Tam1, a telomere-associated meiotic protein, functions in chromosome synapsis and crossover interference

Penelope R. Chua and G. Shirleen Roeder
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520-8103 USA

The TAM1 gene of Saccharomyces cerevisiae is expressed specifically during meiosis and encodes a protein that localizes to the ends of meiotic chromosomes. In a taml null mutant, there is an increase in the frequency of chromosomes that fail to recombine and an associated increase in homolog nondisjunction at meiosis I. The taml mutant also displays an increased frequency of precocious separation of sister chromatids and a reduced efficiency of distributive disjunction. The defect in distributive disjunction may be attributable to overloading of the distributive system by the increased number of nonrecombinant chromosomes. Recombination is not impaired in the taml mutant, but crossover interference is reduced substantially. In addition, chromosome synapsis is delayed in taml strains. The combination of a defect in synapsis and a reduction in interference is consistent with previous studies suggesting a role for the synaptonemal complex in regulating crossover distribution. taml is the only known yeast mutant in which the control of crossover distribution is impaired, but the frequency of crossing over is unaffected. We discuss here possibilities for how a telomere-associated protein might function in chromosome synapsis and crossover interference.

[Key Words: Telomeres; crossover interference; meiosis; chromosome synapsis; recombination]

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During meiosis, a single round of DNA duplication is followed by two successive nuclear divisions to generate four haploid progeny from a single diploid cell. The meiosis II division resembles mitotic chromosome segregation, but the meiosis I division is unique in that homologous chromosomes disjoin from each other. A complex series of events unique to prophase of meiosis I ensures that homologs segregate reductionally at the first division.

One important aspect of meiotic prophase is a high rate of recombination between homologous chromosomes. Crossing over establishes chiasmata, which are physical connections between homologous chromosomes that persist until metaphase. Chiasmata ensure the proper orientation of chromosomes on the meiosis I spindle and therefore promote reductional chromosome segregation (for review, see Carpenter 1994). The distribution of crossovers, and the chiasmata to which they give rise, is nonrandom in two respects. First, two crossovers rarely occur closely together—a phenomenon known as crossover interference. Second, every chromosome pair (no matter how small) almost always sustains at least one crossover—referred to as obligate chiasma. A number of studies suggest that crossover interference and obligate chiasma are different manifestations of the same underlying mechanism (Jones 1967; Kaback et al. 1989, 1992; King and Mortimer 1990; Sym and Roeder 1994; P. Ross-Macdonald, J. Novak, and G.S. Roeder, in prep.).

The pairing of homologous chromosomes during meiotic prophase culminates in the formation of an elaborate proteinaceous structure called the synaptonemal complex (SC), which holds homologs closely apposed along their entire lengths (von Wettstein et al. 1984). In yeast, meiotic recombination and chromosome synapsis occur concurrently. Double-strand breaks (the initiators of exchange) are formed just before the initiation of synapsis (Padmore et al. 1991). Joint molecules consisting of double Holliday junctions are present during pachytene, when chromosomes are synapsed fully (Schwacha and Kleckner 1994, 1995). Mature recombinants are produced near the end of pachytene as chromosomes begin to desynapse (Padmore et al. 1991). A number of studies suggest that the SC has a role in regulating the distribution of meiotic recombination events (Arana et al. 1987; Parker 1987; Havekes et al. 1993; Sym and Roeder 1994; Egel 1995). Perhaps the SC provides a conduit for the transmission of an inhibitory signal from one crossover site to nearby potential sites of crossing over (Egel 1978; Maguire 1988; King and Mortimer 1990).

Little is known about the processes that mediate chromosome pairing before the initiation of chromosome synapsis. In some organisms, meiotic chromosomes form a bouquet, which results from the attachment of
telomeres to the nuclear membrane followed by telomere movements that result in their clustering together on one side of the nuclear membrane (for review, see von Wettstein et al. 1984; Fussell 1987; Loidl 1990; Dernburg et al. 1995). It has been suggested that bouquet formation facilitates chromosome pairing by bringing homologs into approximate register with each other, thereby reducing a three-dimensional homology search to a two-dimensional search (Fussell 1987; Dernburg et al. 1995). The timing of bouquet formation is consistent with a role for the bouquet in promoting the initiation of synopsis (Dawe et al. 1994; Scherthan et al. 1996; Bass et al. 1997).

In addition to the exchange-dependent pathway of chromosome segregation described above, there exists an exchange-independent pathway of reductional chromosome segregation called distributed disjunction [Dawson et al. 1986; for review, see Carpenter 1991; Hawley and Theurkauf 1993]. This alternate pathway was first observed in Drosophila females, where the obligately nonexchange fourth chromosome almost always undergoes proper meiosis I disjunction [Grell 1965]. In yeast, two nonexchange artificial chromosomes usually segregate away from each other at meiosis I, as do two nonhomologous authentic chromosomes that lack their normal pairing partners (Dawson et al. 1986; Guacci and Kaback 1991; Sears et al. 1992, 1994). A high rate of recombination and strong interference in yeast ensure that almost all homolog pairs are chiasmate. Therefore, disjunctive disjunction in wild-type yeast operates as a backup system for segregating rare achiasmate chromosomes (Dawson et al. 1986).

We have characterized a novel meiosis-specific yeast gene, TAM1, whose product localizes to the ends of meiotic chromosomes. The tam1 mutant displays reduced spore viability resulting from chromosome missegregation at meiosis I. Recombination is not impaired in tam1 strains, but there is a defect in crossover interference. In addition, chromosome synapsis and nuclear division are delayed in the tam1 mutant. In this paper we consider models for how a telomere-associated protein might be involved in chromosome synapsis and crossover interference.

Results

Isolation of the TAM1 gene

In a screen for transposon insertions that generate lacZ fusion genes in yeast, a strain [M45] carrying a fusion gene expressed specifically in meiotic cells was identified [Burns et al. 1994]. The DNA immediately adjacent to lacZ in strain M45 was cloned [Burns et al. 1994] and used to probe a blot of ordered phage and cosmids clones of yeast genomic DNA [Olson et al. 1986]. The cloned sequence localizes to cosmids c9348, which maps to the left arm of chromosome XV.

A diploid homozygous for the transposition insertion displays a decrease in spore viability relative to wild type [Burns et al. 1994]. A 1.8-kb Xhol fragment (Fig. 1) subcloned from cosmid c9348 rescues this spore inviability phenotype. The gene present in the subclone has been sequenced and designated YOL104c in the course of yeast genome sequencing. Throughout the rest of this paper, YOL104c will be called TAM1 [for telomere-associated meiotic protein, see below]. Analysis of the sequence reveals that the TAM1 open reading frame (ORF) encodes a protein of 352 amino acids with no significant sequence homology to other proteins in databases. A consensus URS1 regulatory element (TGGCGGCTA), shown previously to be involved in the regulation of meiosis-specific genes [Buckingham et al. 1990], is located at bases -80 to -91.

The Tam1 protein is meiosis-specific

Production of the Tam1 protein was monitored in the original M45 strain heterozygous for a tam1::lacZ translational fusion gene. As a control, an isogenic strain [M85; Burns et al. 1994] heterozygous for a zip1::lacZ translational fusion gene was used. ZIP1 has been shown previously to encode a meiosis-specific component of the SC [Sym et al. 1993]. β-Galactosidase assays were performed in mitosis and at various time points in meiosis. There is no detectable β-galactosidase activity in vegetative cells, and both fusion proteins reach maximal abundance at 12–13 hr after the introduction into sporulation medium [Fig. 2]. At this time, the majority of cells are in prophase, before meiosis 1 chromosome segregation [Sym et al. 1993]. TAM1 therefore belongs to the early class of meiotic genes [Mitchell 1994]. Compared with the Zip1 fusion protein, the Tam1–β-galactosidase fusion protein is produced at a very low level or is very unstable. At the time of maximal expression, there is 10 times more β-galactosidase activity in the zip1::lacZ strain than in the tam1::lacZ strain [Fig. 2].

The Tam1 protein localizes to discrete spots at the ends of meiotic chromosomes

Antibodies against the Tam1 protein were generated [see Materials and Methods] and used to localize Tam1 on
expression of \textit{tam1::lacZ} and \textit{zip1::lacZ} fusion genes throughout meiosis. Production of Tam1 and Zip1 proteins was monitored in strains carrying \textit{tam1::lacZ} (M45) and \textit{zip1::lacZ} (M85) translational fusion genes.  

\textbf{Figure 2.} Expression of \textit{tam1::lacZ} and \textit{zip1::lacZ} fusion genes throughout meiosis. Production of Tam1 and Zip1 proteins was monitored in strains carrying \textit{tam1::lacZ} (M45) and \textit{zip1::lacZ} (M85) translational fusion genes.  

\textbf{Figure 3.} Localization of the Tam1 protein. (A) Spread nucleus from wild type [PC712] stained with a DNA-binding dye (DAPI), shown in blue, and anti-Tam1 antibodies, shown in red. (B) A subset of chromosomes selected from wild-type spreads for quantification of the frequency of Tam1-associated telomeres. (C-E) Nucleus from a strain [V5G12] containing an HA-tagged version of Rap1 stained with anti-Tam1 (C) and anti-HA antibodies (D). The two staining patterns are superimposed in E. (F) Spread nucleus from the \textit{spo11} mutant [PC896] stained with DAPI (blue) and antibodies to Tam1 (red). Spread nucleus from a \textit{tam1} strain [PC664] stained with DAPI (G) and anti-Zip1 antibodies (H). Scale bars, 1 \textmu m.
and the epitope. Previous studies have shown that Rap1 is concentrated at the ends of chromosomes [Klein et al. 1992]. There is extensive co-localization of Tam1 and Rap1 foci in pachytene nuclei (Fig. 3C–E). In addition to a number of foci that stain intensely with antibodies to both Tam1 and Rap1, there are a number of sites that stain faintly with antibodies to Rap1, but contain no detectable Tam1. These presumably correspond to interstitial Rap1-binding sites [Shore and Nasmyth 1987].

Nuclei judged to be in early prophase based on the pattern of anti-Zip1 staining [Smith and Roeder 1997] also contain Tam1 spots, indicating that Tam1 localization occurs before synopsis (data not shown). Tam1 localization must be independent of chromosome synopsis and meiotic recombination, because Tam1 localizes to chromosomes in the spo11 mutant [Fig. 3F], which does not initiate recombination or synopsis [Dresser and Giroux 1988, Cao et al. 1990].

A tam1 null mutation reduces spore viability

Null alleles of TAM1 were generated by deleting most of the coding region and replacing it with yeast selectable markers [Fig. 1]. The tam1 null mutant is viable and does not display any obvious mitotic defects [e.g., tam1 strains exhibit wild-type resistance to the DNA-damaging agent, methylmethane sulfonate]. When introduced into starvation medium, 86% of tam1 diploid cells undergo sporulation to produce asc, compared with 96% in wild type [Table 1]. Spore viability in the homozygous tam1 null mutants is reduced significantly compared with wild type [Table 1]. In one strain background, spore viability is 82% in tam1 compared with 96% in wild type. In another strain background, spore viability is 76% in the mutant compared to 94% in wild type. These differences are statistically significant [P < 0.0001]. Additionally, the pattern of spore death in the tam1 mutant is significantly different from wild type [P < 0.0001]. In tam1, 2-spore-viable and 0-spore-viable tetrads account for more than half of all tetrads containing inviable spores. In contrast, 3-spore-viable tetrads are the major source of inviable spores in wild type.

tam1 mutants are proficient for recombination

Meiotic gene conversion in tam1 strains was assessed in two ways. Prototroph formation was measured at four different heteroallelic loci [Table 2A]. tam1 mutants display approximately wild-type levels of prototroph formation at all loci examined [0.8- to 1.6-fold decrease; differences not significant]. Gene conversion was also measured by tetrad analysis [Table 2B]. At five loci examined, the frequencies of 1:3 and 3:1 segregations in the tam1 mutant are not significantly different from those in wild type (0.3- to 2.6-fold decrease).

Crossing over was measured in five intervals on chromosome III by tetrad analysis [Table 3]. In four of the intervals measured [HML–HIS4, HIS4–CEN3, CEN3–MAT, and MAT–RAD18], the levels of crossing over in tam1 mutants are not significantly different from those in wild type. In the CEN3–MAT and RAD18–HMR intervals, crossing over in tam1 is increased slightly compared to wild type.

tam1 strains undergo both homolog nondisjunction and precocious separation of sister chromatids

In a wild-type meiosis, homologous chromosomes segregate to opposite poles at meiosis I and sister chromatids segregate at meiosis I. This results in the production of four viable spores, each carrying one copy of each chromosome [Fig. 4A]. During meiosis I nondisjunction, both homologs segregate to the same pole, resulting in the production of two viable sister spores that are disomic for the missegregating chromosome and two dead spores that are nullisomic [Fig. 4B]. If there is precocious separation of sister chromatids [PSSC] at meiosis I, one chromosome and one chromatid from the homologous chromosome can segregate to the same pole, resulting in the

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Table 1. Spore viability, distribution of tetrad types, and frequencies of chromosome III segregation errors and Eo tetrads

| Strain     | Spo. efficiency (%) | No. tetrads | 4-s.v. | 3-s.v. | 2-s.v. | 1-s.v. | 0-s.v. | Spore viability (%) | Frequency (%) | Eo tetrads |
|------------|---------------------|-------------|--------|--------|--------|--------|--------|---------------------|--------------|------------|
|            |                     |             |        |        |        |        |        |                     |              |            |
| TAM1 (MY261) | 94                 | 683         | 89.0   | 7.2    | 2.9    | 0.2    | 0.7    | 96                  | 0            | 0.29       |
| tam1 (PC873) | 87                 | 830         | 70.6   | 6.8    | 12.7   | 1.7    | 0.2    | 82                  | 1.2          | 0.48       |
| tam1 (PC872) | 98                 | 825         | 86.5   | 7.9    | 2.8    | 1.2    | 1.6    | 94                  | 0            | 0.24       |
| tam1 (PC871) | 85                 | 1339        | 54.5   | 17.7   | 14.4   | 4.0    | 9.3    | 76                  | 0.3          | 0.82       |

The efficiency of sporulation [Spo. efficiency] and the distribution of 4-spore-viable [4-s.v.], 3-spore-viable [3-s.v.], 2-spore-viable [2-s.v.], 1-spore-viable [1-s.v.], and 0-spore-viable [0-s.v.] tetrads are shown for wild-type and the tam1 mutant in two different sets of isogenic strains. MY261 and PC873 are isogenic to each other, as are PC872 and PC871. The frequency of meiosis I nondisjunction [MI ndj.] for chromosome III is that of 3-spore-viable and 2-spore-viable tetrads containing a single disomic spore among total tetrads dissected [No. tetrads]. The frequency of precocious separation of sister chromatids [PSSC] is that of 3-spore-viable and 2-spore-viable tetrads containing a single disomic spore among total tetrads. "Trp` Ura` disomes in 1-spore-viable tetrads could have resulted either from PSSC, meiosis I nondisjunction of chromosome III accompanied by missegregation of other chromosomes, or random spore death. The total frequency of chromosome III meiosis I missegregation events [misseg.] includes events that could not be categorized in addition to meiosis I nondisjunction and PSSC events. The number of Eo tetrads refers to the number of 4-spore-viable tetrads in which chromosome III is nonrecombinant along its entire length [in strains PC872 and PC871]. The number of Eo tetrads observed [obs.] was adjusted [see Materials and Methods] to account for the expected number of two-strand double crossovers to give the corrected Eo value [cor.].
Table 2. Gene conversion

| Locus         | TAM1   | tam1   | Percent of wild type |
|---------------|--------|--------|----------------------|
|               |        |        |                      |
| A. Prototroph formation |        |        |                      |
| leu2-3,112    | 4.3 (± 0.6) x 10^{-4} | 2.7 (± 1.0) x 10^{-4} | 63 |
| leu2-27       | 6.5 (± 0.25) x 10^{-3} | 6.3 (± 0.9) x 10^{-3} | 97 |
| his4-260      | 1.6 (± 0.3) x 10^{-4} | 1.9 (± 0.4) x 10^{-4} | 119 |
| his4-280      | 5.0 (± 2.1) x 10^{-5} | 4.1 (± 0.9) x 10^{-5} | 82 |
| B. Tetrad analysis |        |        |                      |
| HML           | 0.4% (3/714) | 1.2% (9/730) | 300 (P = 0.09) |
| HIS4          | 0.7% (5/714) | 0.5% (4/730) | 71 (P = 0.71) |
| MAT           | 0.1% (1/714) | 0.4% (3/730) | 400 (P = 0.33) |
| RAD18::ADE2   | 4.8% (34/708) | 4.4% (32/725) | 92 (P = 0.74) |
| HMR           | 1.3% (9/713) | 0.5% (4/730) | 38 (P = 0.15) |

[A.] Meiotic prototroph frequencies at four different heteroallelic loci are shown along with the change in tam1 relative to wild type. Data are from strains BR2625 [TAM1] and PC647 [tam1]. The rate of meiotic prototrophy was determined by subtracting the mitotic (premeiotic) frequency from the meiotic frequency (taken after 24 hr in sporulation medium) for each experiment and averaging the meiotic values. At least three cultures were analyzed for each strain. [B.] Absolute frequencies of gene conversion were measured by tetrads analysis at five different loci. Data are from strains PC872 (wild type) and PC871 (tam1). The number of conversion events relative to the total number of 4-spore-viable tetrads scored is indicated in parentheses. The amount of gene conversion in the tam1 mutant is indicated as a percent of the wild-type level; the probability that the difference observed is statistically significant is indicated in parentheses.

production of one disomic spore, one nullisomic spore, and two haploid spores [Fig. 4C].

The types of missegregation events occurring in tam1 strains, and the influence of recombination on chromosome disjunction, were determined using appropriately marked yeast strains [Fig. 5A]. The centromere of chromosome III is marked with URA3 on one chromosome, and with TRP1 on the homolog; CEN3 is flanked by markers at HIS4 and MAT. A meiosis I nondisjunction event in which both chromosomes III segregate to the same pole (and all other chromosomes segregate correctly) produces a 2-spore-viable tetrads in which both

Table 3. Crossing over and crossover interference

| Strain       | Interval | PD | TT | NPD | Map distance [cM] | Fold decrease | Probability WT C.O. | NPDs expected | NPD ratio | Probability WT int. | Probability no int. |
|--------------|----------|----|----|-----|------------------|---------------|---------------------|---------------|-----------|---------------------|---------------------|
| TAM1 (PC872) | HML-HIS4 | 279 | 409 | 17  | 36.2             | —             | —                   | 58            | 0.29      | —                   | <0.0001*            |
| tam1 (PC871) | HML-HIS4 | 361 | 336 | 22  | 32.5             | 1.1x           | 0.14                | 31            | 0.71      | 0.02*               | 0.11                |
| TAM1 (PC872) | HIS4-CEN3| 244 | 444 | 19  | 39.5             | —             | —                   | 79            | 0.24      | —                   | <0.0001*            |
| tam1 (PC871) | HIS4-CEN3| 292 | 434 | 32  | 41.3             | 0.9x           | 0.48                | 66            | 0.48      | 0.03*               | <0.0001*            |
| TAM1 (PC872) | CEN3-MAT | 349 | 358 | 5   | 27.2             | —             | —                   | 37            | 0.14      | —                   | <0.0001*            |
| tam1 (PC871) | CEN3-MAT | 391 | 313 | 23  | 31.0             | 0.9x           | 0.11                | 25            | 0.92      | 0.0002*             | 0.21                |
| TAM1 (PC872) | MAT-RAD18| 281 | 382 | 12  | 33.6             | —             | —                   | 51            | 0.24      | —                   | <0.0001*            |
| tam1 (PC871) | MAT-RAD18| 339 | 330 | 21  | 33.0             | 1.0x           | 0.82                | 31            | 0.68      | 0.01*               | 0.07                |
| TAM1 (PC872) | RAD18-HMR| 396 | 263 | 8   | 23.3             | —             | —                   | 18            | 0.44      | —                   | 0.02*               |
| tam1 (PC871) | RAD18-HMR| 344 | 322 | 23  | 33.4             | 0.7x           | <0.0001*            | 29            | 0.79      | 0.25                | 0.27                |

Strains PC871 and PC872 are isogenic. Only 4-spore-viable tetrads that did not show gene conversion of the markers indicated were used to calculate map distances. Tetrads were scored as parental ditype (PD), tetratype (TT), or nonparental ditype (NPD). The probability of wild-type crossing over [Probability WT C.O.] refers to the likelihood that the observed differences in the frequency of crossing over between wild type and tam1 are attributable to chance [see Materials and Methods]. NPDs expected are the number of four-strand double crossovers expected in the absence of interference; the NPD ratio refers to the number of NPDs observed relative to the number of NPDs expected. The probability of wild-type interference [WT int.] indicates the likelihood that the difference in NPD ratio between wild type and tam1 is attributable to chance; the probability of no interference [no int.] is the likelihood that the difference between the number of NPDs observed and the number expected in the absence of interference is attributable to chance. Asterisks denote probability values in which the differences observed are statistically significant [P < 0.05].
Intervals monitored. However, none of the 14 pairs of nondisjunction for chromosome III was detected in meiosis I nondisjunction. A possible explanation for these differences is presented in the Discussion Section.

The total frequency of chromosome III missegregation events (meiosis I nondisjunction and PSSC) is increased from 0% in wild type to 0.3% in the mutant, whereas PSSC is increased from 0.24% to 0.82%. Conversely, in the isogenic wild type in both sets of strains. In one set (MY261 and PC871), meiosis I nondisjunction is in-creased from 0.24%-0.29% in wild type to 1.2% in the tam1 mutant, whereas PSSC is increased from 0.29% to 0.48%. In the other set (PC871 and PC872), meiosis I nondisjunction is increased from 0% in wild type to 0.3% in the tam1 mutant, whereas PSSC is increased from 0.24% to 0.82%. The total frequency of chromosome III missegregation events [meiosis I nondisjunction and PSSC] is increased from 0.24%-0.29% in wild type to 1.5%-1.9% in tam1 mutants. These differences between wild type and mutant are statistically significant (P < 0.005). Note that the relative distributions of chromosome missegregation events are different in the two different tam1 strains studied. In one strain (PC873), meiosis I nondisjunction is 2.5-fold more frequent than PSSC. Conversely, in the other strain (PC871), PSSC is 2.7-fold more frequent than meiosis I nondisjunction. A possible explanation for these differences is presented in the Discussion Section.

A total of 14 pairs of disomes indicative of meiosis I nondisjunction for chromosome III was detected in tam1 mutants. Based on the frequency of tetratype tetrads among 4-spore-viable tetrads (Table 3), 7 of the 14 pairs of disomes should be recombinant in at least one of two intervals monitored. However, none of the 14 pairs of disomes detected are recombinant, which is significantly different from the number of recombinants expected (P < 0.001). This result suggests that the failure to recombine is the cause of homolog nondisjunction in the tam1 mutant. Of 15 PSSC events detected, three disomes contain chromosomes that are recombinant in one or both intervals, which is not significantly different from the five recombinant disomes expected based on the frequency of tetratype tetrads observed among 4-spore-viable tetrads (P > 0.2). This result suggests that PSSC occurs irrespective of recombination in tam1 mutants.

The proportion of E0 tetrads is increased in tam1 mutants

The increased frequency of homolog nondisjunction at meiosis I in tam1 strains might be attributable to an increase in the number of tetrads in which one or more pairs of homologous chromosomes fail to receive a crossover (E0 tetrads). The frequency of E0 tetrads for chromosome III was determined in a pair of isogenic strains [PC872 [wild type] and PC871 [tam1]] in which chromosome III has been marked along its entire length (Fig. 5B; Table 1). Both the observed and corrected numbers of E0 tetrads [see Materials and Methods for determination of E0 tetrads] are at least an order of magnitude higher in tam1 than in the isogenic wild type. The frequency of E0 tetrads among the total number of 4-spore-viable tetrads is 0.28% in wild type and 5.3% in the mutant, corresponding to a nearly 20-fold increase in the number of chromosome pairs that fail to cross over. The frequency of E0 tetrads is almost certainly underestimated, as these events are detected only in 4-spore-viable tetrads.

Crossover interference is impaired in tam1 mutants

Crossover interference is the term used to describe the nonrandom distribution of crossovers, such that there is an inhibition of crossing over in regions that have already undergone exchange. One way to assess interference is to determine the NPD ratio (Snow 1979), which is the frequency of NPDs [nonparental ditype tetrads resulting from 4-strand double crossovers] observed divided by the frequency of NPDs expected in the absence of interference. The expected frequency is calculated based on the observed frequency of tetratype tetrads resulting from single crossovers. No interference results in an NPD ratio of 1.0 while absolute interference results in an NPD ratio of zero.

Crossover interference was measured in five different intervals on chromosome III in both wild type (PC872) and tam1 [PC871]. Interference was found to be significantly weaker in the tam1 mutant than in the isogenic wild type (Table 3). For wild type, the NPD ratios for all five intervals are significantly lower than the value of 1.0 expected in the absence of interference. In contrast, the tam1 mutant shows a significant level of interference only in the HIS4-CEN3 interval. In each of the five intervals, the NPD ratio in the mutant is higher than in wild type (differences significant in every interval except...
Figure 5. Relevant configurations of markers in yeast strains used for genetic analysis. (A) Strains MY261 and PC873. The centromere of one chromosome III is marked with \( URA3 \) and the centromere of the homolog is marked with \( TRP1 \). Flanking markers at \( HIS4 \) and \( MAT \) allow crossing over to be monitored. (B) In strains PC871 and PC872, \( CEN3 \) is also marked with \( URA3 \) on one homolog and \( TRP1 \) on the other. Flanking markers on both arms of chromosome III allow crossing over to be monitored almost the entire length of the chromosome. (C) Strains DS101 and PC874 contain nonhomologous YAC pairs, one of which is marked with \( URA3 \) and \( HIS3 \) and the other with \( LYS2 \) and \( TRP1 \). (D) PC875 and PC876 contain homologous YAC pairs, with the centromere marked by \( URA3 \) or \( TRP1 \) and flanked by \( LEU2 \) and \( HIS3 \). Crossing over can be detected on either YAC arm.

RAD18–HMR), but less than 1.0. We conclude that crossover interference is impaired, but not completely abolished, in \( tam1 \) mutants.

Another way to assess interference is to determine whether crossing over in one interval is inhibited by exchange in an adjacent interval. Such inhibition results in a reduced frequency of tetrads that sustain coincident crossovers in two adjacent intervals, compared with the frequency predicted if crossing over in the two intervals is independent.

Table 4 shows the results of tetrad analyses for four pairs of adjacent intervals in wild-type (PC872) and \( tam1 \) (PC871) strains. In wild type, the frequency of coincident crossovers is significantly lower than that predicted assuming no crossover interference in all pairs of intervals except for \( HIS4-CEN3-MAT \). In contrast, the frequencies of coincident events in \( tam1 \) are not significantly different from the predicted frequencies in all pairs of intervals analyzed. Therefore, crossovers in \( tam1 \) strains are not strongly affected by crossovers nearby.

Distributive disjunction is impaired in \( tam1 \) mutants

To examine the effect of \( tam1 \) on the distributive disjunction system, the distributive disjunction of yeast artificial chromosomes (YACs) was measured in wild type and the \( tam1 \) mutant. Two sets of YACs, a nonhomologous pair (Fig. 5C) and a homologous pair (Fig. 5D), were examined.

In wild type, the nonhomologous pair of YACs segregates correctly to opposite poles at meiosis I in 76% of all meioses and incorrectly to the same pole in 12% of meioses (Table 5). This is significantly different from the 1:1 ratio of correct segregations to meiosis I nondisjunctions expected if the YACs segregate randomly. In \( tam1 \), proper segregation occurs 50% of the time and meiosis I

| Strain | Interval | Frequency 1st interval | Frequency 2nd interval | Frequency expected | Frequency observed | Probability |
|--------|----------|------------------------|------------------------|--------------------|--------------------|------------|
| TAM1   | HML–HIS4-CEN3 | 0.578                   | 0.705                  | 0.408              | 0.333              | <0.0001 *  |
| tam1   | HML–HIS4-CEN3 | 0.509                   | 0.648                  | 0.330              | 0.347              | 0.34       |
| TAM1   | HIS4-CEN3-MAT | 0.694                   | 0.381                  | 0.264              | 0.242              | 0.17       |
| tam1   | HIS4-CEN3-MAT | 0.620                   | 0.437                  | 0.271              | 0.258              | 0.44       |
| TAM1   | CEN3-MAT–RAD18 | 0.379                   | 0.581                  | 0.220              | 0.138              | <0.0001 *  |
| tam1   | CEN3-MAT–RAD18 | 0.474                   | 0.507                  | 0.241              | 0.216              | 0.13       |
| TAM1   | MAT–RAD18–HMR | 0.585                   | 0.402                  | 0.235              | 0.174              | 0.0002 *   |
| tam1   | MAT–RAD18–HMR | 0.508                   | 0.502                  | 0.255              | 0.250              | 0.79       |

Data are from strains PC872 (TAM1) and PC871 (tam1). Frequencies in the first and second intervals refer to the frequency of tetrads that are either tetratype or nonparental diatype in that interval. Expected frequency refers to that of tetrads that are recombinant in both intervals and is calculated by multiplying the frequencies in the individual intervals; observed frequency refers to that of tetrads that are recombinant in both intervals. Probability refers to the likelihood that the difference between the expected and observed frequencies is attributable to chance. Asterisks denote probabilities for which the differences observed are statistically significant (\( P < 0.05 \)).
nondisjunction occurs 26% of the time. This nondisjunction frequency is significantly higher than that observed in wild type, but it is still significantly lower than that expected for a random pattern of segregation. Meiosis I PSC also occurs in both wild-type and tam1 strains, but is increased about twofold in the mutant.

A similar result was obtained when homologous pairs of YACs were examined. In this case, only nonrecombinant YACs (~97% of meioses) were considered in calculations of distributive disjunction (Dawson et al. 1986). Nonrecombinant YACs disjoin from each other 92% of the time in wild type, but only 82% of the time in tam1. Therefore, distributive disjunction is impaired, but not abolished, in tam1 mutants.

**Table 5. Distributive disjunction**

|                     | Nonhomologous YACs | Homologous YACs |
|---------------------|--------------------|----------------|
|                     | [YAC12-UH/YAC21.4-LT] | [YLp45/YLp49] |
| Correct disjunction | TAM1 | tam1 | TAM1 | tam1 |
| Meiosis I           |       |       |       |       |
| nondisjunction      | 84 (76%) | 74 (50%) | 119 (92%) | 115 (82%) |
| Meiosis I PSC       | 13 (12%) | 39 (26%) | 10 (8%) | 26 (18%) |
| Total tetrads       | 110 | 148 | 129 | 141 |
| Probability wild type |       |       |       |       |
|                     | <0.0001* | 0.02* | <0.0001* | <0.0001* |
| Probability random  |       |       |       |       |

Distributive disjunction was measured for two different pairs of YACs. Strains DS101 [TAM1] and PC874 [tam1] were used to measure distributive disjunction of nonhomologous YACs. Strains PC875 [TAM1] and PC876 [tam1] were used to measure distributive disjunction of homologous YACs; data shown are for nonrecombinant YACs. Probability wild type indicates the likelihood that the observed differences between wild type and tam1 are attributable to chance alone. Probability random indicates the likelihood that the observed frequencies of disjunction follow a random (1:1) pattern. Only tetrads displaying correct disjunction or meiosis I nondisjunction were included in probability calculations. Asterisks denote probabilities for which the differences observed are statistically significant (P < 0.05).

The meiosis I division is delayed in tam1 mutants

To monitor the timing of the meiotic nuclear divisions, cells from wild-type and tam1 strains were harvested at different time points after the induction of meiosis and stained with DAPI. Cells that have completed the meiosis I division contain two discrete nuclei, whereas cells that have completed both meiotic divisions contain four nuclei. The kinetics of nuclear division were examined in both sets of isogenic strains used to examine chromosome segregation (Table 1).

In tam1 mutants, the generation of binucleate and tetranucleate cells is delayed by at least 2 hr compared with the isogenic wild type. In addition, formation of binucleate cells appears to be less synchronous in tam1. Meiosis II is also delayed in tam1 strains, presumably as a consequence of the delay in meiosis I.

The data also reveal that the two sets of strains examined differ with respect to the timing of the meiotic divisions. Strains MY261 and PC873 [Fig. 6A–C] undergo meiosis faster than strains PC871 and PC872 [Fig. 6D–F]. This difference may account for the different patterns of chromosome missegregation observed in tam1 mutants (see above and Discussion Section).

**Figure 6.** Time course of nuclear divisions. Nuclear divisions in wild-type (○) and tam1 (▲) strains were examined at 1-hr intervals throughout meiosis. Mononucleate cells have not yet completed the meiosis I division. Binucleate and tetranucleate cells have completed meiosis I or both meiotic divisions, respectively. Results shown in A, B, and C are for strains MY261 [wild type] and PC873 [tam1]. Results shown in D, E, and F are for strains PC872 [wild type] and PC871 [tam1].
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Chromosome synapsis is delayed in tam1 mutants

Spread meiotic chromosomes from wild type and a tam1 mutant were stained with silver nitrate and examined in the electron microscope. At the time of maximum synapsis, tam1 nuclei form tripartite SCs that are morphologically similar to those seen in wild type [Fig. 7]. Spread chromosomes from the tam1 mutant were also stained with anti-Zip1 antibodies. Zip1 is a component of the central region of the SC; therefore, Zip1 localization serves as a marker for chromosome synapsis. In the tam1 mutant, Zip1 appears to localize along the length of every chromosome pair, indicating full synapsis [Fig. 3G,H].

To monitor the kinetics of SC assembly, chromosome spreads were prepared from sporulating cultures of tam1 and wild-type strains at various times after the induction of meiosis. Anti-Zip1 and anti-tubulin antibodies were used to visualize the SC and meiotic spindles, respectively. In the wild-type strain, the number of cells in pachytene reaches a maximum at 13 hr after the induction of meiosis [Fig. 8A]. After this time point, nuclei that stain with anti-Zip1 antibodies decrease in frequency, whereas nuclei with spindles increase. Compared with wild type, tam1 mutants display a delay in chromosome synapsis [Fig. 8B]. The number of nuclei in pachytene reaches a maximum after 17 hr of sporulation, ~4 hr later than in wild type. Correspondingly, nuclei with spindles appear much later in the tam1 mutant than they do in wild type.

Discussion
		
tam1 is defective in meiotic chromosome segregation

Disruption of the TAM1 gene confers multiple defects in meiotic chromosome segregation. Compared with wild type, meiosis I nondisjunction and PSSC are increased significantly in the tam1 mutant, and distributive disjunction is less efficient.

The increase in homolog nondisjunction in tam1 strains is the consequence of an increase in tetrads that contain nonrecombinant homologs. There are two possible explanations for this increase in Eo chromosomes. If a tam1 mutation confers a defect in homolog pairing, then homologs might fail to cross over because they fail to find each other. Alternatively, or in addition, the increase in Eo chromosomes might result from a deregulation of crossover distribution. Crossover interference is thought to be important for distributing crossovers among chromosomes so that even small chromosomes undergo at least one crossover in every meiosis [King and Mortimer 1990; Kaback et al. 1992]. Analysis of the zip1 mutant [Sym and Roeder 1994], in which crossover interference is abolished, is consistent with the hypothesis that interference is responsible for distributing crossovers among chromosomes. If a wild-type number of...
crossovers is distributed randomly among chromosomes (as a function of chromosome size), then chromosome III is expected to be nonrecombinant in 9.5% of meioses. Therefore, the frequency of Eo tetrads for chromosome III in tam1 [5.3%] could be accounted for by a deregulation of crossover distribution.

The increase in Eo tetrads probably accounts for the reduced efficiency of distributive disjunction in the tam1 mutant. Because the distributive disjunction pathway does not discriminate chromosomes on the basis of homology, an increase in the number of chromosomes entering this system might cause the YACs to disjoin from authentic chromosomes instead of from each other. In Drosophila, mutations that decrease crossing over cause missegregation of the fourth chromosome, which normally segregates exclusively via the distributive disjunction pathway [for review, see Baker et al. 1976]. Even in wild-type flies, the fourth chromosome is more likely to nondisjoin in ova in which the X chromosome is nonrecombinant [for review, see Baker et al. 1976]. It seems likely that the distributive disjunction pathway in yeast is also sensitive to the number of chromosomes it has to segregate. An alternative or additional explanation for the defect in distributive disjunction is that the Tam1 protein is directly involved in the pairing and/or segregation of chromosomes via the distributive system.

The increase in PSSC in the tam1 mutant might be attributable to the delay in meiotic prophase progression. If kinetochores continue to differentiate during this pause in the cell cycle, then sister chromatids may separate from each other before meiosis I, therefore leading to PSSC. A similar model was proposed by Carpenter [1994] to account for the increase in PSSC observed in strains containing nonrecombinant or dicentric chromosomes [e.g., Rockmill and Roeder 1994; Flatters et al. 1995]. This model can explain the different relative frequencies of meiosis I nondisjunction versus PSSC observed in different tam1 strains. A strain that spends a longer time in prophase would be expected to display more PSSC; a longer prophase might also decrease the probability of homolog nondisjunction by allowing time for nonrecombinant chromosomes to pair and segregate via the distributive system [Loidl et al. 1994]. Of the two strains used to study chromosome segregation, the strain that has a higher frequency of PSSC relative to meiosis I nondisjunction spends a longer time in prophase than the strain in which the relative frequencies of homolog nondisjunction and PSSC events are reversed.

tam1 decreases interference without decreasing crossing over

tam1 mutants display approximately wild-type levels of crossing over in all intervals examined; however, crossover interference is reduced significantly. Therefore, the tam1 mutation functionally separates the control of crossover distribution from the control of crossover frequency. In this respect, tam1 is unique among yeast mutants. In the two other yeast mutants known to be defective in interference, zip1 [Sym and Roeder 1994] and msh4 [Ross-Macdonald and Roeder 1994; P. Ross-Macdonald, J. Novak and G. S. Roeder, in prep.] mutations both decrease crossover interference. However, the phenotype of the tam1 mutant demonstrates that a reduction in crossover interference need not be associated with a decrease in crossing over.

The defect in synthesis may account for the decrease in interference in the tam1 mutant

In addition to the defect in interference, tam1 mutants also display a delay in chromosome synopsis. Based on previous studies [summarized below] suggesting a role for the SC in the regulation of crossover distribution, it is attractive to speculate that the delay in synopsis is the cause of the defect in interference in the tam1 mutant. Many models of interference postulate the transmission of an inhibitory signal along the SC, starting at the site of a crossover and extending to nearby potential sites of crossing-over [Egel 1978; Maguire 1988; King and Merey 1989].
timer 1990). If commitment to crossing over occurs on
time in tam1 strains, or is delayed to a lesser extent than
synapsis, then transmission of the inhibitory signal
might be compromised.

A number of studies have implicated the SC in the
control of crossover distribution. Most recombination-
proficient organisms display interference and also make
SC (for review, see Jones 1987). In contrast, two organ-
isms that do not make SC, Schizosaccharomyces pombe
[Olson et al. 1978] and Aspergillus nidulans [Egel-Mitani
et al. 1982], do not display crossover interference. In
yeast, analysis of the zip1 mutant has provided evidence
for a functional relationship between the SC and inter-
ference. Strains lacking the ZIP1 gene, which encodes a
component of the central region of the SC [Sym et al.
1993; Sym and Roeder 1995], undergo a substantial
amount of recombination, but are completely defective
in crossover interference [Sym and Roeder 1994]. The asl
and asb mutants of tomato also display defects in both
synapsis and interference [Moens 1969, Havekes et al.
1994]. In asb, analysis of partially synapsed chromo-
somes reveals that a recombination node (indicative of
crossing over) is located at each synapsed segment
(Havekes et al. 1993). A chromosome can contain as
many as three n nodules [which is rare for wild type] if it
contains three independently synapsed segments, sug-
gest ing that interference does not extend beyond the
boundaries of the synapsed segment. In addition, in
plants and insects heterozygous for a chromosome rear-
rang ement, interference does not operate across the in-
terruption in the SC that results from rearrangement
(Arana et al. 1987; Parker 1987).

Two different models have been proposed to account
for the role of the SC in crossover interference. One
model supposes that synapsis initiates at the sites of
crossovers. Further exchange is inhibited in regions of
the chromosomes that have synapsed, but crossovers can
still be initiated in regions that have not yet synapsed
[Egel 1978; Maguire 1988]. Therefore, zipping up of the
SC serves as the mechanical basis of interference.
An alternate view is that interference acts after synapsis
is complete and full-length SC acts as a substrate for the
transmission of an inhibitory signal [King and Mortimer
1990; Sym and Roeder 1994]. Signal transduction may
involve a conformational change in proteins associated
with the SC that starts at sites of crossing over and
moves along the chromosome in a domino-like effect, or
it may involve the polymerization of a protein along the
SC starting at the sites of crossing over. According to
both models, the signal that inhibits crossing over would
not be transmitted on time if synapsis is delayed relative
to the timing of commitment to crossing over.

A recent model for crossover interference suggests that
interference is independent of the SC [Kleckner 1996;
Storlazzi et al. 1996]. This model is based on the obser-
vation that a zip1 mutation reduces crossing over in a
red1 mutant and hinges on the assumption that there is
no Zip1 polymerization in the red1 mutant. However, a
recent study demonstrates that there is extensive poly-
merization of the Zip1 protein in red1 strains [Smith and
Roeder 1997]; therefore, Zip1 need not participate in re-
combination in a manner that is independent of its role
in SC formation. In addition, mutational analysis of the
ZIP1 gene has failed to identify any mutations that sepa-
rate the role of Zip1 in chromosome synapsis from its
role in crossover interference [K.-S. Tung and G.S.
Roeder, in prep.].

A role for Tam1 in homolog pairing!

How might a telomere-associated protein participate in
chromosome synapsis and crossover interference? In
eukaryotes, synapsis initiates at or near telomeres
[von Wettstein et al. 1984], raising the possibility that
Tam1 is required for the initiation of synapsis. In yeast,
however, the evidence indicates that telomeres are not
important for synaptic initiation. Loidl [1995] observed
that synapsis can initiate interstitially by studying pair-
ning partner switches in tetraploid yeast. Studies of the
zip1 mutant have identified sites of intimate association
between paired but unsynapsed axial elements. These
connections, which are presumed to be sites of synaptic
initiation [Sym et al. 1993; Rockmill et al. 1995], are not
concentrated at the ends of chromosomes. In addition, in
zip1 non-null mutants in which the rate of synapsis is
delayed, regions near the ends of chromosomes are fre-
quently unsynapsed even when most of the chromosome
pair is engaged in SC formation [K.-S. Tung and G.S.
Roeder, in prep.]. Furthermore, when wild-type spreads
are double-stained with anti-Zip1 and anti-Tam1 antibi-
dies, the Zip1 and Tam1 foci do not overlap in nuclei
presumed to be in zygotene (data not shown). Taken to-
gether, these observations argue against the possibility
that synapsis initiates at telomeric regions in yeast.

It is possible that Tam1 has a role in crossover inter-
ference that is independent of its role in synapsis. Klec-
nner and colleagues [Kleckner 1996; Storlazzi et al. 1996]
have proposed that the attachment of meiotic chromatin
to axial elements [the proteinaceous cores shared by sis-
ter chromatids] induces tension and that recombination
intermediates are sensitive to this stress. When the level
of tension reaches a certain threshold, a crossover event
is initiated. The act of initiation relieves stress in the
immediate vicinity of the crossover, which then reduces
the probability that additional crossing over will be ini-
tiated nearby. Tam1 might function in this context if
attachment of chromosome ends to the nuclear mem-
brane [or some other structure] is necessary to impose
tension. In the absence of an attachment that confines
the movement of a stressed chromosome, the tension
required for crossover control might be more easily dis-
sipated, resulting in reduced interference. This model
does not easily account for the delay in synapsis in the
tam1 mutant.

We favor the view that the Tam1 protein has a role in
homolog pairing, and that the delay in chromosome syn-
apsis is the consequence of an earlier defect in chromo-
some alignment. The attachment of telomeres to the
nuclear membrane and their clustering together during
early meiotic prophase have been postulated to be im-

important for bouquet formation (for review, see von Wettstein et al. 1984; Fuselli 1987; Loidl 1990; Dernburg et al. 1995). Formation of the bouquet may facilitate chromosome pairing by bringing homologous chromosomes into approximate alignment [Fuselli 1987; Dernburg et al. 1995]. Because the bouquet is a meiotic phenomenon, it is reasonable to suppose that a meiosis-specific protein like Tam1 is involved. Tam1 might be important for the early steps in chromosome pairing by promoting the attachment of chromosome ends to the nuclear membrane or by promoting their subsequent clustering.

Materials and methods

Strain constructions and media

Yeast manipulations were performed and media were prepared according to Sherman et al. [1986]. Subtitutive and integrative transformations [Rothstein 1991] were carried out by the lithium acetate procedure [Ito et al. 1983]. Genotypes of yeast strains are listed in Table 6. Strain BR2625 was obtained from Beth Rockmill (Yale University, New Haven, CT). Strain MY261 was obtained from Mary Sym (Sym and Roeder 1994). Strain DS101 was obtained from Phil Hieter [Sears et al. 1994]. Strain BR2495, a pHSS6 [Seifert et al. 1986] derivative in which the EcoRI site in the polynucleotide has been destroyed, to generate pCB241. The Ty1 sequences immediately upstream of TAM1 were then removed by cutting with BsalI [which cuts between the Ty1 element and the TAM1 promoter region] and HindIII (which cuts in the polynucleotide), filling in the overhanging ends with the Klenow fragment of DNA polymerase I, and religating to generate pCB243. pCB243 was used to make all tam1 dele-

Table 6. Yeast strains

| Strain | Genotype |
|--------|----------|
| BR2625 | his4-260 leu2-3,112 MATa ARG4 thr1-4 trp1-289 ura3-1 ade2-1 CYH10 spo13::ura3-1 |
| PC674  | BR2625, but homozygous tam1::URA3 |
| MY261  | his4-260 leu2::hisG CEN3::TRP1 MATa lys2 ho::lys2 trp1-3 H3 ura3 |
| HIS4   | leu2::hisG CEN3::URA3 MATa lys2 ho::LYS2 trp1-3 H3 ura3 |
| PC873  | MY261, but tam1::LYS2 |
| PC872  | HML his4 leu2 CEN3::TRP1 MATa RAD18 hmr::LYS2 ade2 arg4-npa ura3 lys2 trp1-3 H3 tam1::ARG4 |
| PC871  | BS::LEU2 his4-260 leu2::hisG CEN3::URA3 MATa RAD18::ADE2 HMR ade2 arg4-4 npa ura3 lys2 trp1-3 H3 TAM1 |
| PC875  | BS::LEU2 leu2::hisG CEN3::TRP1 MATa lys2 ho::lys2 trp1-3 H3 ura3 |
| PC876  | BS::LEU2, but homozygous tam1::ARG4 |
| DS101  | MA::leu2-31 ura3-52 ade2-101 his3-200 trp1-1 lys2-801 CEN6 YAC214-4-LT [LYS2 TRP1] |
| PC874  | BS::LEU2 leu2-31 ura3-52 ade2-101 his3-200 trp1-1 lys2-801 CEN6::LEU2-CEN11 YAC12-UH [URA3 HIS3] |
| PC712  | BS::LEU2 leu2-31 leu2-3,112 MATa ARG4 thr1-4 trp1-289 ura3-1 ade2-1 CYH10 |
| PC6644 | BS::LEU2, but homozygous tam1::URA3 |
| PC896  | BS::LEU2, but homozygous spo1::ADE2 |
| M45    | leu2-388 MATa cry1 ade2-101 HIS3 ura3-52 lys2-801 can1 trp1-31 CYH2 tam1::lacZ Cir |
| M45    | VSG12 & M45, but homozygous TAM1 and heterozygous zip1::lacZ |

Strains BR2625, PC647, PC712, and PC664 were constructed in a background related to BR2495 [Rockmill and Roeder 1990]. Strain PC874 is isogenic to DS101 [Sears et al. 1994]. Strains M45, M55, and VSG12 were derived from Y800 [Burns et al. 1994]. tam1::lacZ and zip1::lacZ fusions were created by mutagenesis with Tn3::lacZ::LEU2 [Burns et al. 1994], whereas the RAP1::HA fusion was created by mutagenesis with mTn3::3XHA::lacZ [Ross-Macdonald et al. 1997] followed by Cre-mediated excision of most of the transposon. Strains MY261 and PC873 were constructed in an SK1 strain background [Kane and Roth 1974; Williamson et al. 1983]. Strains PC872, PC871, PC875, and PC876 are congenic with SK1. The ADE2 gene was inserted upstream of RAD18 in strains PC872 and PC871.
Antibodies generate pR940. Then, the 863-bp region between the was replaced with a 1.6-kb Roeder (1990) was inserted into the fragment (in which the fragment containing sequences in and up-stream of the 2.3-kb ClaI-XbaI fragment containing sequences in and up-stream of the 2.3-kb ClaI-XbaI fragment containing fragments were performed. Expression studies

β-Galactosidase assays were performed as described by Engbrecht and Roeder (1990). Y800 strains (Burns et al. 1994) heterozygous for taml::lacZ and zip1::lacZ were assayed in duplicate, and the averages are shown in Figure 2.

Cytology

Spreads of meiotic chromosomes were prepared for electron microscopy according to Dresser and Giroux (1988), with modifications made by Engbrecht and Roeder (1990). Immunofluorescence procedures were performed according to Sym et al. (1993). A Leitz DMRB microscope equipped with fluorescence and a PL APO 100× objective was used to observe antibody-stained preparations. Images were captured using a Photometrics Imagepoint CCD camera. Meiotic cells were stained with DAPI as described by Thompson and Roeder (1989). Cells were grown and induced for meiosis as described by Sym and Roeder (1994) for SKI strains, and by Bhargava et al. (1991) for all other strains.

Antibodies

A glutathione S-transferase–Tam1 fusion protein was overproduced in bacteria using the plasmid pCB250 described above. The fusion protein was produced and purified according to Smith and Johnson (1988) and Guan and Dixon (1991). Rabbit antibodies against the fusion protein were generated by the Pocono Rabbit Farm and Laboratory (Canadensis, Pennsylvania) according to their fusion protein protocol. Anti-glutathione S-transferase–Tam1 antibodies were affinity purified according to Snyder (1989) and used at a 1:100 dilution. Cy3- or Texas red-conjugated secondary antibodies against rabbit IgG (Jackson Laboratories) were used at a 1:200 dilution to detect anti-Tam1 antibodies. Mouse anti-α-tubulin antibodies (16B12, Babco (Richmond, CA)] were detected with fluorescein-conjugated secondary antibodies against mouse IgG (Jackson Laboratories). Anti-Zip1 antibodies (Sym et al. 1993) were detected with fluorescein-conjugated secondary antibodies against rabbit IgG [Jackson Laboratories]. Anti-tubulin antibodies [YOLI/34; (Kilmartin et al. 1982); Sera-lab, 1:100 final dilution of monoclonal supernatant] were detected with fluorescein-conjugated secondary antibodies against rat IgG (Jackson laboratories).

Genetic procedures and analysis

Recombination frequencies among disomes were calculated according to Ross-Macdonald and Roeder (1994). Interference was determined according to Sym and Roeder (1994). To determine the NPD ratio [NPDobserved/NPDexpected] the Papazian equation (Papazian 1952), NPD = \(\frac{12}{10} - \frac{3}{2} \times \frac{1}{2} \times \frac{3}{4} \), was used to calculate the proportion of NPDs expected, where TT is the proportion of tetratypes observed. To compare the number of NPDs observed with that expected in the sample, a \(x^2\) test was performed for each interval. To compare the NPD ratios between wild type and tam1, a contingency \(x^2\) test was performed for each interval, using the observed and expected NPD values for wild type and tam1. To compare crossover frequencies between wild type and tam1, a contingency \(x^2\) test was performed for each interval, using the number of nonrecombinant and recombinant tetrads (where the number of nonrecombinant tetrads = PD – NPD + 1/2[TT – 1/2[TT – 1/2[TT – 2NPD] + 1/2[TT – 2NPD]]) was used to compare the expected and observed frequency of coincident crossovers in adjacent intervals, a \(x^2\) test was performed for each pair of intervals. \(x^2\) tests and contingency \(x^2\) tests were performed to compare spore viability and the pattern of spor death, respectively, between wild type and tam1.

The observed frequency of Eo tetrads for chromosome III in strains PC872 and PC871 was determined as the number of 4-spore-viable tetrads in which all four spores retained the parental configuration of markers along the chromosome, divided by the total number of 4-spore-viable tetrads. The corrected Eo value was obtained by subtracting from the raw number of Eo tetrads the estimated number of tetrads within the Eo population that had sustained a two-strand double crossover in one or more intervals. Assuming no chromatid interference, the number of two-strand double crossovers in an interval is equal to the number of four-strand double crossovers detectable as NPDs. For each interval, [PD – NPD]/PD is the probability that an apparent PD is real. The sum of the probabilities of two-strand double crossovers in one, two, or more intervals along the entire chromosome was subtracted from the observed number of Eo tetrads to generate the corrected frequency. To compare Eo frequencies between wild type and tam1, contingency \(x^2\) tests were performed.

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Note added in proof

The TAM1 gene is identical to NDII, which was recently described by Conrad et al. (1997) in Science 276: 1252–1255.

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