1/sae2* mutants compared to wild type as judged by colony size on YPD plates at 15, 30 and 37°.

Synapsis of axial elements is strongly reduced in *com1/sae2*: Synapsis is severely reduced in *com1-1* (Y248) as well as in the *com1/sae2* disruption mutants Y235 and Y236. Whereas in wild type we can demonstrate by transmission electron microscopy of silver-stained spread preparations the formation of tripartite SC during pachytene stage (Figure 7a and b), no nuclei with completely synapsed sets of chromosomes were found in the *com1/sae2* mutants. Instead nuclei with long or short axial element fragments but lacking any synapsis appear frequently (Figure 7e). They are extremely rare in wild type. However, in many nuclei some synapsis is initiated and may proceed to involve a considerable part of the chromosome complement (Figure 7c). The typical tripartite SC structure is also formed during this partial synapsis (Figure 7d).

The anti-Zip1p antibody binds strongly only to synapsed regions of meiotic chromosomes (SYM *et al.*

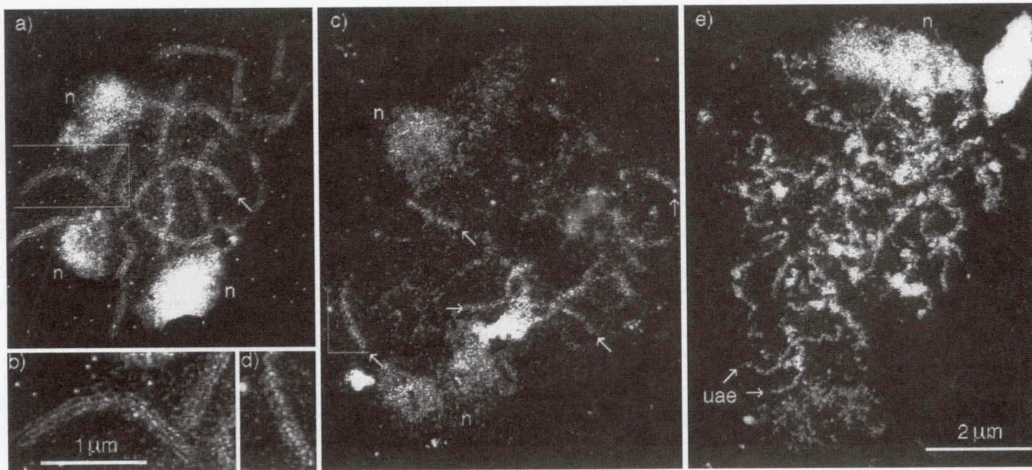

Figure 7.—Synapsis of axial elements is initiated but not completed in *com1/sae2* mutants. Nuclei from cells that were 4 or 6 hr in SPM were spread and analyzed by transmission electron microscopy after silver staining. Negative representation of silver staining was chosen to optimize contrast. (a) Wild-type SK1. (b) Enlargement of a wild-type SC fragment from panel a demonstrating its tripartite structure. (c) Partial synapsis in strain Y236 (*com1-tn3/20*). (d) Tripartite structure of synapsed mutant chromosomes. (e) Nucleus with long axial elements, but devoid of any synapsis in Y236. Arrows point at sites of synapsis, where tripartite structures are visible; n, nucleolus; uae, unsynapsed axial elements.

1993). Therefore this antibody was used in order to monitor synapsis in the mutant. This analysis confirmed that partial but not complete synapsis occurs in the *com1-1* point mutant (Y248), in the disruption mutant *com1-tn3/10* (Y235) and in the homozygous *com1/sae2*-null mutant (Y359). Three selected nuclei are shown in Figure 8 and compared to wild type. Due to excess Zip1 protein not accommodated within the SCs, bright

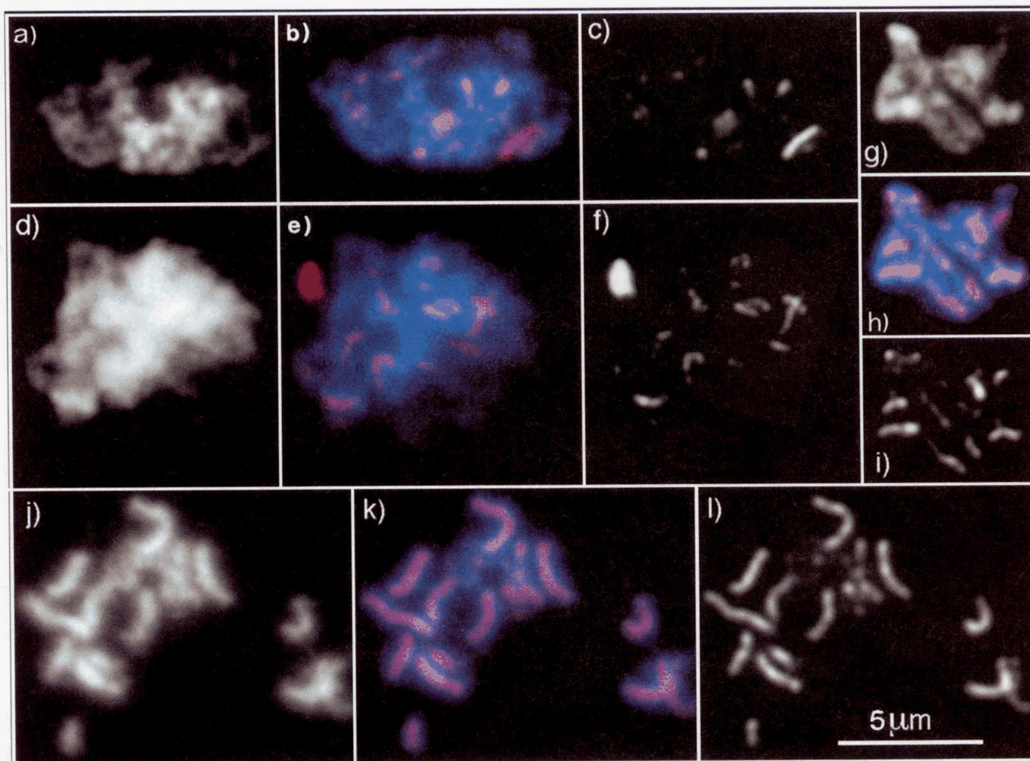

Figure 8.—Demonstration of partial synapsis in *com1/sae2* mutants using synapsis-specific anti-Zip1p antibody. Nuclei from cells that were 4 or 6 hr in SPM were spread and analyzed by indirect immunofluorescence, using FITC-conjugated anti-rabbit antibody to detect the rabbit-anti-Zip1p antibody. (a—c) Nucleus of strain Y248 (*com1-1*) with axial elements, but devoid of any synapsis. (a) DAPI, (b) overlay of DAPI (blue) and FITC (red), (c) FITC. The bright signal represents a Zip1p aggregate typical for a defective meiosis. (d–f) Nucleus of strain Y248 (*com1-1*) with partial synapsis. (d) DAPI, (e) overlay of DAPI and FITC, (f) FITC, showing a bright Zip1p aggregate as in c. (g–i) Extensive synapsis in a nucleus of strain Y235 (*com1-tn3/10*). (g) DAPI, (h) overlay of DAPI and FITC, (i) FITC. (j–l) Complete SC in nucleus of strain Y219 (*COM1/SAE2*). (j) DAPI, (k) overlay of DAPI and FITC, (l) FITC.

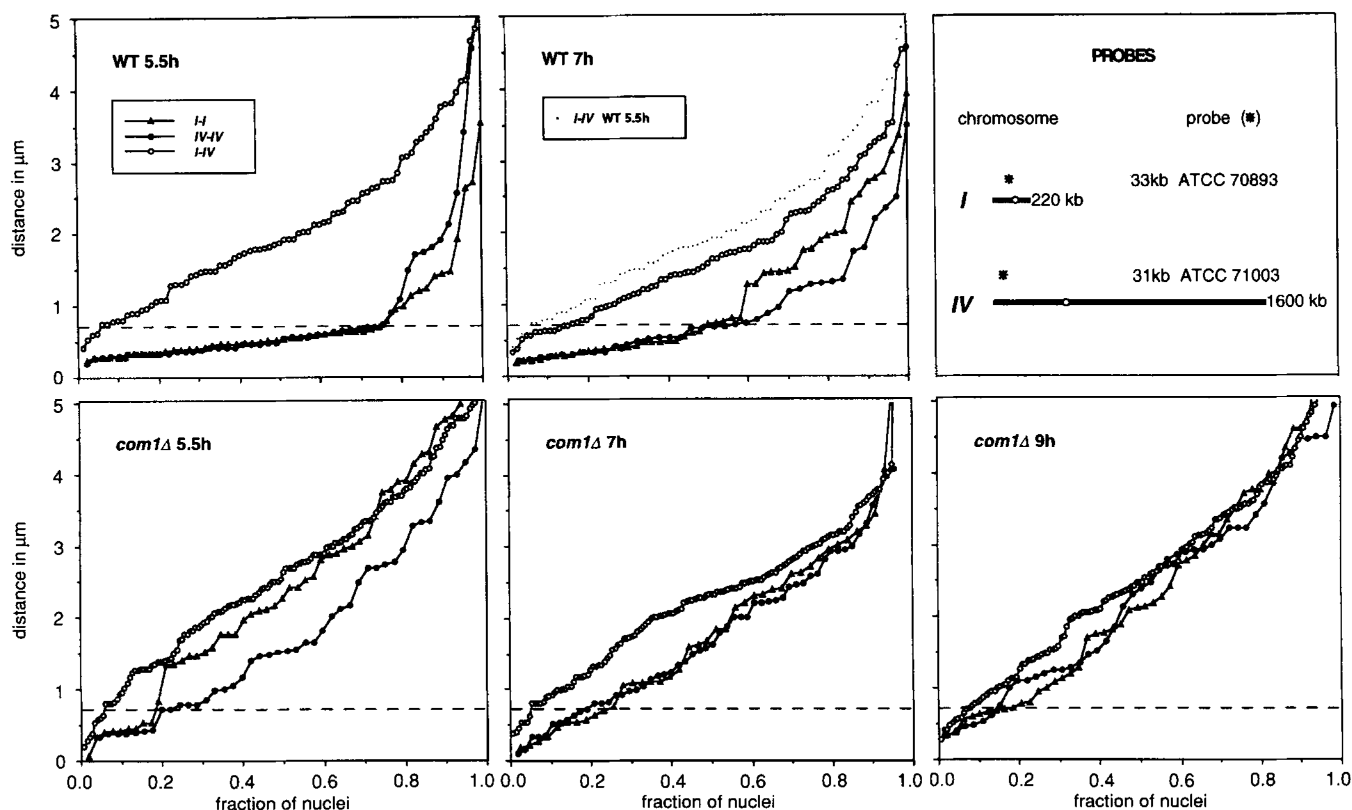

FIGURE 9.—Distance distributions of homologous sequences in *com1-1 vs.* wild type during pachytene. Results of FISH analysis, performed as described in the MATERIALS AND METHODS section. Strain Y361 is wild type; strain Y359 is *com1/sae2*-null. Distances between homologous signals (filled symbols) and heterologous signals (open symbols) were measured using a CCD camera (Photometrics) and appropriate software (IPLab Spectrum, from Signal Analytics). Presentation was chosen similar to that of WEINER and KLECKNER (1994). Distances are given in μm and every data point represents a single measurement. Data are presented in ascending order. Fifty nuclei were analyzed for homologous signals, resulting in 50 measurements for homologous signals, but up to 200 for nonhomologous signals. To compare distributions with different sample sizes the sum of samples was set to 1, making the *x*-axes the fraction of nuclei analyzed. The distribution of random distances is quite superimposable between the different panels except for wild-type 7 hr, where distances are a factor of 1.2 smaller than all the others, most likely because of a slightly lower degree of spreading. The random distances of wild-type 5.5 hr are superimposed on wild-type 7 hr to illustrate this fact. The factor of 1.2 was used to correct all measured distances of wild type at 7 hr in Figure 10. The location of the probes on the two chromosomes is illustrated in one panel. The dashed line marks 0.7 μm, the distance under which signals are assumed to be synapsed.

Zip1p aggregates not associated with chromatin can be frequently observed in the mutant. Up to 30% of the mutant nuclei contained SC fragments (compare Figure 10).

We do not know whether synapsed homologous chromosomes are joined or whether there is random synapsis of axial elements, as can be observed in haploid meiosis (LOIDL *et al.* 1991). To investigate whether there is some pairing of homologous chromosomes in a *com1/sae2* mutant, we performed quantitative FISH analysis on spread meiotic nuclei.

Associations between homologous DNA sequences at a time corresponding to wild-type pachytene are reduced in *com1/sae2* mutants: The results of FISH analysis show a strong shift toward a random distribution of signal distances between homologues in the *com1/sae2Δ* strain (Y359) as compared to the wild type (Y361) (Figure 9). However complete randomization of homologous sequences was not observed in *com1/sae2Δ*. Twenty percent of signals were found closely paired (<0.7 μm) for chromosome I and chromosome IV (see also Figure 10). Apart from this the distributions of signals from the two chromosomes differed for distances > 0.7 μm in two respects. First, a distinct gap in the distribution of distances caused by the underrepresention of distances between 0.55 and 1.35 μm ($p_{\chi}2 < 0.01$) was observed for chromosome I, most strongly pronounced at the 5.5-hr time point for the mutant, but was not found for chromosome IV signals. Second, signals > 0.7 μm from chromosome IV exhibited a concave deviation from the expected random, linear distribution at 5.5 hr for the mutant, indicating the presence of long distance associations. This deviation was not observed for chromosome I signal distances > 1.35 μm, which were distributed essentially at random. Neither the gap nor the concave deviation from linearity were seen at any time for the nonhomologous signals.

The gap in the chromosome I distribution may un-

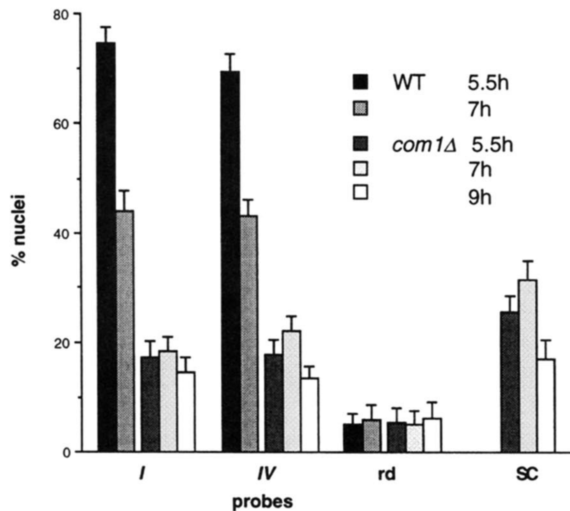

FIGURE 10.—Close homologous pairing is reduced to 25% of wild-type levels in the *com1/sae2*-null mutant. Percent of nuclei with synapsed signals (distance < 0.7 μm) are represented as bars. Groups of bars are labeled I, IV or rd according to the probes used to measure the distances. Distances between the chromosome I probes yield nearly identical values as those between chromosome IV probes for all experiments. rd (random) indicates that distances between nonhomologous signals (*i.e.*, between a chromosome I and a chromosome IV signal) were measured. It represents the internal control for close random chromosomal associations. Up to 20% of homologous signals are synapsed in the *com1/sae2*-null mutant Y359, while only 6% of nonhomologous signals are closer than 0.7 μm. The right group of bars represent the number of nuclei containing partial synapsis in the mutant, as assayed by the synapsis-specific anti-Zip1p antibody (staining was carried out on the same preparation, but prior to FISH). The fraction of cells containing SCs parallels the fraction of homologous associations, suggesting a causal relation between them in the mutant.

cover a critical distance, under which closer pairing occurs very rapidly. Only the smallest chromosomes may be pulled together completely, thus causing underrepresentation of homologous signals within the critical distance. For longer chromosomes the gap may be obscured because the signals may sit within a variable distance from the paired region. As an alternative to the rapid pairing model one may assume that homologous sequences may be excluded from the critical distance. If DNA sequences within the critical distance were under some sort of tension, the release of one component of the forces during spreading could result in their snapping back to a larger distance. This model would be compatible with a more pronounced gap for very small chromosomes as well. Others have observed a very similar gap in the distribution of homologous signals for *rad50S* cells (B. Weiner and N. Kleckner, personal communication).

A possible explanation for the long distance associations is that homologous chromosome IV signals sometimes are held together by partial synapsis or other close associations of sequences not encompassing the site of the signal. The 10-fold shorter chromosome I on the other hand can only be completely paired or not at all. Our data show a decrease of long distance associations at the 7- and 9-hr time points relative to 5.5 hr in the mutant. This could reflect a slight decrease in condensation of the chromosomal linkers that connect the chromosome IV signals or a reduction of the number of close associations per nucleus.

Figure 10 demonstrates that the *com1/sae2*Δ mutant has the capacity to closely pair (<0.7 μm) and possibly synapse a given pair of homologous sequences in ~20% of the nuclei, which is clearly above background (6%). The percentage of nuclei with closely paired signals is identical for both chromosomes and similar to the observed percentage of nuclei containing partial SCs (on average 25% for the three time points taken). In addition the kinetics of nuclei exhibiting paired sequences parallels the kinetics of nuclei containing SC fragments, as assayed by anti-Zip1p antibody staining.

DISCUSSION

We have developed a screen allowing the identification of mutations in essential meiotic genes required after initiation of meiotic recombination by formation of meiosis-specific DSBs. When applied in the absence of *SPO13*, as in the work described here, mutations are recognized that have an essential role in meiosis apart from just ensuring a reductional mode of segregation. However, the screen can be easily broadened to characterize all meiotic lethal mutations involved in recombination as well as reductional segregation by carrying *SPO13*, *SPO11* and *ADE2* on the same plasmid. Our search was complicated by the fact that heterozygous chromosomal mutations apparently also fulfill the requirements of the screen, since they cause recombination-dependent meiotic lethality. Due to these complications, we have not yet saturated the screen and have not yet identified alleles of *rad50S*, *dmc1*, *rad58* or *mei5*. We have, however, discovered an allele of *mre11* that causes a novel, *rad50S*-like meiotic phenotype (K. Nairz and F. Klein, unpublished results).

Applying the described screen, a mutation in a new gene called *COM1/SAE2* was identified. We will refer to mutations as *com1/sae2* when no major differences between our different alleles were observed or otherwise specify the allele. The *COM1/SAE2* gene was cloned and encodes a small, hydrophilic protein with a predicted molecular weight of 40 kD. Com1/Sae2p is thus small enough to enter the nucleus without a nuclear localization signal. It exhibits no significant homology to other proteins and does not contain informative sequence motifs. The *COM1/SAE2* message seems to be transcribed at a very low level, suggesting that the protein is required in small amounts. No regulatory sites known to govern other early meiotic genes could be recognized in the upstream region of *COM1/SAE2*.

The defect caused by *com1/sae2* closely resembles that of *rad50S* (Alani *et al.* 1990) in a number of phenotypes. We have observed a significant delay of both meiotic

divisions in both mutants in SK1 strains. Whereas the delay of meiosis I was quite variable and lasted 2–8 hr, suggesting that its duration depends on exogenous factors, the second meiotic division was less variable (2–4.5 hours). Alternatively, the meiosis II delay may not be variable at all, but a fraction of the cells may have been unable to complete meiosis II division in the experiment shown in Figure 3. Our results suggest that the recombination intermediates accumulating in *com1/sae2* and *rad50S* mutants cause the observed delay in meiotic progression. There is no delay when recombination is not induced, as in *spo11* or *rad50* mutants (F. KLEIN, unpublished results). Therefore the complete absence of DSBs, recombination intermediates, synapsis, recombination products or chiasmata is not sufficient to cause a delay.

On the other hand it seems to be a general phenomenon that mutations that accumulate DSBs form at least partial SCs, such as *rad50S* (ALANI *et al.* 1991; LOIDL *et al.* 1994), *dmc1* (BISHOP *et al.* 1992), *sep1* (BAEHLER *et al.* 1994), *rad51* (ROCKMILL *et al.* 1995) and, as we show in this article, also *com1/sae2*. This could mean that partial synapsis can occur in the absence of recombination or that residual recombination in the mutants is sufficient to induce in some cases quite extensive synapsis. Two possible models explain the observed delay (or arrest). In model 1 accumulation of recombination intermediates possibly together with interacting proteins causes a signal resulting in the delay or arrest of the division. Alternatively, the delay may be a direct consequence of the initiation of slow or nonhomologous synapsis caused by defective recombination. The very recent result by LYDALL *et al.* (1996) that three mitotic DNA-damage checkpoint genes are required for the meiotic arrest seen for *dmc1* and *zip1* mutants in some strains strongly argues for model 1. In addition, meiosis-specific proteins, namely Red1p and Mek1/Mre4p, are needed for the arrest to occur in *rad51* and *zip1* mutants (XU *et al.* 1996).

The fact that *com1/sae2* mutants never or rarely produce morphologically normal spores even in a *spo13* background cannot be explained just by failure of reductional segregation. Aborted or inappropriate recombination must directly or indirectly cause this effect. As the accumulated DSBs decreased only slightly (20%) at the 14-hr time point when 50% of the cells had completed the first meiotic division, the majority of the breaks were still present in these cells. This raises the possibility that only fragments of chromosomes are segregated to the daughter nuclei, which could explain the severe spore formation defect.

In studying the meiotic DSBs close to *THR4* we found the same fragments accumulating at the same time for different *com1/sae2* mutants, as well as for *rad50S*. The bands are not fuzzy, indicative of a block in processing of the DSBs. When compared, the signals accumulating in *com1/sae2*-null mutants were stronger than those in *com1-1* or *rad50S*. One possible explanation is that some DSBs are lost exclusively at early time points in *com1-1* or *rad50S* cells due to residual repair or nonspecific degradation, implying the existence of at least two different species of recombination intermediates. As no decrease of *com1-1* or *rad50S* signal intensities relative to *com1/sae2*-null is observed for the later time points, reduced formation of DSBs but not constant loss can also account for the different levels observed. The *rad50S* allele could cause reduced DSB initiation because the Rad50 protein is involved in DSB formation. If *com1-1*, on the other hand, has a negative effect on DSB formation compared to the null mutant, this would mean that the wild-type *COM1/SAE2* gene antagonizes DSB initiation to a similar extent (because the DSB levels are the same in *rad50S* and *rad50S, com1-1* double mutants). Alternatively the observed differences may reflect a variable efficiency of meiotic induction of the particular cultures not caused by the mutated alleles under investigation.

We did not observe any effect of the *com1/sae2* mutations on the mitotic growth rate on YPD plates monitored at three different temperatures. The frequency of mitotic gene conversion was also not altered in *com1-1* in our experiments, which is again similar to *rad50S* (ALANI *et al.* 1990), thus giving us no indication for mitotic expression of Com1/Sae2p. γ-Irradiation revealed no enhanced radiation sensitivity of the *com1/sae2Δ* mutant. However, others have noticed a slight growth defect and a slight methyl-methane-sulfonate (MMS) sensitivity caused by *com1/sae2* (MCKEE and KLECKNER, 1997).

We have observed early loss of viability of a *com1-1* strain in return to growth experiments at a time corresponding to the zygotene or early pachytene stage and concomitantly with the appearance of DSBs. We find also that *rad50S* is not rescued by return to growth. In contrast no loss of viability was found in *dmc1* mutants (BISHOP *et al.* 1992), which is only partially due to the reversible meiotic arrest preventing entry into the first meiotic division. We rather explain the difference as a consequence of the different recombination intermediates accumulated by these mutants. Whereas *dmc1* blocks with intermediates that have unusually long 3′ single-stranded ends (BISHOP *et al.* 1992), a possible substrate for various repair pathways, *com1/sae2* and *rad50S* mutants were shown to have protected 5′ termini with a protein covalently attached at the DSB sites (KEENEY and KLECKNER 1995). In spite of the dramatic loss of viability due to this 5′ modification an ~40-fold induction of meiotic gene conversion at two sites was found in the small surviving fraction of *com1-1* cells during return to growth. In our hands *rad50S* behaves similarly. We conclude that the 5′ modifications, which obviously can be removed, impede but do not completely prevent recombinational repair during return to growth in *com1-1* and *rad50S* mutants. For the majority of cells, however, the protein-conjugated DNA ends may either exceed the capacity of the repair system and

may remain unrepaired or, alternatively, may lead to lethal errors in the course of repair.

We and others have shown that mutants homozygous for *spo11*, *rad50* or *rad50S* are still capable of residual homologous pairing (Loidl *et al.* 1994; Weiner and Kleckner 1994). The pairing detected in *com1/sae2*Δ further confirms the notion that homologous pairing is at least partially independent of DSB processing. The amount of close homologous pairing in *com1/sae2*Δ is about one third that of wild type at a time corresponding to wild-type pachytene and at the same time clearly different from random. The extensive pairing observed in *com1/sae2*Δ or in *rad50S* compared to mutants not initiating DSBs (such as *rad50*Δ or *spo11*) could be explained by SC-stabilized homologous associations that had been established earlier. This might also explain why the extent of pairing at different times in the *com1/sae2*Δ mutant parallels the extent of SC formation. Alternatively, the pairing process itself may be affected differently in these mutants.

With the exception of strains carrying mutations in potential SC components such as Zip1p, Red1p and Hop1p synapsis of axial elements can be observed in all strains able to induce meiotic DSBs. In contrast no synapsis at all is found in strains where meiotic DSBs are absent. We have recently found evidence that meiotic DSBs act as a trigger, which is necessary to initiate synapsis (F. Klein, A. Malkova and J. Haber, unpublished results). Our results are in agreement with these findings, constituting yet another example in which SC is formed, while only unprocessed DSBs can be detected, suggesting that their presence is a prerequisite for partial synapsis. We further conclude that *COM1/SAE2* is not required for synapsis and tripartite SC formation, whereas full levels of SC formation as well as of homologous pairing are very well dependent on *COM1/SAE2* function.

Based on the observed phenotypes of the *com1/sae2* mutants, we propose that Com1/Sae2p is either part of, or activating a component of, the recombination machinery involved in processing meiotic DSBs, beginning with the removal of the 5′-attached protein. Thus an obvious possibility is that the Com1/Sae2p protein actually represents the enzyme required to release this protein from the 5′ terminus of the DSB (in conjunction with Rad50p and possibly additional factors). However, we favor the idea that Com1/Sae2p acts indirectly, because the *com1/sae2*-null mutant does not inhibit DSB formation. In contrast null mutants of genes presumed to be physically present at meiotic DSB hotspots, because they physically or genetically interact with each other (*i.e.*, *RAD50*, *MRE11*, *XRS2*, and *MER2*; Ogawa *et al.* 1995) all completely abolish DSB formation. For these reasons Com1/Sae2p is probably not a component of the early recombination complex defined by the above genes and clearly is not necessary for their expression. It could still join the complex at a later stage to exert its function directly. More likely, however, it activates one or more essential components of the recombination complex through modifications. We intend to solve this question by analysis of suppressors of *com1/sae2* and by a two hybrid approach. Should functional homologues of *COM1/SAE2* exist in other organisms, it would be of interest to investigate whether a knockout would also result in accumulation of meiotic DSBs in organisms other than *S. cerevisiae.*

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Note added in proof: Recently the protein covalently attached to the 5′ ends of the DSBs in *rad50S* mutants has been shown to be Spo11 (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). *rad58* (*xrs4*) is an allele of *MRE11* (H. Tsubouchi and H. Ogawa, unpublished results).

LITERATURE CITED

Ajimura, M., S. H. Leem and H. Ogawa, 1993 Identification of new genes required for meiotic recombination in *Saccharomyces cerevisiae.* Genetics **133:** 51–66.

Alani, E., R. Padmore and N. Kleckner, 1990 Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell **61:** 419–436.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers and D. J. Lipman, 1990 Basic local alignment search tool. J. Mol. Biol. **215:** 403–410.

Arbel, T., A. Sherman, M. Goldway, S. Klein and G. Simchen, 1992 Multiple sites for double-strand breaks in whole meiotic chromosomes of *Saccharomyces cerevisiae.* EMBO J. **11:** 3441–3447.

Baehler, J., G. Hagens, G. Holzinger, H. Scherthan and W. D. Heyer, 1994 *Saccharomyces cerevisiae* cells lacking the homologous pairing protein p175SEP1 arrest at pachytene during meiotic prophase. Chromosoma **103:** 129–141.

Bishop, D. K., D. Park, L. Xu and N. Kleckner, 1992 *DMC1*: a meiosis-specific yeast homolog of *E. coli recA* required for recombination, synaptonemal complex formation, and cell cycle progression. Cell **69:** 439–456.

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.

Chepurnaya, O. V., S. A. Kozhin, V. T. Peshekhonov and V. G. Korolev, 1995 *RAD58* (*XRS4*)—a new gene in the *RAD52* epistasis group. Curr. Genet. **28:** 274–279.

Coglievina, M., I. Bertani, R. Klima, P. Zaccaria and C. V. Bruschi, 1995 The DNA sequence of a 7941 bp fragment of the left arm of chromosome *VII* of *Saccharomyces cerevisiae* contains four open reading frames including the multicopy suppressor gene of the *pop2* mutation and a putative serine/threonine protein kinase gene. Yeast **11:** 767–774.

De Massy, B., and A. Nicolas, 1993 The control in *cis* of the position and the amount of the *ARG4* meiotic double-strand break of *Saccharomyces cerevisiae.* EMBO J. **12:** 1459–1466

De Massy, B., V. Rocco and A. Nicolas, 1995 The nucleotide map-

ping of DNA double-strand breaks at the *CYS3* initiation site of meiotic recombination in *Saccharomyces cerevisiae*. EMBO J. **14:** 4589–4598.

ESPOSITO, M. S., and R. E. ESPOSITO, 1974 Genetic recombination and commitment to meiosis in Saccharomyces. Proc. Natl. Acad. Sci. USA **71:** 3172–3176.

GAILUS-DURNER, V., J. X. XIE, C. CHINTAMANENI and A. K. VERSHON, 1996 Participation of the yeast activator Abf1 in meiosis-specific expression of the *HOP1* gene. Mol. Cell. Biol. **16:** 2777–2786.

GALBRAITH, A., and R.E. MALONE, 1992 Characterization of *REC104*, a gene required for early meiotic recombination in the yeast *Saccharomyces cerevisiae*. Dev. Genet. **13:** 392–402.

GAME, J. C., 1992 Pulsed-field gel analysis of the pattern of DNA double-strand breaks in the *Saccharomyces* genome during meiosis. Dev. Genet. **13:** 485–497.

GIETZ, D., A. ST. JEAN, R. A. WOODS and R. H. SCHIESTL, 1992 Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. **20:** 1425.

GIROUX, C. N., S. L. MISRA, M. MODESTI and J. P. GUERREIRO, 1993 Decision points and the genetic control of meiotic progression in yeast, pp. 1–17 in *Meiosis II: Contemporary Approaches to the Study of Meiosis*, edited by F. P. HASELTINE and S. HEYNER. AAAS Press, Washington, D.C.

GOLDWAY, M., A. SHERMAN, D. ZENVIRTH, T. ARBEL and G. SIMCHEN, 1993 A short chromosomal region with major roles in yeast chromosome *III* meiotic disjunction, recombination and double-strand breaks. Genetics **133:** 159–169.

GOYON, C., and M. LICHTEN, 1993 Timing of molecular events in meiosis in *Saccharomyces cerevisiae*: stable heteroduplex DNA is formed late in meiotic prophase. Mol. Cell. Biol. **13:** 373–82.

HOLLINGSWORTH, N. M., and B. BYERS, 1989 *HOP1*: a yeast meiotic pairing gene. Genetics **121:** 445–462.

HOLLINGSWORTH, N. M., L. PONTE and C. HALSEY, 1995 MSH5, a novel MutS homolog, facilitates meiotic reciprocal recombination between homologs in *Saccharomyces cerevisiae* but not mismatch repair. Genes Dev. **9:** 1728–1739.

INTERTHAL, H., C. BELLOCQ, J. BAHLER, V. I. BASHKIROV, S. EDELSTEIN *et al.*, 1995 A role of Sep1 (= Kem1, Xrn1) as a microtubule-associated protein in *Saccharomyces cerevisiae*. EMBO J. **14:** 1057–1066.

KANE, S. M., and R. ROTH, 1974 Carbohydrate metabolism during ascospore development in yeast. J. Bacteriol. **118:** 8–14.

KEENEY, S., and N. KLECKNER, 1995 Covalent protein-DNA complexes at the 5′ strand termini of meiosis-specific double-strand breaks in yeast. Proc. Natl. Acad. Sci. USA **92:** 11274–11278.

KOLODNER, R., D. H. EVANS and P. T. MORRISON, 1987 Purification and characterization of an activity from *Saccharomyces cerevisiae* that catalyzes homologous pairing and strand exchange. Proc. Natl. Acad. Sci. USA **84:** 5560–5564.

LIU, J., T. C. WU and M. LICHTEN, 1995 The location and structure of double-strand DNA breaks induced during yeast meiosis: evidence for a covalently linked DNA-protein intermediate. EMBO J. **14:** 4599–4608.

LOIDL, J., K. NAIRZ and F. KLEIN, 1991 Meiotic chromosome synapsis in a haploid yeast. Chromosoma **100:** 221–228.

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.

LYDALL, D., Y. NIKOLSKY, D. K. BISHOP and T. WEINERT, 1996 A meiotic recombination checkpoint controlled by mitotic checkpoint genes. Nature **383:** 840–843.

MALONE, R. E., S. BULLARD, M. HERMISTON, R. RIEGER, M. COOL *et al.*, 1991 Isolation of mutants defective in early steps of meiotic recombination in the yeast *Saccharomyces cerevisiae*. Genetics **128:** 79–88.

MCKEE, A. H. Z., and N. KLECKNER, 1997 A general method for identifying recessive diploid-specific mutations in *Saccharomyces cerevisiae*, its application to the isolation of mutants blocked at intermediate stages of meiotic prophase and characterization of a new gene, *SAE2*. Genetics **146:** 797–816.

MITCHELL, A., 1994 Control of meiotic gene expression in *Saccharomyces cerevisiae*. Microbiol. Rev. **58:** 56–70

NICOLAS, A., D. TRECO, N. P. SCHULTES and J. W. SZOSTAK, 1989 An initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae*. Nature **338:** 35–9.

OGAWA, H., K. JOHZUKA, T. NAKAGAWA, S. H. LEEM and A. H. HAGIHARA, 1995 Functions of the yeast meiotic recombination genes, *MRE11* and *MRE2*. Adv. Biophys. **31:** 67–76.

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

PRINZ, S., F. KLEIN, H. AUER, D. SCHWEIZER and M. PRIMIG, 1995 A DNA binding factor (UBF) interacts with a positive regulatory element in the promoters of genes expressed during meiosis and vegetative growth in yeast. Nucleic Acids Res. **23:** 3449–3456.

ROCKMILL, B., and G. S. ROEDER, 1988 *RED1*: a yeast gene required for the segregation of chromosomes during the reductional division of meiosis. Proc. Natl. Acad. Sci. USA **85:** 6057–6061.

ROCKMILL, B., and G. S. ROEDER, 1990 Meiosis in asynaptic yeast. Genetics **126:** 563–574.

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.

ROSE, D., W. THOMAS and C. HOLM, 1990a Segregation of recombined chromosomes in meiosis I requires DNA topoisomerase II. Cell **60:** 1009–1017.

ROSE, M. D., F. WINSTON and P. HIETER, 1990b *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

ROTHSTEIN, R. J., 1983 One step gene disruption in yeast. Methods Enzymol. **101:** 202–211.

SCHWACHA, A., and N. KLECKNER, 1994 Identification of joint molecules that form frequently between homologs but rarely between sister chromatids during yeast meiosis. Cell. **76:** 51–63.

SEIFERT, H. S., E. Y. CHEN, M. SO and F. HEFFRON, 1986 Shuttle mutagenesis: a method of transposon mutagenesis for *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **83:** 735–739.

SHERMAN, F., and H. ROMAN 1963 Evidence for two types of allelic recombination in yeast. Genetics **48:** 255–261.

SHINOHARA, A., and T. OGAWA, 1995 Homologous recombination and the roles of double-strand breaks. Trends Biochem. Sci. **20:** 387–391.

SHUSTER, E. O., and B. BYERS, 1989 Pachytene arrest and other meiotic effects of the start mutations in *Saccharomyces cerevisiae*. Genetics **123:** 29–43.

SIKORSKY, R. S., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.

SUGAWARA, N., E. L. IVANOV, J. FISHMAN-LOBELL, B. L. RAY, X. WU *et al.*, 1995 DNA structure-dependent requirements for yeast RAD genes in gene conversion. Nature **373:** 84–86.

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, 1994 Crossover interference is abolished in the absence of a synaptonemal complex protein. Cell **79:** 283–292.

SYM, M., ENGEBRECHT, J.A. and G.S. ROEDER, 1993 ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. Cell **72:** 365–378.

TISHKOFF, D. X., A. W. JOHNSON and R. D. KOLODNER, 1991 Molecular and genetic analysis of the gene encoding the *Saccharomyces cerevisiae* strand exchange protein Sep1. Mol. Cell. Biol. **11:** 2593–2608.

TISHKOFF, D. X., B. ROCKMILL, G. S. ROEDER and R. D. KOLODNER, 1995 The *sep1* mutant of *Saccharomyces cerevisiae* arrests in pachytene and is deficient in meiotic recombination. Genetics **139:** 495–509.

WACH, A., A. BRACHAT, R. PÖHLMANN and P. PHILIPPSEN, 1994 New heterologous modules for classical or PCR-based gene disruptions. Yeast **10:** 1793–1808.

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., B. M. WEINER and N. KLECKNER, 1996 Meiotic cells monitor the status of the interhomolog recombination complex. Genes Dev. **11:** 106–118.

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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