Mutations in *Saccharomyces cerevisiae* That Block Meiotic Prophase Chromosome Metabolism and Confer Cell Cycle Arrest at Pachytene Identify Two New Meiosis-Specific Genes *SAE1* and *SAE3*

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Manuscript received September 18, 1996
Accepted for publication March 27, 1997

ABSTRACT

Two new meiosis-specific genes, *SAE1* and *SAE3*, have been identified in a screen for mutations that confer an intermediate block in meiotic prophase. Such mutations confer a block to spore formation that is circumvented by addition of a mutation that eliminates meiotic recombination initiation and other aspects of chromosome metabolism, *i.e.*, *spo11*. We show that *sae1-1* and *sae3-1* mutations each confer a distinct defect in meiotic recombination. *sae1-1* produces recombinants but very slowly and ultimately to less than half the wild-type level; *sae3-1* makes persistent hyper-resected meiotic double-strand breaks and has a severe defect in formation of recombinants. Both mutants arrest at the pachytene stage of meiotic prophase, *sae1-1* temporarily and *sae3-1* permanently. The phenotypes conferred by *sae3-1* are similar to those conferred by mutation of the yeast RecA homologue *DMC1*, suggesting that *SAE3* and *DMC1* act at the same step(s) of chromosome metabolism. These results provide further evidence that intermediate blocks to prophase chromosome metabolism cause cell-cycle arrest. *SAE1* encodes a 208-residue protein homologous to vertebrate mRNA cap-binding protein 20. *SAE3* corresponds to a meiosis-specific RNA encoding an unusually short open reading frame of 50 codons.

DURING meiosis, a single round of DNA synthesis is followed by two rounds of chromosome segregation, the meiosis I and meiosis II divisions. The first division has an aspect that is unique to meiosis: homologous pairs of chromosomes disjoin from one another. This process, which reduces the genetic complexity of the two daughter nuclei, does not occur during the mitotic cell cycle. Sister chromatids disjoin in the second meiotic division (MII), a process that resembles mitosis.

A number of processes that are unique to meiosis occur in a cell that is preparing for a reductional division. Most of these events occur during meiotic prophase and can be divided roughly into four categories: homologous chromosome pairing, recombination, synaptonemal complex (SC) formation and cell cycle progression. In diploid yeast cells, homologous chromosomes are paired as a regular feature of vegetative growth and in G1/G0 cells preparing to enter meiosis (WEINER and KLECKNER 1994; B. WEINER and N. KLECKNER, unpublished results). Pairing interactions are disrupted during meiotic S-phase and are restored in early prophase (WEINER and KLECKNER 1994; GOLDMAN and LICHTEN 1996), independent of and prior to initiation of recombination by double-strand breaks (DSBs) (WEINER and KLECKNER 1994; XU and KLECKNER 1995; BULLARD *et al.* 1996; ROCCO and NICOLAS 1996).

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Most or all meiotic recombination in yeast is initiated at the chemical level by DSBs, which occur at preferred "hot spots" (LICHTEN and GOLDMAN 1995; ROEDER 1995; KLECKNER 1996). DSBs are converted to double Holliday junctions and thence to mature recombination products, likely both crossovers and noncrossovers, which appear coordinately late in prophase (PADMORE *et al.* 1991; SCHWACHA and KLECKNER 1995; STORLAZZI *et al.* 1996).

The SC is a prominent structural aspect of meiotic prophase common to virtually all organisms (VON WETTSTEIN *et al.* 1984). Bulk SC formation occurs approximately concomitant with the transition from DSBs to double Holliday junctions (SCHWACHA and KLECKNER 1994). Furthermore, mutations that are defective in making this transition exhibit a delay in SC formation. These and other observations suggest that a transition through this point in the interhomologue interaction pathway is required for efficient nucleation of SC formation (ROEDER 1990; KLECKNER *et al.* 1991; STORLAZZI *et al.* 1996). Mature recombinants appear at about the time that SC disassembly begins, but SC disassembly is not required for maturation of most and perhaps all crossovers (ROSE and HOLM 1993; XU *et al.* 1997).

Finally, cell cycle progression out of prophase is subject to important regulatory controls. A mutation in the catalytic subunit of maturation promoting factor (*cdc28*) causes chromosomes to arrest in the pachytene SC configuration (SHUSTER and BYERS 1989); an identical phenotype is conferred by mutations in a meiosis-

specific gene *NDT80,* proposed to encode a meiosis-specific modulator of Cdc28p function (Xu and Kleckner 1995). Furthermore, the transition out of prophase is subject to regulatory arrest; if appropriate prior conditions have not been met, separation of spindle pole bodies is blocked or delayed and, in many cases, spore formation is defective (*e.g.,* Byers 1981; Alani *et al.* 1990; Rose *et al.* 1990; Bishop *et al.* 1992; Sym *et al.* 1993; Tishkoff *et al.* 1995).

Of particular relevance to the current work is the fact that mutations that cause defects in intermediate stages of prophase chromosome metabolism cause arrest at the end of prophase, usually but not always with chromosomes at the pachytene stage. It seems that cells are sensing that some process is aberrant, rather than the absence of some important process, because mutations that cause severe defects in initiation of recombination and development of prophase chromosomes do not cause arrest; moreover, such "early block" mutations alleviate the arrest caused by mutations that confer an intermediate block (*e.g.,* Alani *et al.* 1990; Bishop *et al.* 1992; Sym *et al.* 1993).

The weight of current evidence suggests that the cell cycle monitors the status of the meiotic recombination process and delays progression out of prophase if this process is aberrant, although more complicated possibilities are not rigorously excluded (Lydall *et al.* 1996; Storlazzi *et al.* 1996; Xu *et al.* 1997). Both meiosis-specific features and components of the mitotic DSB repair monitoring process are involved (Lydall *et al.* 1996; Xu *et al.* 1997). It has been proposed however that, rather than detecting the existence of a defect *per se,* the meiotic recombination machinery emits a signal that inhibits progression out of pachytene until the recombination process has completed certain critical steps and/or is entirely finished (Xu *et al.* 1997).

Genetic approaches to the identification of additional intermediate block mutations were initiated at a time when the only such known mutations were *rad52* and "S" alleles of *RAD50,* whose cell cycle arrest and spore formation defect are alleviated by an upstream early block mutation, such as *spo11*Δ or *rad50*Δ (Malone 1983; Alani *et al.* 1990). We reasoned that a search for additional mutations that failed to make spores in an otherwise wild-type background but not in a *spo11*Δ background would identify additional intermediate block recombination mutations. The details of our search are described in McKee and Kleckner (1997); Prinz *et al.* (1997) independently developed a similar approach. Our analysis has identified not only new *rad50S* alleles but also alleles of the *DMC1* gene (found in the interim to be of the intermediate block type; Bishop *et al.* 1992) and alleles of three new genes, named *SAE1, SAE2* and *SAE3* (for sporulation in the absence of *spo eleven*). *SAE2* was also identified independently by Prinz *et al.* (1997) using a logically analogous strategy and was named *COM1* by this group. *SAE2/COM1* is not a meiosis-specific gene and is described in McKee and Kleckner (1997) and in Prinz *et al.* (1997).

Mutations in *SAE1* or *SAE3* confer defects in meiotic recombination as well as transient (*sae1-1*) or permanent (*sae3-1*) arrest in the pachytene stage of meiotic prophase. Phenotypic analysis of these mutants and transcription analysis of *SAE3* suggest that both genes are meiosis specific. Molecular analysis of the *SAE1* and *SAE3* genes is described.

MATERIALS AND METHODS

Media, strains and plasmids: All yeast strains used in this work are of the SK1 strain background (Kane and Roth 1974) (Table 1). Strains containing *sae* mutations were backcrossed by wild type from the original isolates: NKY2673, five times; NKY2655 and NKY2664, four times; NKY2745, four times; and NKY2328, twice. Media preparation and genetic procedures generally followed Sherman *et al.* (1986). Sporulation medium used in agar plates (SPM) contained 1% potassium acetate, 0.1% yeast extract, 0.1% glucose, 2% bacto agar and 100 mg/liter adenine sulfate. Sporulation medium used in time course experiments and to make meiotic RNA was 0.3% potassium acetate, 0.02% raffinose, and, if the strain in use had any amino acid auxotrophies, 50 mg/liter of the corresponding amino acid.

Isolation of *sae* mutants: *sae* mutants were found by a two-step process. Diploid cell lines that failed to make spores when incubated on sporulation medium were identified. The *SPO11* gene was removed from the cell lines in this pool, and the resulting cells were rescreened for spore formation proficiency. Mutants that failed to make spores except in the absence of the *SPO11* gene were named *sae* (sporulation in the absence of *SPO eleven*). The diploid cell lines used in this screen were made by treating a homothallic diploid strain with EMS, sporulating it, isolating random spores and growing these to colonies. Crosses and other genetic manipulations of homothallic cells were simplified by inclusion of a conditional sterile mutation, *ste7-1.* Sporulation was determined using a UV fluorescence assay. These techniques and the screen are described in McKee and Kleckner (1997).

RNA isolation: Total cellular RNA was isolated for use in Northern analysis and primer extension reactions. All solutions used for preparation of RNA were treated with 0.01% diethylpyrocarbonate (DEPC) except those containing Tris, which were made by adding solid Tris to DEPC-treated water. From vegetative or sporulating cultures of density 1.5×10^7 cells/ml, 10-ml aliquots were cooled on ice, pelleted at 5000 $\times$ *g* and washed once with 10 ml of ice-cold water and once with 2 ml of cold RNA buffer (50 mM Tris-Cl, 100 mM $MgCl_2$, 10 mM EDTA, pH 7.4). The cell pellet was resuspended in 300 μl of RNA buffer and was transferred to a 1.5-ml screw-cap tube. These tubes were frozen at −70° until subsequent steps were performed. After thawing the cells, glass beads (212–300 μm, Sigma) were added to the level of the meniscus, and cells were lysed at highest speed for six rounds of 15 sec, with cooling on ice between each round. To this was added 300 μl of RNA buffer and 60 μl of 10% SDS followed by 0.5 volume 65° phenol. This tube was vortexed four times, 20 sec each, with heating to 65° between vortexing. The resulting mixture was centrifuged at 12,000 $\times$ *g* and the aqueous phase was recovered. Both the phenol and aqueous phases were re-extracted with 500 μl RNA buffer and the aqueous phases were pooled. The pooled aqueous phases were extracted with phenol and then twice extracted with 94% $CHCl_3$, 4% isoamylalcohol. The RNA was then precipitated using 0.1 volume 2 M sodium acetate, 2 volumes ethanol.

TABLE 1

Yeast strains

Strain	Relevant genotype
NKY1551	*MATa his4X::LEU2(Bam)-URA3 ho::LYS2* *MATα his4B::LEU2 ho::LYS2*
NKY1552	Same as NKY1551
NKY1879	*MATa dmc1Δ::LEU2 his4X::LEU2(Bam)-URA3 ho::LYS2* *MATα dmc1Δ::LEU2 his4B::LEU2 ho::LYS2*
NKY2328	*MATa sae1-1 ste7-1 HO ura3* *MATα sae1-1 ste7-1 HO ura3*
NKY2655	*MATa sae3-1 ste7-1 HO his4X::LEU2(Bam)-URA3* *MATα sae3-1 ste7-1 HO his4B::LEU2*
NKY2664	*MATa sae1-1 ste7-1 HO his4X::LEU2(Bam)-URA3* *MATα sae1-1 ste7-1 HO his4B::LEU2*
NKY2673	*MATa sae3-1 dmc1Δ::LEU2 his4X::LEU2(Bam)-URA3 ho::LYS2* *MATα sae3-1 dmc1Δ::ARG4 his4B::LEU2 ho::LYS2*
NKY2745	*MATa sae3-1 ste7-1 HO ura3* *MATα sae3-1 ste7-1 HO ura3*
NKY2973	*MATa sae3Δ::hisG-URA3-hisG ste7-1 ura3 his4X::LEU2(Bam)-URA3* *MATα sae3-1 ste7-1 ura3 his4B::LEU2*
NKY2975	*MATa SAE1::URA3 HO ste7-1 ura3Δ::hisG* *MATα sae1-1 HO ste7-1 ura3*

Primer extension: Primer extensions were performed as follows: 50 μg of RNA was mixed with 2 ng ^{32}P-kinased oligo in 15 μl of 1.5 M KCl, 0.12 M Tris-Cl pH 8.0, 1.2 mM EDTA and was heated to 90° for 3 min. The primer was then allowed to anneal at its calculated melting temperature for 30 min. Liquid was then collected at the bottom of the tube by a brief centrifugation and 50 μl of 70 mM Tris-Cl pH 8.0, 14 mM $MgCl_2$, 0.7 mM EDTA, 0.7 mM DTT, and 1.0 mM of each of the four dNTPs were added. Twenty microliters of avian myeloblastosis virus (AMV) reverse transcriptase was added and the mixture was incubated at 45° for 30 min. Following this extension reaction, RNA was hydrolyzed by the addition of 2 μl of 2.5 M NaOH, 25 mM EDTA and was incubated at 90° for 3 min. The liquid in the tube was collected by centrifugation and then filtered through a 1.0-ml Sephadex G25 column equilibrated in 10 mM Tris-Cl, 1 mM EDTA, pH 7.4 (TE). Liquid was evaporated in a evacuated speedvac, and the resulting pellet was resuspended in 20 μl of formamide, 0.25% xylene cyanol and 0.25% bromophenol blue. The extension products were analyzed on a 6% polyacrylamide gel containing TBE buffer and 50% w/v urea using a DNA sequencing ladder made from the same primer as size markers.

Northern analysis: RNA was size fractionated by formaldehyde gel (SAMBROOK *et al.* 1989) and was transferred to Nytran nylon membrane (S&S) according to manufacturer's instructions. Probes were prepared by random hexanucleotide priming of double-stranded plasmid DNA and incorporation of α-^{32}P dCTP (800 Ci/mmol) or by treating oligonucleotides with T4 polynucleotide kinase and ^{32}P-γATP (>6000 Ci/mmol). Hybridization to the Nytran membrane using double-stranded probes was done according to the manufacturer's instructions using the "standard protocol" in 50% formamide. Hybridization with oligonucleotide probes was performed after soaking the membrane in prehybridization solution (6× SSC, 10× Denhardt's solution, 0.2% SDS) for 2 hr at 65°. Hybridization solution was the same except the Denhardt's solution was reduced to 5×. Hybridization solution was filtered through a Millipore 0.22-μm filter before addition to the blot. One nanogram of ^{32}P-kinased oligo was added to the hybridization solution, and the blot was incubated with shaking at 22° for 18 hr. Following this incubation the membrane was washed three times in 6× SSC, 0.2% SDS, twice at 22° and finally at 10° below the calculated melting temperature of the oligonucleotide. For both oligo and double-stranded probes the blot was exposed to X-ray film with an intensifying screen at −70°.

Time course analysis: Southern blots, DAPI staining of meiotic nuclei and return to growth analysis are described in MCKEE and KLECKNER (1997). Intensity of radioactivity on Southern blots was determined using a Fuji phosphoimager. To examine meiotic nuclei for SC using silver stain and the light microscope, meiotic nuclei were prepared and spread on glass microscope slides by the method of KLEIN *et al.* (1992) with the following changes: To prepare cells for spheroplasting, 10 ml of meiotic culture was centrifuged at 800 × *g* for 2 min and the cells were resuspended in 2.5 ml of 200 mM Tris-Cl, pH 7.5. To this was added 50 μl of 1.0 M DTT. This suspension was incubated for 2 min at room temperature, centrifuged as above and resuspended in 1.5 ml of 0.5 M KCl, 50 mM Tris-Cl pH 7.5. To this was added 10 μl of 20 mg/ml Zymolase 100T (ICN Biochemicals) prepared in a semidissolved suspension in a buffer of 50 mM Tris-Cl pH 7.5, 2% glucose. Cells were incubated with slow rolling at 30°. Aliquots were removed and were examined with a microscope for spheroplasting, which generally took 10 min. Subsequent steps are the same as KLEIN *et al.* (1992) to the point when spheroplasts were spread on a microscope slide and were treated with fixative. Following addition of fixative and detergent the spheroplasts were monitored at the microscope and when ~90% had lysed the second addition of fixative (80 μl) was applied and the solution was spread evenly over the slide using a glass rod. These slides were then dried at ambient temperature for

12 hr. To prepare the specimens for staining, the sucrose was removed by immersing the slides in 0.2% Photo-Flo (Kodak) for 5 min, and then slides were dried at ambient temperature for 3 hr. SCs were stained by applying 100 μl of 33% silver nitrate solution (1 g silver nitrate, 2 g water) to the surface of the slide and then overlaying a piece of nitex membrane that had previously been immersed in the silver nitrate solution. The slides were incubated at 65° in a humid chamber for 45 min and then the excess silver was washed off using H_2O. The DNA on the slides was stained by immersing the slide in 4× SSC, 1 μg/ml DAPI for 5 min. Excess liquid was removed from the slide and then 1 drop of 2× antifade solution (Molecular Probes Inc.) and a cover glass were applied. The slides were examined at ×1000 magnification using a light/fluorescence microscope.

Location of the *SAE3* locus: The location of the *SAE3* locus was determined by probing a blot of λ and cosmid clones acquired from the American Type Culture Collection. A nonradioactive probe DNA consisting of the 1.2-kilobase (kb) *Hin*dIII-*Xho*I fragment at the 3′ end of YHR080c was made using a Boehringer Mannheim Genius kit, and specific hybridization was detected by the recommended fluorescent method.

Segregation analysis of *SAE1* locus: A 2.5-kb *Hin*dIII fragment containing *SAE1* was cloned into pRS306 (Sikorski and Hieter 1989) to make pNKY538. This plasmid also carries a *URA3* gene. This construct was targeted to the *SAE1* locus by cutting with *Eco*NI prior to transformation. Proper integration was confirmed by Southern blot. A derivative of the original transformant was used to construct strain NKY2975 (*SAE1::URA3/sae1-1*). Tetrads from this strain were germinated and grown at 22° to allow the homothallic *ste7-1* spore clones to diploidize, and the resulting clones were tested for spore production by incubating on sporulation medium and for segregation of the integrated *URA3* gene.

Construction of *sae3* deletion: A 3.9-kb *Eco*RI fragment containing the *SAE3* locus was introduced into a derivative of pBluescript II KS+ that had had its *Hin*dIII site removed. A deletion spanning from 90 base pairs (bp) into the 5′ end of the *SAE3* open reading frame (ORF) to 1600 bp upstream of the ORF was made by cutting with *Hin*dIII and *Mun*I, filling in with T4 DNA polymerase and adding a *Bam*HI linker. At this *Bam*HI linker a *hisG-URA3-hisG* cassette was inserted (Alani *et al.* 1987) to make pNKY513. This plasmid was used to transform yeast after cutting with *Eco*RI. Correct integration at the *SAE3* locus was confirmed by Southern blot.

Subcloning, exonuclease III deletions, DNA sequencing and transformation of yeast by electroporation: Subcloning and manipulation of plasmid DNA was done by standard practices, generally using Sambrook *et al.* (1989) as a guide. DNA sequencing was done on double-stranded DNA using a Taq sequencing kit from BRL. Electroporation of SK1 yeast was done according to Becker and Guarente (1991).

Site-specific mutations: Mutations were made according to the Kunkel method as reported in Sambrook *et al.* (1989). Single-stranded DNA of plasmid pNKY1216, which is a derivative of pRS316 (Sikorski and Hieter 1989) containing the *SAE3* gene, was made by infecting *Escherichia coli* CJ236 (*dut⁻ ung⁻ F′*) with phage M13rv1. To create several different stop codon mutants in one procedure, a single 29-residue oligonucleotide complementary to the 5′ end of the putative *SAE3* ORF was made that contained mixed bases in codons 3, 4 and 5. Each of these codons could be changed to a nonsense codon by a single base change. Forty-eight independent plasmids from the mutagenesis were purified and transformed into *sae3-1* mutant strain NKY2745. Complementation of the spore formation defect by the plasmid was tested by incubating the transformants on sporulation plates and inspecting for the formation of ascospores. All 48 of the plasmids were sequenced across the 5′ end of the putative *SAE3* ORF to determine if a stop codon had been created. Plasmid 37 contained a fortuitous frameshift mutation.

The GenBank accession numbers for the *SAE1* and *SAE3* gene sequences are Z73534 and U82546, respectively.

RESULTS

Identification of *sae* mutants: A general screen for mutations that confer a *SPO11*-dependent block to spore formation has been described (McKee and Kleckner 1997). In brief, a homothallic diploid strain carrying the *SPO11* gene on a plasmid marked with *ADE2* was mutagenized and sporulated; haploid spores were allowed to germinate and the resulting self-diploidized spore clones were tested for spore formation by a UV fluorescence test (Briza *et al.* 1990). For mutants defective in spore formation, derivatives lacking the *ADE2 SPO11* plasmid were isolated and tested for spore formation. Mutants in which loss of the plasmid restored spore formation were analyzed further. Genetic analysis was facilitated by inclusion of a temperature-sensitive conjugation mutation (*ste7-1*) as described (McKee and Kleckner 1997).

This screen yielded mutations in three new genes: *SAE1, SAE2* and *SAE3* as well as nonnull "S" alleles of the *RAD50* gene and mutations in the *DMC1* gene. Phenotypes of *sae2* mutants and molecular analysis of the *SAE2* gene have been described (McKee and Kleckner 1997). Analysis of *sae1* and *sae3* mutations and their corresponding genes is described below.

Phenotypic characterization of the *sae1-1* mutant: *The sae1-1 mutant has a defect in meiotic recombination:* Liquid cultures of yeast derived from the SK1 strain can be induced into a highly synchronous meiosis (Alani *et al.* 1990; Padmore *et al.* 1991). Recombination between homologous chromosomes can be assayed directly in these cultures by physical analysis of the DNA at a recombination hot spot *HIS4LEU2* (Cao *et al.* 1990). In suitably marked strains (Figure 1) the two homologues differ at specific *Xho*I restriction sites flanking the locus of interest. In such strains, formation of DSBs and appearance of recombinant products can be monitored by the appearance of novel diagnostic DNA fragments (Figure 1). In *Xho*I-digested genomic DNA the two homologues yield different "parental" bands on a Southern blot. Novel restriction fragments can also be identified that are diagnostic of complete recombination reactions between homologues (Figure 1).

In wild-type cells crossover recombinants are first detectable at the 5-hr time point of a meiotic time course and occur at maximal levels by $t = 6–7$ hr; ultimately, ~25% of all chromatids have undergone a crossover at this locus (Figure 1B). In a *sae1-1* mutant, in contrast, the first crossover products are not evident until 10 hr. Furthermore, even at a very late time point, 48 hr after initiation of meiosis, the level of recombinants is only

one third to one half that of wild type. These results show that the *sae1-1* mutant is delayed for the formation of mature recombinants and that the final level of recombination is depressed, even after prolonged incubation in sporulation medium.

Similarly, in wild-type cells, DSBs occur at maximal levels at ~3.5 hours, are sometimes detectable as late as 5 hr and are not detectable thereafter (*e.g.*, see below). In a *sae1-1* mutant, in contrast, high levels of DSBs are still present at 10 hr and are detectable as late as 16 hr; the degree of DSB resection, however, seems normal (data not shown). Further physical analysis is required to determine whether DSBs appear at the normal time in the mutant case.

When recombination in a *sae1-1* strain is assayed genetically in a return to growth experiment (SHERMAN and ROMAN 1963; Figure 2) "commitment to heteroallelic recombination" is seen to occur with normal timing and at normal levels. In a wild-type strain containing *his4* heteroalleles, which can recombine to give a functional *HIS4* gene, the per-generation rate of recombination to *HIS4* is elevated 3000-fold in meiotic cells over the spontaneous mitotic rate (MCKEE and KLECKNER 1997). In the time-course experiment presented in Figure 2, as in previous analyses (CAO *et al.* 1990), many wild-type cells were already committed to recombination at 3 hr, the first time point examined here and, by $t = 4$ hr, the frequency of recombinants is at its maximum level with 1% of all cells giving rise to a histidine prototroph. Strain NKY2664, a *sae1-1* mutant, is indistinguishable from wild type in all respects (Figure 2).

The precise nature of the molecular events required to commit a meiotic cell to heteroallelic recombination in a return to growth assay is unclear. A reasonable possibility, however, is that DSB formation, in combination with conditions appropriate to ensure interhomologue recombination, is sufficient; progression to the double Holliday junction stage may not be required. According to this idea, DSBs may form at normal levels, with normal timing and in a qualitatively normal way in a *sae1-1* mutant, with progression out of the DSB stage (and perhaps later transitions) being delayed.

Cytological analysis of the sae1-1 mutant: Synchronous meiotic cultures were monitored for the occurrence of the two meiotic divisions by fluorescence microscopy of DAPI-stained cells. In a wild-type culture, approximately half of the cells have completed the first meiotic division (MI) 6 hr after introduction into sporulation medium; the percentage of cells that have achieved MI reaches a maximum of 90% at 10 hr; the second nuclear division (MII) follows shortly thereafter (Figure 2; see also ALANI *et al.* 1990; XU *et al.* 1997).

sae1-1 cells, in contrast, exhibit a delay of ~6 hr in the onset of MI as compared to the wild-type culture examined in parallel (Figure 2). In this case 50% of the cells in the culture have completed MI by 12 hr after the cells have been transferred to sporulation medium. Approximately 90% of the mutant cells eventually complete MI, although the synchrony of the division is somewhat reduced relative to wild type, as signified by the reduction in the slope of the accumulation curve (Figure 2). Furthermore, nearly all of the cells that completed the first division also completed MII, 80% in the experiment shown. Moreover, for the cells that complete both divisions, the position of the accumulation curve for MII indicates that the division follows shortly after MI after a period similar to, or perhaps slightly longer than, that of wild-type cells. Thus the delay in progression of meiosis observed in the *sae1* mutant is rather specific to MI.

sae1-1 was isolated as a mutation that caused a sporulation defect that could be eliminated by the presence of a *spo11*Δ mutation. Strains mutant in the *SPO11* gene alone execute MI and MII ~1 hr earlier than wild-type strains (data not shown; see also KLAPHOLZ *et al.* 1985). A *spo11*Δ *sae1-1* double-mutant strain performs MI simultaneously with the *spo11*Δ single mutant (data not shown), thus the *spo11*Δ mutation is epistatic to *sae1-1* with respect to the delay in meiotic prophase.

In wild-type meiosis, nuclei containing SC are present in significant numbers from 3 to 6 hr after initiation of meiosis (Figure 3). This corresponds to the time between the onset of the disappearance of resected DSBs and the first appearance of mature recombinants (CAO *et al.* 1990; SCHWACHA and KLECKNER 1994). In *sae1-1* mutant cells the onset of pachytene is delayed and its length is greatly extended. SC containing nuclei are not observed until 8 hr after transfer to sporulation medium and are still observed in >50% of the nuclei at 16 hr (Figure 3).

To measure the survival of cells as they proceed through meiosis, cells are removed from sporulation medium at various time points and plated for colonies on complete medium. Wild-type cells maintain complete viability throughout a meiotic time course (Figure 2). The *sae1-1* mutant retains viability for 6–8 hr but then exhibits a marked decrease in colony-forming units at times later than 10 hr, with 20% viability at $t =$ 48 hr. This level of survival corresponds to the frequency of cells that form ascospores, also ~20%. The viability of spores in tetrads was determined by dissecting 13 *sae1-1* tetrads, each containing four spores, on YEPD medium. Fifty of 52 spores grew to colonies, a level of viability similar to that of wild-type ascospores.

Two points regarding *sae1-1* meiosis are notable. First, in liquid sporulation conditions, more than half the cells accumulate with chromosomes in pachytene morphology but only 20% yield four-spored asci, in which all spores are viable. Moreover, the frequency of crossovers is also reduced to 20% of wild type. Thus it is conceivable that 80% of cells contain no crossovers and never emerge from arrest, while 20% of cells containing normal levels of crossovers emerge from arrest and give normal levels of viable spores. It will be interesting to

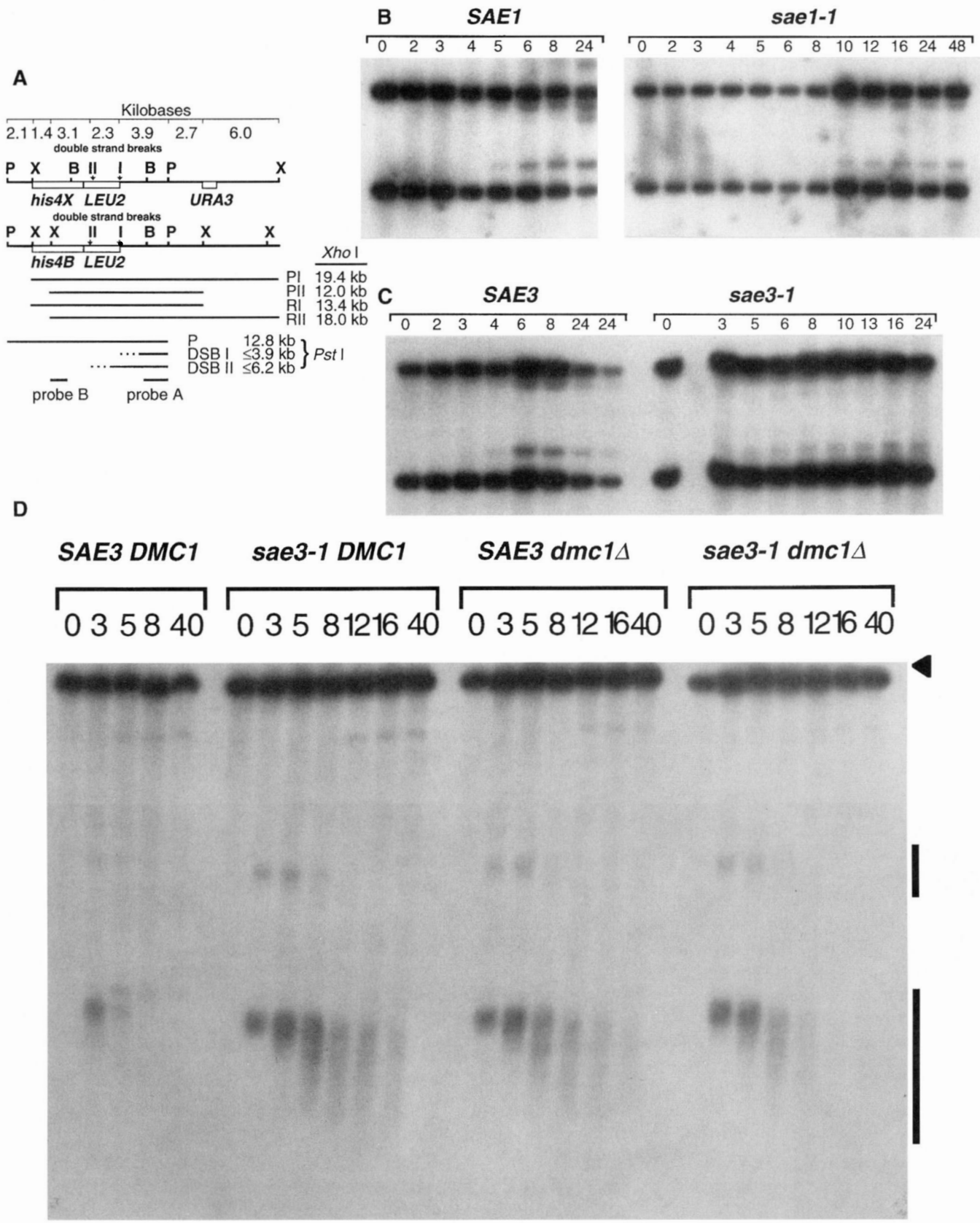


FIGURE 1.—Map of *HIS4* locus, physical analysis of recombination in *sae1-1* and *sae3-1* mutants. Physical analysis of meiotic recombination and meiotic-induced DSBs was performed by probing Southern blots of restriction enzyme-digested genomic DNA isolated from cells in meiotic time course experiments. The *HIS4LEU2* locus contains a "hot spot" for meiotic-induced DSBs and recombination. Parental and recombinant chromatids as well as DSBs can be detected at this locus. (A) Map of the *HIS4LEU2* locus. The two homologous chromosomes differ in restriction enzyme sites and in inserted ectopic DNA. Each

determine whether recombination is in fact normal in viable *sae1-1* spore products, not only with respect to the level of crossovers but with respect to the presence or absence of crossover interference. Second, the spore formation defect is much more severe when sporulation is carried out on solid media, as during mutant isolation (McKee and Kleckner 1997), than during liquid sporulation. The reason for this difference is unknown.

Phenotype of the *sae3-1* mutant: The effects of the *sae3-1* mutation on meiosis were analyzed by the same tests discussed above for *sae1-1*.

Physical analysis of recombination: Physical analysis of genomic DNA from meiotic cells was carried out on cells of a *sae3-1* mutant and on a parallel wild-type control culture. In the wild-type culture meiotically induced DSBs are seen at 3 and 5 hr (Figure 1D). The *sae3-1* mutant makes DSBs and these are resected. The extent of resection is more extensive in the mutant than in wild type and appears to become progressively more extensive as meiosis proceeds as indicated by the extent of the "fuzzy" band signal on the Southern blot and the progression of material in this band to a higher mobility in later time points (Figure 1D). DNA fragments indicative of recombination are observed in the later time points, but the level of accumulation is reduced to ~10% of that seen in equivalent wild-type strains.

Cytological analysis of sae3-1: sae3 mutant cells arrest permanently in meiosis. Cells of a synchronized meiotic culture were stained with DAPI and examined by fluorescence microscopy for nuclear division at the time of entry into sporulation medium and at regular intervals thereafter for 24 hr. At all time points, every cell examined contained only one nuclear body, indicating complete arrest prior to telophase of MI (Figure 4).

In the same time course, using silver stain and light microscopy (MATERIALS AND METHODS), aliquots of wild-type and *sae3-1* cells were examined for the presence of SCs. In wild-type SK1 yeast SCs appear transiently and are normally present in a given cell for ~1 hr (Padmore *et al.* 1991). In the wild-type control culture examined here, cells containing SCs were abundant (30%) at 3.5 hr and were discernible through the 6.5-hr sampling. In the *sae3-1* mutant culture examined in parallel, in contrast, SC-containing nuclei appeared later than normal, with 30% of cells containing such structures at 5 hr; furthermore, the fraction of nuclei containing SC increased with time until 10 hr, when a maximum of 90% was reached and maintained until the final time point assayed, $t = 16$ hr (Figure 4). At the low level of resolution afforded by this light microscope analysis, the SC structures in the *sae3-1* mutant are identical to those observed in wild-type cells. We conclude that *sae3-1* mutants are delayed in reaching pachytene but that, once having reached this point, the chromosomes arrest in the pachytene configuration.

The sae3-1 mutant exhibits a modest defect in commitment to heteroallelic recombination: Commitment to recombination was assayed in wild-type and *sae3-1* cultures in parallel (Figure 4). At $t = 2$, $t = 4$ and $t = 5$ hr, the times during which commitment occurs in wild-type cells, the *sae3-1* strain exhibits three- to fivefold fewer recombinants than wild type. We interpret this result to mean that there is a severalfold reduction in the number of commitment events in the mutant strain. At later times, the frequency of recombinants per viable cell rises in the mutant culture, ultimately reaching the wild-type level. This result might imply that commitment to recombination ultimately reaches normal levels in the mutant strain. However, since cell viability drops about 15-fold during this same period (Figure 4), it is also possible that the frequency of commitment is not changing and that cells that have not undergone commitment are preferentially lost.

The phenotype of sae3-1 closely resembles that of dmc1Δ: The *DMC1* gene codes for a yeast homologue of the bacterial RecA protein. The characteristics of the DSBs in the *sae3-1* mutant are strikingly similar to those

homologue can be detected as a different-sized fragment on a Southern blot. Recombinant chromosomes appear as intermediate-sized bands. Meiotic-induced DSBs occur at two sites in the *LEU2* DNA. Each homologue broken at these sites can be detected as a unique band. Fragments from *Xho*I digests were detected using probe B and fragments from *Pst*I digests were detected using probe A. The fragments created by each of these digests are drawn beneath the map, and their sizes are listed. (B) Physical analysis of recombination in wild-type and *sae1-1* mutant strains. *Xho*I-digested genomic DNA reveals crossover recombinants as bands of molecular weight intermediate between the two parental bands. These first appear at 5 hr in *SAE1* strains and at 10 hr in *sae1-1* strains. Darker exposures of this blot and analysis using a Fugi phosphoimager machine fail to detect any recombinant in *sae1-1* strains at the 8-hr time point. Other cultures in this same time course experiment do not have detectable recombinant bands until the 12-hr time point (data not shown). The final level of recombinant chromosomes in the *sae1-1* mutant is one third that observed in the wild-type strain. Strains: *SAE1*, NKY1552; *sae1-1*, NKY2664. (C) Physical analysis of recombination in wild-type and *sae3-1* mutant strains. *Xho*I-digested genomic DNA reveals crossover recombinants as bands of intermediate molecular weight between the two major parental bands. Crossover recombinants are observed in the *sae3-1* mutant at ~10% the level seen in the wild type. The lower recombinant band in the *sae3-1* mutant overlaps with the signal from a minor DSB site on the upper parental chromosome. This causes this area of the Southern blot to present as a diffuse dark band, over representative of the level of recombinant chromosomes. Strains: *SAE3*, NKY1551; *sae3-1*, NKY2655. (D) Analysis DSBs at the *HIS4LEU2* hot spot in *sae3-1*, *dmc1*Δ and *sae3-1 dmc1*Δ double mutants. Genomic DNA from a meiotic time course was digested with the enzyme *Pst*I. In wild type (NKY1551), DSBs appear transiently. The location on the Southern blot of the DSB signals from site II and site I is indicated to the right of the figure by short and long bars, respectively. Intact restriction fragment is marked by a triangle. In the three mutant strains the DSBs persist and become increasingly resected at later time points. Strains: *SAE3 DMC1*, NKY1551; *sae3-1 DMC1*, NKY2655; *SAE3 dmc1*Δ, NKY1879; *sae3-1 dmc1*Δ, NKY2673.

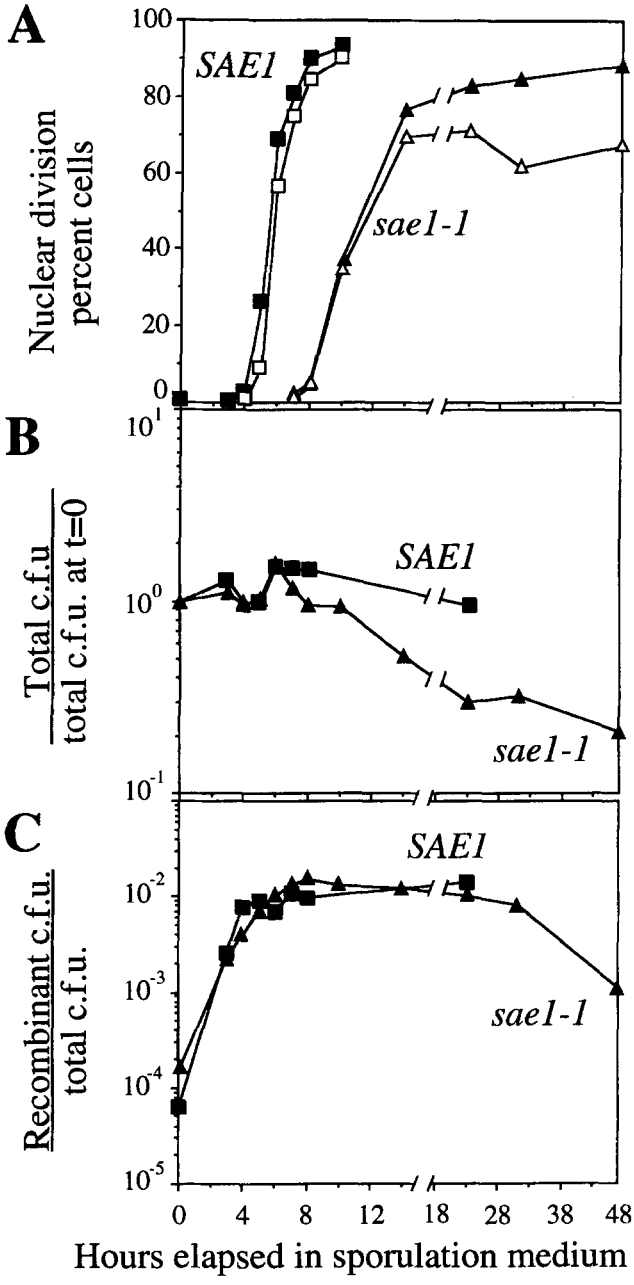


FIGURE 2.—Meiotic divisions, survival and return to growth recombination in the *sae1-1* mutant NKY2664 (△, ▲) compared to wild-type NKY1551 (□, ■). (A) The meiotic divisions are delayed in *sae1-1* by several hours compared to wild-type cells. In wild-type strains MI (■) and MII (□) occur at approximately 6 hr after introduction into sporulation medium. For *sae1-1* the midpoint of the accumulation of MI nuclei does not occur until later than 12 hr. In both wild-type and in *sae1-1* mutant strains MII follows quickly after MI. (B) Survival of cells in meiotic medium. Wild-type cells maintain complete viability to the end of the time course experiment, at which point >90% of the yeast are ascospores. Strains mutant for *SAE1* begin to lose viability after 12 hr. At ≥24 hr survival is ~20%, equal to the frequency of ascospores in cultures at this point. (C) Frequency of prototrophs made by return to growth recombination between heteroalleles at the *HIS4* locus. Wild type and *sae1-1* are indistinguishable: both strains become committed to an identical level of meiotic recombination with identical kinetics.

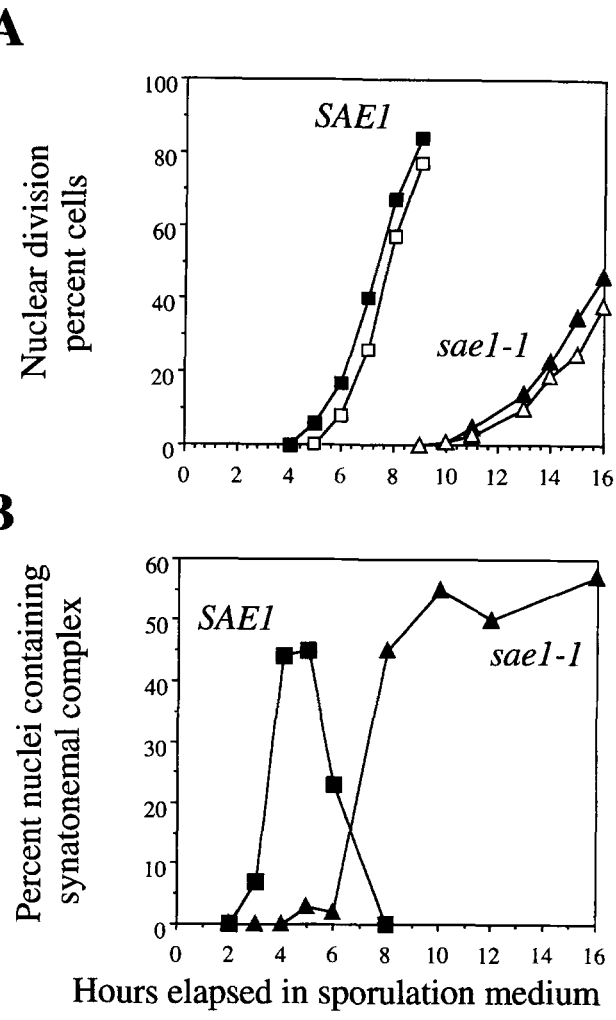
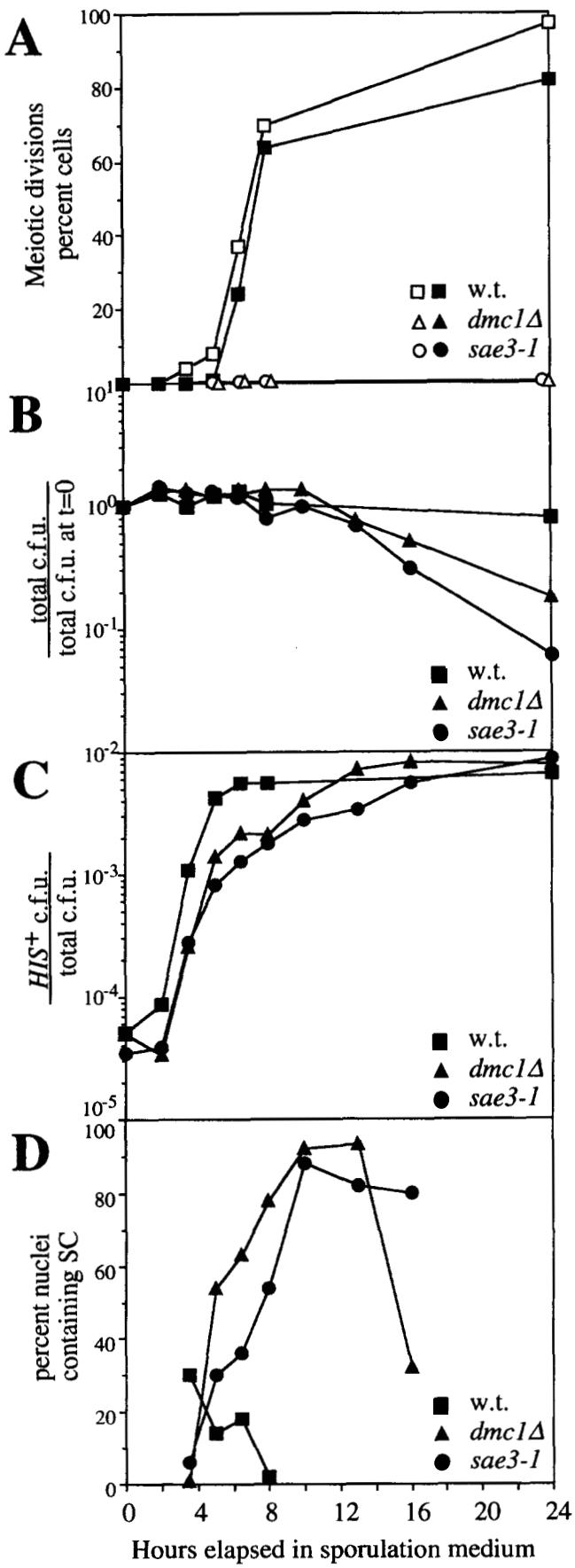


FIGURE 3.—Strains mutant in *SAE1* are arrested in pachytene. (A) Half the cells in a wild-type culture have completed MI by 7 hr (■) and have completed MII (□) shortly thereafter. MI occurs many hours later in a strain mutant in *sae1-1* (▲), followed rapidly by MII (△). Strains: wild type, NKY1551; *sae1-1*, NKY2664. (B) Synaptonemal complex, indicative of pachytene stage, is transient in wild type (■); it first appears in cells at 3 hr, peaks at 4 and 5 hr and is gone by 8 hr. The *sae3-1* mutant (▲) does not enter the pachytene stage until 8 hr, and >50% of the cells remain in pachytene at the final time point tested, 16 hr.

observed in a *dmc1*Δ mutant. *dmc1* mutant cells make meiotic DSBs that persist and are hyperresected and only 10% of the mature level of crossover recombinants is produced (BISHOP *et al.* 1992; XU *et al.* 1997). Furthermore, initiation of SC formation is delayed in *dmc1*Δ strains but, because cells arrest at the pachytene stage, nearly all cells eventually contain substantial or even apparently normal SC (ROCKMILL *et al.* 1995; XU *et al.* 1997).

Because of these similarities, *dmc1* and *sae3* mutant phenotypes were compared directly by including a culture of a *dmc1*Δ mutant in the *sae3-1* time course experiment. The nuclear division phenotype, the DSB phenotype, the return to growth phenotype both for survival



and production of recombinants and the SC phenotype are all extremely similar for the two mutant strains (Figures 1 and 4). In individual nuclei of a late time point (10 hr for instance), stained with silver and observed with the light microscope, the SC of a *dmc1*Δ mutant is identical in shape and quantity to those observed in a similar time point of a *sae3-1* mutant or to those observed in an earlier time point of a wild-type strain (4 hr). *dmc1*Δ mutant strains are late entering and arrest in pachytene just like *sae3-1* mutant strains.

Considering the degree of variability observed among different cultures of any given wild-type or mutant strain in such experiments, the correspondence between *dmc1* and *sae3-1* phenotypes observed in these experiments is remarkable. In addition, a Northern blot reveals that as cells proceed through meiosis the induction of transcription of *SAE3* is similar to that of *DMC1* (Figure 5). We conclude that the two mutations likely confer essentially indistinguishable phenotypes.

To see if the effects of the two mutant genes are in any way additive, a double-mutant strain containing both *sae3-1* and *dmc1*Δ was constructed and analyzed by time course experiment. The double mutant is essentially indistinguishable from either single mutant in terms of SC accumulation and meiotic arrest (data not shown) and DSB morphology up to 8 hr into meiosis (Figure 1D). At 12 hr and later into the time course the signal on the Southern blot that represents resected molecules appears fainter in the double mutant than in either single mutant. The biological relevance at these later time points is, however, suspect. It is therefore likely that these two mutations affect the same step in recombination and chromosome metabolism.

FIGURE 4.—Meiotic time course analysis of wild-type, *sae3-1* and *dmc1*Δ strains. In all parts of the figure the strains are represented as follows: □ and ■, wild type, (NKY1551); ○ and ●, *sae3-1*, (NKY2655); △ and ▲, *dmc1*Δ, (NKY1879). (A) Meiosis I and meiosis II. The wild-type cells have completed MI (□) by 8 hr and MII (■) ~30 min later. Both the *sae3-1* and *dmc1*Δ mutants arrest prior to the first meiotic division. (B) Cell viability. Wild-type cells maintain complete viability throughout the time course. The *sae3-1* mutant and the *dmc1*Δ mutant maintain viability until the 14-hr time point, at which time survival begins to decrease. At 24 hr survival has dropped to ~10% in both mutant strains. (C) Frequency of prototrophs made by return to growth recombination between heteroalleles of the *HIS4* gene. The *sae3-1* strain commits to recombination as early as wild-type cells, but the frequency of prototrophs that arise from aliquots removed in the first 8 hr of sporulation is approximately one third that of wild type. At time points after 8 hr the frequency of prototrophs among surviving cells approaches that of wild-type cells. The *dmc1*Δ mutant is similar to the *sae3-1* mutant. (D) Synaptonemal complex, indicative of the pachytene, appear transiently in wild type, from 3 to 6 hr. In the *sae3-1* mutant SC is first observed at 5 hr. By 10 hr >90% of the *sae3-1* nuclei contain SC structures. The mutant remains arrested in pachytene until the last time point analyzed, 16 hr. Appearance of SC in the *dmc1* mutant exhibits similar kinetics to that of the *sae3-1* mutant.

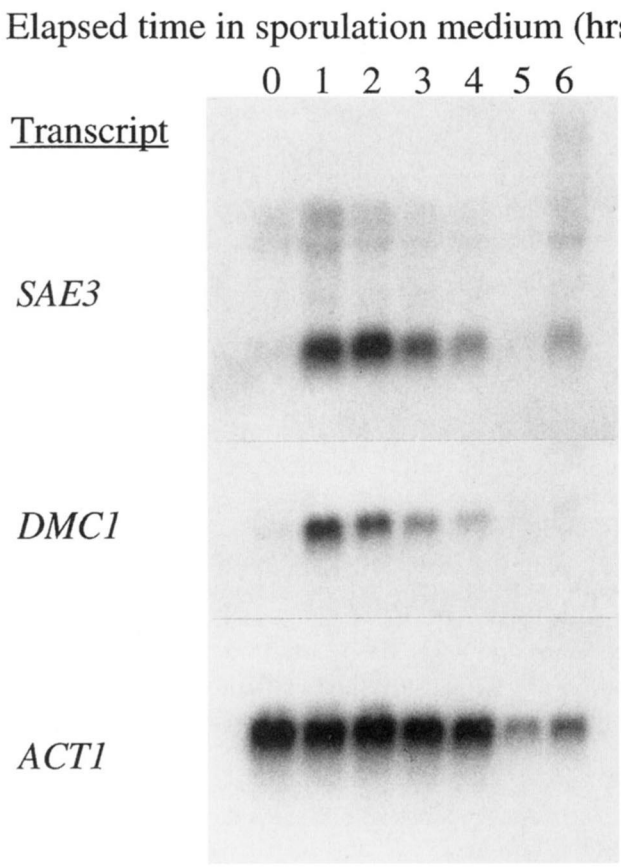


FIGURE 5.—Transcription of the *SAE3* gene is induced in meiotic cells. A Northern blot of total cellular RNA isolated at hourly time points from a meiotic culture was probed for *SAE3* transcripts. No signal was observed in premeiotic cells ($t = 0$). At all later time points, from 1 to 6 hr, a transcript was present. The Northern blot was stripped and reprobed for *DMC1* transcripts. Induction of the *DMC1* gene followed identical kinetics. The same blot was stripped again and was probed for the *ACT1* transcript, a gene transcribed at steady levels in both meiotic and mitotic cells, as a control for RNA concentration. Strain used for RNA isolation: NKY1551.

The sae3 mutant has no apparent mitotic defect: Meiotic recombination, repair of DNA damage in vegetative cells and switching of information at the mating type locus require overlapping sets of genes. Repair of damage caused by ionizing radiation (X-rays, γ-rays) requires recombinational repair functions, including the *RAD51* gene, mutations in which result in a meiotic DSB phenotype similar to *sae3-1* (SHINOHARA *et al.* 1992). To see if *SAE3* is necessary for repair of this type of damage, *sae3-1* mutants were tested for sensitivity to methyl methane sufonate (MMS), a chemical whose effects mimics those caused by ionizing radiation.

Vegetative cells containing a *sae3-1* mutation were plated on a range of concentrations of MMS and were incubated at 18 and 30° following the method described in MCKEE and KLECKNER (1997). The *sae3-1* mutants were indistinguishable from wild-type cells both in colony size and in efficiency of plating, at both temperatures and at all concentrations of MMS (data not shown). Thus *sae3-1* mutants have no detectable deficit compared to wild-type cells in dealing with the damage created by MMS.

Mating type switching appears normal in *sae3-1* mutant cells. Homothallic haploid cells switch mating type virtually every generation (STRATHERN and HERSKOWITZ 1979). A homothallic *sae3-1* haploid strain that contains a sterility mutation *ste7-1*, which allows homothallic haploid cells to switch mating type but prevents mating (HARTWELL 1980; MCKEE and KLECKNER 1997), has a growth rate indistinguishable from wild-type cells and grows into a colony of mixed *MAT***a** and *MAT*α cells (data not shown). This reveals that *sae3-1* mutants can switch mating type and, if the process does contain a defect, it is not so severe as to cause a decrease in colony growth rate as happens in a *rad50*Δ mutant (MCKEE and KLECKNER 1997). Normal *MAT* switching and resistance to MMS are consistent with the virtual absence of mRNA transcript of the *SAE3* gene in vegetative cells (Figure 5).

Cloning of the *SAE1* and *SAE3* genes: The *SAE1* and *SAE3* genes were cloned from a yeast genomic library (ROSE *et al.* 1987) on the basis of complementation of their spore formation defects. A *sae1-1* strain (NKY2328) and a *sae3-1* strain (NKY2745) were transformed with the genomic library DNA by electroporation, and approximately 10^4 transformants were obtained for each strain. The transformed colonies were replica stamped to SPM, and incubated at 30° for 4 days. The SPM plates were then screened for sporulation proficient transformants using a UV fluorescence assay (MCKEE and KLECKNER 1997). Two sporulation-proficient transformants were identified for both the *sae1-1* and the *sae3-1* mutant strains, and plasmid DNA was recovered from each of the four. Confirmation that the plasmids conferred sporulation proficiency was provided by retransforming them into the corresponding *sae* mutant strains, NKY2338 or NKY2745, and observing spore formation by the transformed strains on SPM agar.

Localization of *SAE1*: Restriction enzyme mapping of the plasmid yeast genomic inserts revealed that the two *sae1-1* complementing plasmids have overlapping maps, as do the two *sae3-1* complementing plasmids. To identify the exact location of the *SAE1* gene, subclones of various restriction fragments were tested for complementation of the sporulation defect. By this method the minimum complementing region was narrowed down to a 2.6-kb *Eco*NI-*Msc*I fragment. Partial sequencing of this fragment revealed the *CUP9* gene at one end and a delta sequence and the *SAL6* gene just beyond the other end. The *SAL6* gene is located on the left arm of chromosome XVI (VINCENT *et al.* 1994), and this identifies the chromosomal location of both *SAE1* and *CUP9* (Figure 6). The remainder of the DNA sequence between *CUP9* and *SAL6* was retrieved from the Saccharomyces Genome Database at Stanford Univer-

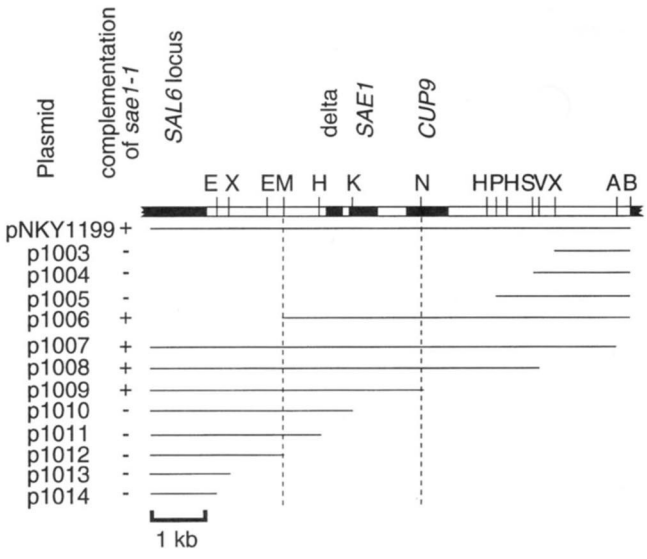


FIGURE 6.—Mapping of *sae1-1* complementing activity by deletion analysis. Restriction enzyme fragment subclones of pNKY1199 were tested for complementation of the spore formation defect of a *sae1-1* mutation in strain NKY2745. At this resolution the minimum complementing fragment consists of a 2.2-kb *Mst*I-*Eco*NI fragment whose endpoints are marked by dashed lines. Partial sequencing of this region revealed that *SAE1* is located between the genes *SAL6*, which is located on the left arm of chromosome XVI (VINCENT *et al.* 1994), and *CUP9*. A delta remnant is immediately adjacent to *SAE1*. Restriction enzyme sites: E, *Eco*RI; X, *Bst*XI; M, *Mst*I; K, *Kpn*I; N, *Eco*NI; H, *Hin*dIII; P, *Hpa*I; S, *Sac*II; V, *Avr*I; A, *Age*I; B, *Bam*HI.

sity. At the location of the *sae1-1* complementing DNA, between *CUP9* and the delta element, the sequence contains an ORF of 208 codons.

This locus corresponds to the site of the *sae1-1* mutation, as confirmed by segregation analysis in homothallic strains. In a *SAE1* strain a *URA3* gene was integrated next to this ORF and was then crossed by a *sae1-1 ura3* strain (MATERIALS AND METHODS). Eleven tetrads from this strain were dissected and the resulting 44 diploid spore clones were incubated on sporulation medium. In each tetrad the two *URA3* clones were sporulation proficient, and the *ura3* clones made <10% ascospores, the phenotype of *sae1-1*. Thus the integrated *URA3* gene is <2.5 cM from the *sae1-1* mutation.

The predicted protein translated from this ORF contains a ribonucleoprotein (RNP) type RNA-binding consensus sequence (MATTAJ 1993; BURD and DREYFUSS 1994) and is highly similar to CBP20, RNA cap-binding protein, of human and xenopus (IZAURRALDE *et al.* 1995; KATAOKA *et al.* 1995). The sequence from residue number 44 to 155 contains 60% identical matches and 75% identical or similar amino acid matches to the human CBP20 and only slightly lower similarity to xenopus CBP20—56% identical and 75% identical or similar matches (Figure 7). It is therefore likely that Sae1p is a nucleic acid-binding protein.

Localization of *SAE3*: The location of the *SAE3* gene on the genomic clones was identified by subcloning and complementation analysis as described above for *SAE1*. This analysis identified a minimum complementing region of 4.5 kb. The complementing region was further narrowed down by making unidirectional deletions with enzyme exonuclease III on the 6-kb *Hin*dIII restriction fragment that spans the complementing region (Figure 8). The endpoints of the deletions delimited a 1200-bp segment of the chromosome necessary for complementation of *sae3-1*.

The *SAE3*-complementing DNA was sequenced beginning at the left-hand *Hin*dIII site shown in Figure 8, was continued through the minimum complementing region and was concluded when a match to the 5′ end of the *IRE1/ERN1* gene (NIKAWA and YAMASHITA 1992; MORI *et al.* 1993) was found.

To map the physical location of *SAE3* in the yeast genome, a membrane of contiguous overlapping lambda and cosmid clones of the yeast genome obtained from the ATCC was probed using the 1200-bp *Hin*dIII-*Xho*I fragment that partially overlaps the complementing area. Lambda clone 70715 reacted with a positive hybridization signal, placing *SAE3* on chromosome VIII.

Subsequent to our sequencing of this region the sequence of the entire chromosome VIII became available (JOHNSON *et al.* 1994). This sequence revealed that the left-hand end of the fragment we sequenced is the 3′ end of a very large ORF, which extends ~4 kb beyond the *sae3-1* complementing region. Between this ORF, designated YHR080c, and the start of the *IRE1/ERN1*

FIGURE 7.—Deduced amino acid sequence of Sae1p aligned with human CBP20 (KATAOKA *et al.* 1995) and partial sequence of xenopus CBP20 (IZAURRAIDE *et al.* 1995). Identical and similar residues between the proteins are indicated by printing on black or shaded background, respectively. Conserved residues of the RNP RNA binding domains are boxed. S, Sae1p; N, human CBP20; X, xenopus CBP20.

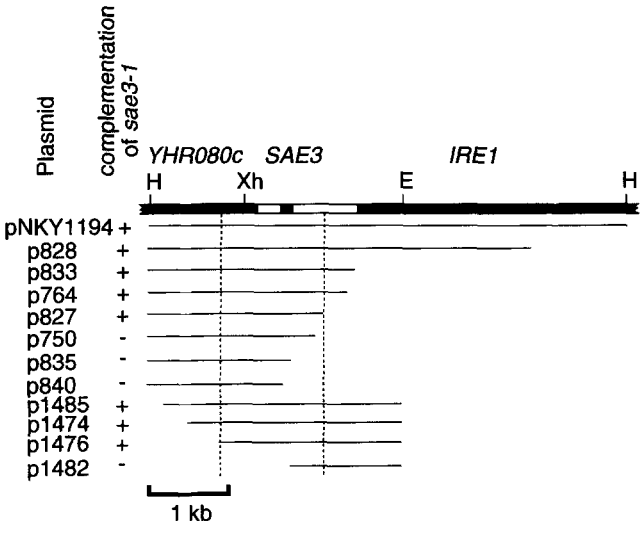


FIGURE 8.—Location of the *SAE3* locus. The *SAE3* gene was cloned by complementation of the spore formation defect. The location of *sae3-1* complementing activity was mapped by making deletions with restriction enzymes or with exonuclease III on pNKY1194 and testing these for complementation of the *sae3-1* spore formation defect in strain NKY2328. The minimum region necessary for complementation, as defined by this set of deletions, is marked by dashed lines. The *SAE3* ORF is located on chromosome VIII between *IRE1* and the ORF YHR080c, all of which are indicated by thick bars.

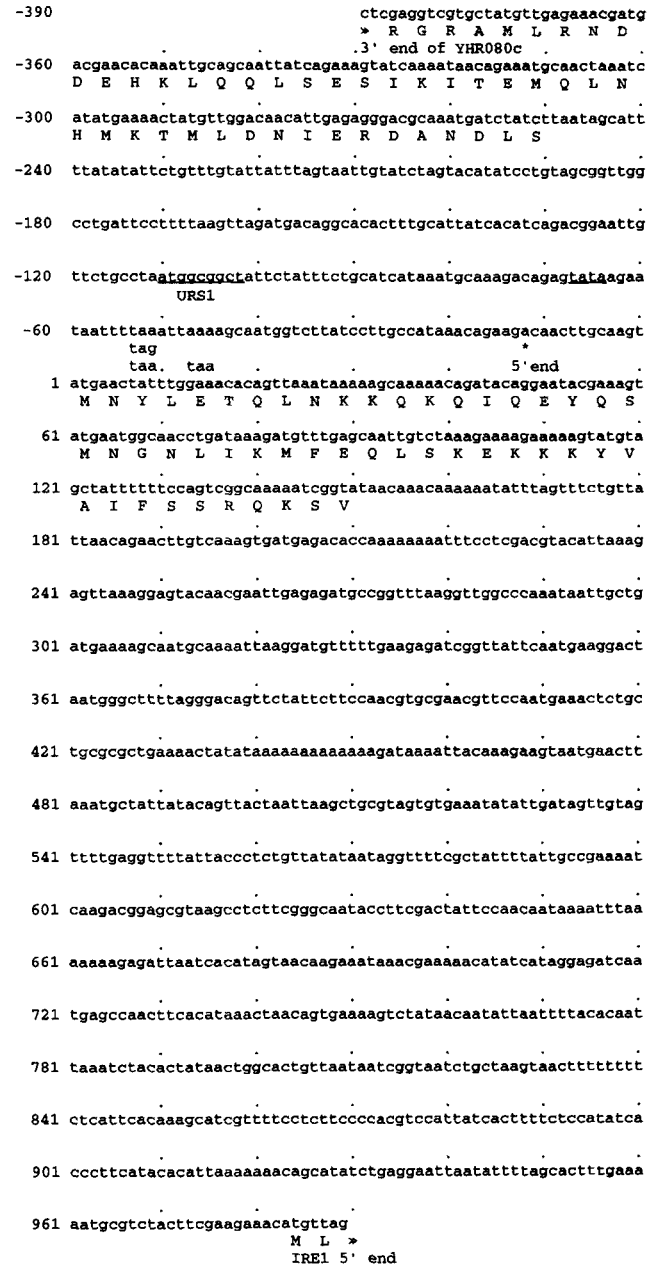


FIGURE 9.—DNA sequence of the *SAE3* locus. The *SAE3* locus is found between the ORF *YHR080c* and the gene *IRE1*. The numbering of the sequence begins at the first nucleotide of the 50-codon *SAE3* ORF. The 5′ end of the meiotically induced transcript is marked with an asterisk, and a mitotic repressing sequence, *URS1*, is underlined. The translation of the ORF is inscribed under its corresponding DNA sequence. Nonsense codons created by site-directed mutagenesis in the third and fifth positions of the ORF are written above the sequence. A frameshift mutation made by insertion of a T after nucleotide 8 results in a truncated ORF of nine codons before the first stop codon. The mutant peptide sequence deviates from that of Sae3p beginning at the fourth position and reads MNYFGNTVK stop. All four mutant versions of *SAE3* fail to complement the *sae3-1* mutation of strain NKY2328.

ORF is a 900-bp stretch of DNA wherein the *sae3-1* complementing activity lies. This region encodes several small ORFs that contain initiator methionine codons; the largest of these ORFs has 50 codons.

Further analysis demonstrates that the 50-codon ORF is required for *SAE3* gene function. A strain deleted for the first 90 bp of this ORF was constructed (MATERIALS AND METHODS) and then mated to a *sae3-1* mutant strain. The resulting diploid NKY2973 failed to make spores when incubated on sporulation medium, indicating that the deletion mutation fails to complement the *sae3-1* mutation. This result confirms that the complementing clone does in fact contain the *SAE3* gene itself and demonstrates that the region encoding the 50-codon ORF is relevant to *SAE3* gene function.

Site-specific mutations: To further document that the 50-codon ORF was part of the *SAE3* gene, four mutations were created that should interfere with its function. These consisted of three nonsense codons and one frameshift mutation near the beginning of the ORF. Two different nonsense codons were made in the third codon of the ORF (TAT was converted to TAA and to TAG), and another was made in the fifth codon (GAA converted to TAA). The frameshift was created by the introduction of a "T" in the fourth codon. This mutation causes the translation of the predicted amino acid sequence to change, starting at the fourth codon and terminating with a nonsense codon at the 10th position (Figure 9). A *sae3-1* diploid strain (NKY2745) was transformed with each one of these mutant genes, along with vector alone and *SAE3* plasmid controls. Transformed colonies were incubated on SPM agar and were examined after 4 days for spore formation. To

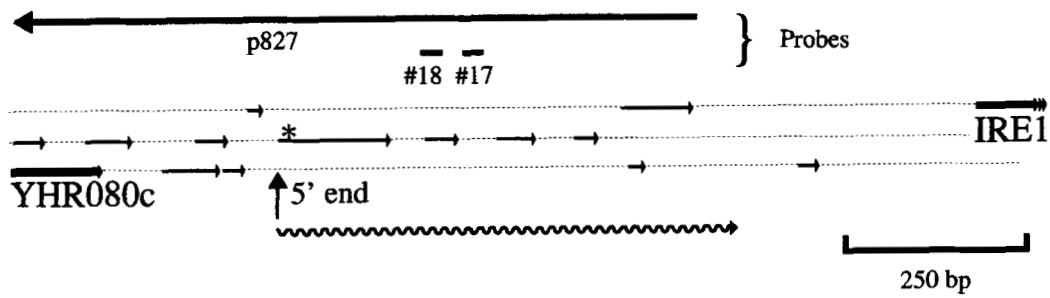


FIGURE 10.—ORFs at the *SAE3* locus. All ORFs on the transcribed strand that contain a methionine codon are indicated by an arrow that begins at the methionine codon. The putative *SAE3* ORF is marked with an asterisk. The 5′ end of the *SAE3* transcript determined by primer extension experiment is also indicated. The approximate size of the transcript, as estimated from the location of the signal on the Northern blot, is shown by the wavy line. Probes used on northern blots include the 24 base oligonucleotide 17, which was used to confirm which strand was transcribed, and the DNA probe made from p827. This probe covers most of the *SAE3* locus and extends into the ORF *YHR080c* to a *Hin*dIII site, ~1 kb to the left. Primer extension analysis used oligonucleotides 17 and 18.

guard against false positives caused by recombination between the plasmid and chromosomal alleles of *sae3* to produce a wild-type allele, 10 independent colonies were tested for each mutant.

Unlike the wild-type allele that complemented *sae3-1*, all four site-specific mutant versions failed to complement the spore formation defect. These results again indicate that the sequences encoding the 50-codon ORF comprise at least part of the *SAE3* gene.

The *SAE3* locus is transcribed in meiotic cells: To determine if the DNA between *IRE1* and YHR080c is transcribed, total cellular RNA was extracted from vegetatively growing yeast, and meiotic yeast samples were taken at hourly intervals through the first 6 hr of a meiotic time course. The RNA was separated on a gel and was analyzed by Northern blot using a radioactive probe (p827, see Figure 10). The Northern blot of this time course exhibited a meiotically induced transcript: in the 0-hr time point there was virtually no detectable signal, at 1 hr the transcript steady-state level was at its peak and it trailed off at later time points (Figure 5). The size of the transcript was of significantly lower molecular weight than the 18 S rRNA molecule, setting an upper size limit of <1700 nucleotides (nt). This blot was stripped and reanalyzed with a probe specific for the *DMC1* transcript. The mRNA of *DMC1* showed identical induction kinetics to that of *SAE3*. Hybridization of the blot with a ^{32}P-labeled oligonucleotide probe (probe 18, see Figure 10) determined that transcription of *SAE3* proceeded in the direction toward the *IRE1/ERN1* gene (data not shown). These observations suggested, as a working model, that this meiosis-induced transcript is a product of the *SAE3* gene.

Primer extension analysis: The 5′ end of the putative *SAE3* transcript was mapped by performing primer extensions from synthetic oligonucleotides on total cellular meiotic RNA using AMV reverse transcriptase. To find a meiosis-specific extension product, two sets of reactions were performed on RNA made from cells harvested from a meiotic time course culture at 3 hr after entry into meiosis using primers 17 and 18 (Figure 10). As a control, extension reactions were done on RNA prepared from an aliquot of cells from the same culture taken prior to transfer of cells to sporulation medium. An extension end point common to both primers was found in the meiotic RNA, and no signal was observed at the corresponding site in the premeiotic RNA (data not shown). The end point is located 13 bp upstream of the initiator methionine codon of the 50-codon ORF.

Analysis of the sequence of the *SAE3* locus: Inspection of the DNA sequence between the *IRE1* gene and YHR080c reveals a *URS1* consensus sequence 90 bp upstream of the RNA initiation site. The *URS1* element is found upstream of several meiosis-specific genes and is required for repression of these genes in vegetative cells (MITCHELL 1994).

Sequence inspection also revealed a potential intron in this gene. Matches occur in the sequence for 5′ and 3′ intron splice sites, *e.g.*, GTATGT and CAG, at bp 114 and 185, respectively, following the numbering in Figure 9. This would create an intron of 74 nt. An imperfect match to the branch site consensus sequence is located at bp 179. The sequence of the putative branch site is TATTAAC, which differs from the consensus by a T in the third position rather than C. To assess whether the *SAE3* message is spliced, an RT-PCR amplification of the *SAE3* message from meiotic RNA was compared to a PCR product made from the DNA of a plasmid carrying the *SAE3* gene. Both amplifications used the same primers: intron removal from the transcript should result in a smaller product amplified from the RNA than from the plasmid DNA. Analyzed by agarose gel, both amplification products were the same size. The resolution of this analysis dictates that no intron of size >10 nucleotides is removed from the RNA transcript (C. KLEIN, this laboratory, unpublished results).

The *DMC1* transcript is spliced in *sae3-1* mutants: The transcript from the *DMC1* gene contains an intron (BISHOP *et al.* 1992). One explanation for the similarity between *sae3-1* and *dmc1*Δ phenotypes would

be that the *SAE3* gene product is involved in removal of the *DMC1* gene intron. To investigate this possibility, primer extension analysis was performed on the *DMC1* transcript in wild-type and *sae3-1* mutant cells. Extensions to the 5′ end were primed from an oligonucleotide that hybridized to the *DMC1* transcript just downstream of the intron, in the second exon. Failure to remove the intron would produce a higher molecular weight signal than that produced from the properly excised transcript. RNA from vegetative cells and from cells that had been in sporulation medium for 3 hr was analyzed. In the RNA made from meiotic wild-type cells (NKY1551) a signal representative of properly spliced *DMC1* transcript was present. No extension products were seen in the vegetative RNA, as expected since the gene is not transcribed. The results were identical in the meiotic and vegetative RNA of the *sae3-1* mutant (NKY2655), revealing that a splicing defect of *DMC1* transcript is not responsible for the *sae3-1* phenotype (data not shown).

DISCUSSION

The *sae* screen identifies mutants having an intermediate block in prophase chromosome metabolism and an arrest or delay in leaving prophase: *sae* mutants are defined by the fact that they are defective in making spores in an otherwise wild-type strain background, but they do make spores in a *spo11* mutant background. When we initiated this screen, the only such mutants known were *rad50S*, which confers an interesting intermediate block in recombination (ALANI *et al.* 1990) and *rad52*, which also causes an intermediate recombination block (MALONE and ESPOSITO 1981; BORTS *et al.* 1986; RESNICK *et al.* 1986; SCHWACHA 1996). The screen was thus motivated initially by the notion that it would identify additional mutations that confer intermediate recombinational blocks. This prediction has been fulfilled. *sae* mutants identified in this screen included alleles of five genes: three new *rad50S* alleles, two alleles of *DMC1* (identified independently by another approach, BISHOP *et al.* 1992) and single alleles of the three new *SAE* genes (MCKEE and KLECKNER 1997). All of these mutations, including a *dmc1* null mutation, permit normal DSB formation but block the recombination process at some subsequent step(s) (above; BISHOP *et al.* 1992; MCKEE and KLECKNER 1997). We are also aware of subsequent unpublished work that has identified four additional genes in which mutations cause both the *sae* phenotype and an intermediate recombinational block.

Among the *sae* mutants identified in our analysis, only three recombinational phenotypes are represented: (1) *sae2* and *rad50S* mutations confer the same phenotype, accumulation of unresected DSBs (MCKEE and KLECKNER 1997); (2) *dmc1* and *sae3* mutations result in identical accumulation of hyper-resected DSBs and (3) the *sae1-1* mutant exhibits a unique phenotype. Taken together, the existing data suggest that the *sae1-1* mutant probably accumulates normal numbers of normally resected DSBs with essentially normal timing and that progression beyond the DSB stage to crossovers is both slow and inefficient. It is possible that a subset of mutant cells finally carries out qualitatively and quantitatively normal meiosis while another subset remains stalled at the DSB stage. The only other mutant that we know of with a related phenotype is *sep1* (BAHLER *et al.* 1994; TISHKOFF *et al.* 1995), whose spore formation defect is, however, not alleviated by a *spo11* mutation, perhaps because of additional defects in some other aspect(s) of meiosis.

Interestingly, among the *sae* mutants, the recombination defect and the cell cycle perturbation are correlated (at least in the SK1 strain background). *rad50S* and *sae2* mutants are blocked earliest, at the DSB resection step, and exhibit delays in the occurrence of the two meiotic divisions (MCKEE and KLECKNER 1997). *dmc1* and *sae3-1* mutants, in contrast, arrest after DSB resection, at the DSB to double Holliday junction transition, and both mutants exhibit essentially permanent arrest at the end of prophase (this work; BISHOP *et al.* 1992). The *sae1-1* mutant phenotype seems to be somewhat different from either of these, with respect to both recombination and the cell cycle, which appear to be affected coordinately. These findings support the view that progression of recombination and progression of the cell cycle are intimately coupled processes.

It appears that defects in the progression of meiotic recombination trigger cell cycle arrest or delay at the end of prophase. This notion evolved originally from the analysis of *dmc1* mutant phenotypes (BISHOP *et al.* 1992). Additional, direct evidence for regulatory monitoring of meiotic recombination has recently been provided by other studies as well (LYDALL *et al.* 1996; XU *et al.* 1997). The phenotypes of the *sae* mutants are fully consistent with this hypothesis. Conversely, the *sae* mutant screen may work as it does because of this coupling.

It is not rigorously excluded that prophase arrest in the recombination-defective mutants actually reflects an aberrancy other than in recombination, *i.e.*, in SC formation. For example, no mutant exhibiting normal recombination and cell cycle progression, but exhibiting aberrant SC formation, has yet been identified.

It has recently been suggested that the cell cycle might actually drive the recombination process, in addition to monitoring its status (KLECKNER 1996). That is, progression of the cell cycle might be required for progression of the recombination reaction. If this were true, a *sae* mutant phenotype might result from a regulatory defect rather than a defect in the biochemistry of recombination *per se*. This possibility is especially intriguing for the *sae1-1* mutant. This is the only meiotic recombination mutant analyzed thus far in which re-

combination progresses relatively normally, just very slowly.

Molecular nature of the Sae1 protein: The putative Sae1 protein contains an RNP nucleic acid-binding domain. Among proteins that contain RNP motifs are found those that bind particular RNAs and others that bind hnRNA and mRNA nonspecifically (reviewed in BURD and DREYFUSS 1994). The RNP domain is found in fungal, plant, animal and bacterial proteins, suggesting an ancient origin for the structure.

Surrounding the RNP motif of Sae1p is a region highly similar to vertebrate CBP20 protein, a component of nuclear RNA cap binding complex (CBC). The similarity spans 112 residues of the 208-codon sequence (Figure 7). At both the C- and N-terminal ends the Sae1p sequence diverges significantly from the two CBP20 proteins. Sae1p is also ~30% larger than these two proteins. Nevertheless, Sae1p could be a yeast homologue of CBP20.

The function of CBP20 in HeLa cells has been examined by depleting the protein from cellular extracts and assaying for splicing activity. Splicing of exogenous *in vitro* synthesized capped mRNAs was radically but not completely eliminated in these extracts. The transcript remained unmodified, suggesting that CBP20 is necessary for an early step in the splicing reaction (IZAURRAIDE *et al.* 1994). The small amount of spliced message that was made was ascribed to either incomplete depletion of the extract or to the action of another splicing pathway. Based on this and other studies (JARMOLOWSKI *et al.* 1994), the authors hypothesize that CBC plays an essential role in export of small nuclear RNAs from the nucleus and plays a nonessential role in the export of mRNA. In accordance with this idea, addition to xenopus nuclear extracts of antibodies specific to CBP20 inhibited but did not completely eliminate splicing, and addition of antibodies to whole cells inhibited nuclear export of U1 and U5 RNA (IZAURRAIDE *et al.* 1995). By analogy with CBP20, yeast Sae1 protein could be a cap-binding protein required for efficient splicing and nuclear export of certain transcript(s) specific to meiotic cells. Alternatively, Sae1 protein could be involved in the processing of some noncoding RNA that is important for meiosis.

Two meiotic recombination genes are known whose transcripts are spliced during meiosis and thus might be targets for *SAE1* action, *MER2* and *DMC1*. The *MER2* transcript is spliced under the direction of the Mre2 and Mer1 proteins (ENGEBRECHT *et al.* 1991; OGAWA *et al.* 1995), while the *DMC1* transcript is spliced independent of these two proteins (NAKAGAWA and OGAWA 1997; T. NAKAGAWA and H. OGAWA, personal communication). However, the phenotype of a *sae1-1* mutant does not resemble that of *mer2, mre2, mer1* or *dmc1* mutants. A *mer2* mutant gives no DSBS; *mre2* and *mer1* mutants give reduced levels of normally resected DSBs, which are converted with normal timing into reduced levels of recombinants; a *dmc1* mutant gives accumulation of hyper-resected DSBs, most of which do not progress further (ENGEBRECHT and ROEDER 1989; BISHOP *et al.* 1992; OGAWA *et al.* 1995). Thus, in these cases, it would be necessary to suppose that a reduction in the level of Mer2p or Dmc1p causes a qualitative change of phenotype.

Molecular nature of the *Sae3* gene product: *SAE3* complementing activity occurs in a 1231-bp segment between the *IRE1* gene and another large ORF YHR080c (JOHNSON *et al.* 1994). More specifically, the complementing locus overlaps a meiosis-specific transcript and a 50-codon ORF located between the *IRE1* gene and the YHR080c ORF.

It seems highly probable that the meiosis-specific transcript made from this region corresponds to the *SAE3* gene. (1) The absence of this transcript in vegetatively growing cells is consistent with the lack of *sae3-1* phenotypes in vegetatively growing cells. (2) This transcript is made in the same direction as the 50-codon ORF, from YHR080c toward *IRE1*. The start site for this transcript is 237 bp downstream of the end of the upstream gene and the approximate size of the transcript (~700 nt) places its 3′ end before the *IRE1* gene (barring extensive intron removal, which seems unlikely, see below). (3) A URS1 sequence, known to be involved in repressing transcription of meiosis-specific genes in vegetative cells and found upstream of many such genes (MITCHELL 1994), occurs in an appropriate position 97 bp upstream of the transcription start site of the putative *SAE3* transcript.

The DNA segment corresponding to this transcript contains several small ORFs with methionine initiator codons of which the 50-codon ORF targeted for mutagenesis is the largest. Since complementing activity is eliminated by several different mutations that should disrupt translation of this ORF, three nonsense and one frameshift mutations, the DNA sequence corresponding to this ORF is part of the *SAE3* gene.

The simplest possibility is that the *SAE3* gene product is the 50-amino acid peptide corresponding to this ORF. Alternatively, the *SAE3* gene product could be the meiotically induced RNA itself. In that case, mutations in the ORF could affect function either because translation of the RNA is required for its functioning or because the mutations affect the functioning of the RNA irrespective of translation.

If the *SAE3* gene product is a protein, that protein would be unusually small, with a molecular weight of <5.7 kD. There are several precedents for small active proteins and peptides in yeast. The gene for the Ost4 protein consists of an ORF that codes for 36 amino acids (CHI *et al.* 1996). This protein is necessary for the normal level of oligosaccharyltransferase activity in yeast. The *MAT***a** and *MAT*α mating factor pheromones are 12 and 13 amino acids, respectively (STOTZLER *et al.* 1976; TANAKA *et al.* 1977; BETZ *et al.* 1987). The

pheromone peptides are, however, made from larger pro-peptides encoded by redundant genes. Mating factor **a** is encoded by two genes, one of which encodes a 36-amino acid peptide and the other a 38-amino acid peptide (MICHAELIS and HERSKOWITZ 1988). Mating factor α precursors are also made from two genes, and their sizes are 165 and 120 amino acids (KURJAN and HERSKOWITZ 1982; SINGH *et al.* 1983). Each of the two pro-peptides contain four and two tandem repeats of the mating factor, respectively. The active version of the Ost4 protein has not been purified, so it is unknown if it undergoes posttranslational processing. More generally, small peptides are known to be associated with certain enzyme complexes (notably cation-dependent ATPases found in fungi and mammalian cells; NAVARRE *et al.* 1992, 1994 and references therein).

An alternative possibility is that the *SAE3* gene product is the RNA itself; the mutations that abolish complementing activity might do so either because translation of the RNA might be required for its functioning or because they affect the structure of the RNA. A precedent for such a possibility exists in fission yeast *Schizosaccharomyces pombe* where an untranslated RNA species is essential for execution of MI (WATANABE and YAMAMOTO 1994).

If the 50-codon ORF is the *SAE3* gene and if it is translated, it is likely that such translation is relatively inefficient. *Saccharomyces cerevisiae* ribosomes always begin translation at the first methionine codon in a transcript. The leader sequence before the first methionine codon of efficiently translated mRNAs is usually 25–30 nt long. The *SAE3* leader is only 13 nt before the initiator methionine codon. Leaders of such diminutive size often result in twofold or greater reductions in the rate of translation initiation compared to longer leaders (reviewed in YOON and DONAHUE 1992).

Since the observed meiosis-specific transcript is considerably larger than that minimally required to encode a 50-codon ORF, we have also considered the possibility that splicing of this transcript (or an even larger precursor RNA) might result in production of a larger protein. To investigate this possibility, a DNA corresponding to the observed *SAE3* transcript was generated by RT-PCR and the size of this DNA was compared to a DNA amplified with the same primers from a genomic clone containing *SAE3* (C. KLEIN, this laboratory, unpublished results). The sizes of the two fragments were identical, suggesting that the observed transcript is unspliced.

What does *SAE3* do? As for Sae1 protein, the function of the *SAE3* gene product is undefined except that it does not encode a factor required for splicing of the *DMC1* transcript (above). Since the phenotype of a *sae3* mutant is indistinguishable from that of a *dmc1* mutant and since Dmc1p appears to be a direct component of the meiotic recombination complex (*e.g.*, BISHOP 1994; TERASAWA *et al.* 1995), Sae3 protein (or RNA) might be such a component as well. Preliminary immunolocalization experiments failed to reveal any discernible signal on the chromosomes, however (C. KLEIN, this laboratory, unpublished results). Alternatively, as for Sae1p, the *SAE3* gene product could be involved in gene expression, chromosome structure and/or cell cycle regulation (even, *e.g.*, as a membrane-associated ATPase complex; above).

We gratefully acknowledge the contribution of PRANAV LOYALKA in the identification of the *sae1-1* mutant. This research was supported by a grant to N.K. from the National Institutes of Health (GM-44794).

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Communicating editor: S. JINKS-ROBERTSON