A Conditional Allele of the *Saccharomyces cerevisiae* HOP1 Gene Is Suppressed by Overexpression of Two Other Meiosis-Specific Genes: RED1 and REC104

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Manuscript received August 4, 1992

Accepted for publication December 9, 1992

ABSTRACT

The *HOP1* gene of *Saccharomyces cerevisiae* is believed to encode a protein component of the synaptonemal complex, the structure formed when homologous chromosomes synapse during meiotic prophase. Five new mutant alleles (three conditional, two nonconditional) of *HOP1* were identified by screening EMS-mutagenized cells for a failure to complement the spore viability defect of a *hop1* null allele. Two high copy plasmids were found that partially suppress the temperature-sensitive spore inviability phenotype of one of these alleles, *hop1*-628. The suppression is allele-specific; no effect of the plasmids is observed in *hop1* null diploids. Mutation of either of the two suppressor genes results in recessive spore lethality, indicating that these genes play important roles during meiosis. The DNA sequence of one high copy suppressor gene matched that of *RED1*, a previously identified meiosis-specific gene. Our data strongly support the idea that *RED1* protein is also a component of the synaptonemal complex and further suggest that the *RED1* and *HOP1* gene products may interact. The second suppressor maps to the right arm of chromosome VIII distal to *CDC12* and is *REC104*, a meiosis-specific gene believed to act early in meiosis.

MEIOSIS is a fundamental process that sexually reproducing organisms undergo in order to reduce by half the chromosome number in germ cells. This reduction is necessary so that when two gametes fuse at fertilization the diploid chromosome number of the cell is reconstituted. The meiotic process must be very accurate so that one and only one member of each chromosome pair is distributed to each gamete. How does the cell keep track of which chromosomes to send to each pole during the first meiotic division? This is accomplished through a coordinated process involving the pairing of homologous chromosomes and their recombination.

The long range interactions that determine which chromosomes are homologous are still unknown, although a variety of mechanisms have been proposed (LOIDL 1990). In many organisms the pairing of homologous chromatids condense along protein cores called axial elements. These axial elements then become aligned and are held together by the assembly of a central element to form a tripartite proteinaceous structure called the synaptonemal complex (SC) [reviewed in VON WETTSTEIN, RASMUSSEN and HOLM (1984)]. In yeast axial element formation and synopsis occur concomitantly (ALANI, PADMORE and KLECKNER 1990). The SC is present along the length of each chromosome pair with the majority of the DNA extending out from the SC in chromatin loops (WEITH and TRAUT 1980). SC formation is complete by pachytene, the stage of meiotic prophase at which crossing over between homologous chromosomes is believed to occur. The bulk of the SC is shed at diplotene and at this time chiasmata, the chromosomal structures derived from crossovers, can be visualized. Homologs proceed to segregate reductionally (sister centromeres to the same pole) at meiosis I and then equational (sister centromeres to opposite poles) at meiosis II, resulting in four haploid meiotic products. In yeast the four meiotic nuclei are packaged into spores which, in turn, are contained in a sac called an ascus.

The role of the SC in meiosis is not yet completely understood. Because a strong correlation exists between the presence of SC and wild-type levels of crossing over (VON WETTSTEIN, RASMUSSEN and HOLM 1984), one function proposed for the SC is in recombination. However, the SC is not absolutely necessary for recombination, since both *hop1* and *red1* mutants in yeast exhibit 10–20% of the wild-type levels of exchange despite a complete absence of SC (HOLLINGSWORTH and BYERS 1989; ROCKMILL and ROEDER 1990). The reduction in the level of recombination by *hop1* and *red1* indicates that while SC formation is not required for exchange, it does appear to facilitate crossing over. A second possible role of the SC is in maintaining the chiasmata generated by crossing over until anaphase of the first meiotic division (MAGUIRE 1978). It is well established that chiasmata are necessary for proper orientation of the homologs on the metaphase plate at meiosis I, probably by
providing a means by which tension can be exerted on the chromosomes from opposite spindle poles (for review, see Hawley 1987). Experiments by Rockmill and Roeder (1990) and Engebrecht, Hirsch and Roeder (1990) have demonstrated that, in yeast, crossovers must occur in the context of the SC if they are to be effective in directing the segregation of homologs to opposite poles at meiosis I. In addition to functioning in synapsis and chromosome segregation, the SC has also been proposed to play a part in the homology search which aligns homologous chromosomes (Carpenter 1987).

Despite extensive cytological studies of the SC, the components that make up this structure are still mostly unknown. Although some non-meiosis-specific proteins such as topoisomerase II have been localized to the complex through immunocytochemical methods (Moens and Earnshaw 1989; Klein et al. 1992), the bulk of the SC is likely formed by assembly of a set of meiosis-specific proteins (Offenberg, Dietrich and Heyting 1991). A useful approach in identifying SC proteins has been the isolation of monoclonal antibodies against either purified SCs or rat spermatocyte fractions enriched in SC material. These antibodies, when screened for cross-reactivity with SC, revealed the presence of several antigens which localize to either the lateral or central elements of the SC (Heyting et al. 1987; Moens et al. 1987; Offenberg, Dietrich and Heyting 1991; Smith and Benavente 1992). While these studies have the advantage of high resolution cytology of the SC, it has been difficult to determine whether any of these antigens are required for SC function. In the yeast Saccharomyces cerevisiae the complementary situation exists. A number of different genes required for SC formation have been identified [SPO11 (Giroux, Dresser and Tiano 1989); RAD50 (Alani, Padmore and Kleckner 1990); MEI4 (Menees, Ross-MacDonald and Roeder 1992); HOP1 (Hollingsworth and Byers 1989); RED1 (Rockmill and Roeder 1990); REC102 (Bhargava, Engebrecht and Roeder 1992); MER1 (Engebrecht and Roeder 1990); DMC1 (Bishop et al. 1992)]. Of these, only the HOP1 protein is known to be localized to meiotic chromosomes. In addition, HOP1 is expressed only in cells undergoing meiosis (Hollingsworth, Goetsch and Byers 1990). No SC is formed in hop1 diploids, and the resulting nondisjunction of homologous chromosomes causes high levels of spore inviability. Finally, diploids deleted for HOP1 show reduced levels of recombination between, but not within, chromosomes (Hollingsworth and Byers 1989). The recombination machinery therefore appears to be intact in hop1 strains. These facts make HOP1 a strong candidate for a gene that encodes an SC component while genes such as RAD50 have been proposed to be involved in earlier events such as homology searching (Alani, Padmore and Kleckner 1990).

Given the idea that HOP1 encodes a component of the SC, one of our goals is to use HOP1 as a tool for the identification and isolation of additional SC proteins. Toward this end we have generated several new conditional alleles of HOP1 in order to isolate high copy plasmids capable of suppressing the hop1 phenotype. Two high copy suppressors were isolated by their ability to correct (at least partially) the temperature-sensitive defect in spore viability conferred by hop1-628. The stronger of these suppressors was found to be RED1, a previously described meiosis-specific gene. Mutations in this gene confer a phenotype similar to that of null alleles of hop1 (Rockmill and Roeder 1990). Mutation of the second suppressor results in a recessive spore inviability phenotype demonstrating that it is essential for meiosis as well. Analysis of this suppressor revealed it to be another meiosis-specific gene, REC104 (A. Galbraith and R. E. Malone, personal communication). These results suggest that RED1 and REC104 may either encode SC components or be involved in its formation. Furthermore they suggest that these genes may interact with HOP1, either directly or indirectly through the protein complex of the SC.

MATERIALS AND METHODS

Yeast strains and media: The genotypes of strains used in this work are listed in Table 1. Standard yeast genetic methods were employed (Mortimer and Hawthorne 1969). Liquid and solid media have been described (Sherman, Fink and Lawrence 1979). The concentration of cycloheximide and canavanine used in plates is 10 μg/ml and 60 μg/ml, respectively. SPm (liquid sporulation medium) is 0.3% potassium acetate and 0.02% raffinose. Sporulation (Spo) plate medium consists of 1% potassium acetate, 0.1% yeast extract, 0.05% dextrose and 2% agar.

NH9-3 was constructed in two steps. First a Cyh' derivative of 4939-1 was obtained by plating 4 × 10^6 cells on each of 10 YEPD plates containing 10 μg/ml cycloheximide, a concentration of the drug that specifically selects for mutations in the GH2 gene (L. Hartwell, personal communication). A Cyh' derivative (4939-1Cyh') was then transformed with a 7.9-kb BglII fragment which has the LEU2 gene inserted into HOP1 (hop1-3:LEU2; Hollingsworth and Byers 1989). Leu' transformants were backcrossed to a Hop- tester, 5815-1-4(21-46), to test for the spore inviability phenotype expected if the HOP1 gene in the transformed strain has been disrupted. One of the 11 Leu' Hop- transformants was designated NH9-3.

The Hop' and Hop' tester strains 5815-1-4(21-46), 5815-7-1(21-46), 5815-1-4(S31-5) and 5815-7-1(S31-5) were generated by transforming 5815-1-4 and 5815-7-1 with either pNH21-46 or pNH31-5 and selecting for Ura' transformants. Both integrating plasmids were digested with SalI prior to transformation to target the recombination events to HOP1 (Orr-Weaver, Szostak and Rothstein 1981). pNH21-46 contains a 5.6-kb BamHI fragment cloned into the BamHI site of Ylp5 (Hollingsworth and Byers 1989). This fragment fails to complement because a portion from the 5' end of the HOP1 gene has been deleted. Because the transformed strains contain the hop1-1 allele, the resulting

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TABLE 1
Saccharomyces cerevisiae strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
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<tr>
<td>4939-1a</td>
<td>MATa leu2 ura3-52 can1 trp1 his7 ade2-1</td>
</tr>
<tr>
<td>4939-1cyh4a</td>
<td>MATa leu2 ura3-52 can1 trp1 his7 ade2-1 cyh2</td>
</tr>
<tr>
<td>NH33a</td>
<td>MATa leu2 ura3-52 can1 trp1 his7 ade2-1 cyh2 hop1-3::LEU2</td>
</tr>
<tr>
<td>7859-10-2a</td>
<td>MATa leu2 ura3-52 trp1 his7</td>
</tr>
<tr>
<td>MR1-51a</td>
<td>MATa leu2 ura3-52 can1 cyh2 ade1</td>
</tr>
<tr>
<td>5815-1-4(21-46)</td>
<td>MATa hop1-1::pNH21-46(URA3 hop1) his3 ura3-52</td>
</tr>
<tr>
<td>5815-7-1(21-46)</td>
<td>MATa hop1-1::pNH21-46(URA3 hop1) his3 ura3-52</td>
</tr>
<tr>
<td>5815-7-1(31-5)</td>
<td>MATa hop1-1::pNH31-5(URA3 HOPl) his3 ura3-52</td>
</tr>
<tr>
<td>NH33b</td>
<td>MATa LEU2 ura3-52 can1 his3 ADE2 hop1-628 HIS7 CYH2 trp1</td>
</tr>
<tr>
<td>NH35b</td>
<td>MATa leu2 ura3-52 can1 HIS3 ade2-1 hop1-628 his7 cyh2 trp1</td>
</tr>
<tr>
<td>NH41b</td>
<td>MATa LEU2 ura3-52 CAN1 his3 ADE2 hop1-842 HIS7 CYH2 TRP1</td>
</tr>
<tr>
<td>NH43b</td>
<td>MATa leu2 ura3-52 CAN1 his3 ADE2 hop1-4088 HIS7 CYH2 TRP1</td>
</tr>
</tbody>
</table>

All the strains described above were constructed by the authors with the exception of 4939-1, 7859-10-2 (from L. HARTWELL) and MR1-51 (from M. REDD). Lines indicate synteny in diploid strains. Insertion of a circular plasmid (X) is indicated by ::pX followed in parentheses by plasmid-borne markers that become inserted between elements duplicated by the integration.

* These strains are isogenic with the A364A strain background.
* These strains are hybrids between A364A and a background which has been backcrossed four times to A364A.

transformants are still Hop- but have a duplication at the HOPl locus marked with URA3. Plasmid pNH31-5 consists of the complementing 5.5-kb BglII fragment from pNH33-2 (HOLLINGWORTH, GOETSCH and BYERS 1990) also cloned into the BamHI site of YIp5. Integration of this plasmid at the HOPl locus results in a Hop+ duplication marked with URA3. (In all cases the Hop phenotype refers to the ability of a diploid to make high levels of viable spores (Hop+) or not (Hop-).)

The hop1 diploids NH33, NH35 and NH43 were constructed in two steps. First rare viable spores were selected on -arg +can +cyh medium from sporulated cultures of the diploids B628 (hop1-628/hop1-3::LEU2), B4686 (hop1-4686/hop1-3::LEU2) and B4088 (hop1-4088/hop1-3::LEU2). Leu+ spores carrying the new hop1 alleles were recovered and crossed to the Hop+ haploid 5815-1-4(31-5). The hop1-628/HOPl::URA3, hop1-4686/HOPl::URA3 and hop1-4088/HOPl::URA3 heterozygous diploids were sporulated and tetrads dissected. As expected all the Ura+ spores tested were Hop+ . A spore clone containing hop1-628 CAN1 and CYH2 was then backcrossed to the parental hop1-628 can1 cyh2 strain to generate NH33. (It was discovered after the bulk of the experiments described in this paper using NH33 were performed that the strain had become homozygous for can1. Therefore only cyh2 was used to select for spores from NH33.) A similar backcross was performed using hop1-842 and hop1-4088 haploids to generate the diploids NH41 and NH43, respectively. For NH35 an hop1-4686 CAN1 CYH2 spore from the outcross was mated to NH19-3.

Construction of 1b-1 and 1g-2 homozygous mutant diploids: As described below, the 1b-1 suppressor is RED1 and the 1g-2 suppressor is REC104. To avoid confusion, these names will be used throughout the paper. The RED1 suppressor was disrupted by cloning a 2.2-kb SalI/Xhol fragment from YEpl3 into the unique Xhol site of pBl-1 to create pNH119. A 4.9-kb BglII/PstI fragment containing the LEU2 disruption of RED1 from pNH119 was gel purified and used to transform either 7859-10-2 or MR1-51 to substitute the mutant gene for the wild-type copy (ROTHSTEIN 1985). A deletion of REC104 was created by digesting pNH128 with EcoRV and CiaI to remove a 1.7-kb fragment containing the REC104 suppressor. The larger fragment was gel purified and the ends were filled in with T4 DNA polymerase. After similarly filling in the ends of the 2.2-kb SacI/Xhol LEU2 fragment, the two were ligated together to form pNH131. This plasmid was digested with XbaI and BamHI and the unfraccionated digest was used to transform either 7859-10-2 or MR1-51. Deletion of REC104 was confirmed by southern blot analysis of the transformants (data not shown).

Liquid sporulation protocol: Single colonies were inoculated into 5 ml YEPlD and grown at 30 °C overnight to stationary phase. Aliquots of 0.2 ml cells were washed with water and resuspended in 2.5 ml SPM. The sporulating cultures were aerated at either 25 °C or 30 °C for 3 days. A measure of spore viability was determined by counting the number of colonies formed on -arg +can +cyh plates relative to the viable colony forming units measured on YEPlD.

Plasmid construction: Plasmids for this study were constructed by standard procedures (MANIATIS, FRITSCH and SAMBROOK 1982), using Escherichia coli strains[JA194 and BS72]. Names, genotypes and sources of plasmids are listed in Table 2. pNH83-2 contains the HOPl gene cloned into YEpl9 and was constructed in two steps. First the 1.4-kb EcoRI/BamHI fragment from A62-3 was ligated into EcoRI/BamHI digested pH42-2 to generate pNH72-2. The 4.2-kb BglII/SalI fragment from pNH72-2 was then subcloned into BamHI/SalI-cut YEpl9. pNH96-1 contains the hop1-3
allele cloned into YEpl4 and was generated by substituting the 1.6-kb BamHI/SalI fragment from pNH48-1 for the wild-type fragment in pNH83-2.

pib-1 derivatives were constructed as follows: pNH112–digestion of pib-1 with SalI and XhoI followed by dilute ligation resulting in a deletion of the 3.2-kb SalI/XhoI fragment from pib-1; pNH116–ligation of the 3.2-kb SalI/XhoI fragment from pib-1 in YEpl4; pNH119–insertion of 2.2-kb SalI/XhoI LEU2 fragment into XhoI site of pib-1; plg-2–8.4-kb fragment containing REC104 in YEpl4; pNH113–deletion of 2.2-kb BamHI fragment from plg-2; pNH114–deletion of 6.2-kb between BamHI and BglII sites in plg-2; pNH117–2.2-kb BamHI fragment from plg-2 in YEpl4; pNH118–4.0-kb BamHI/BglII fragment from plg-2 in YEpl4; pNH121–deletion of 2.2-kb SphI fragment from plg-2; pNH123–2.2-kb SphI fragment from plg-2 in YEpl4; pNH131–Substitution of 2.2-kb LEU2 fragment for 1.7-kb EcoRV/SalI fragment from plg-2.

pVZ1–None; pBS+ vector from Stratagene with expanded polylinker; plg-2 derivatives were constructed as follows: pNH112–digestion of plg-2 with BamHI and SalI followed by dilute ligation resulting in a deletion of the 3.2-kb SalI/XhoI fragment from plg-2 into SalI-digested pib-1.

plg-2 subclones were constructed as follows: pNH113–digestion of plg-2 with BamHI followed by dilute ligation to delete the 2.2-kb BamHI fragment; pNH114–digestion of plg-2 with BamHI and BglII followed by dilute ligation resulting in loss of 6.2 kb of DNA between the BglII site and the outermost BamHI site; pNH117–ligation of the 2.2-kb BamHI fragment from plg-2 into BamHI-digested YEpl4; pNH118–ligation of the 4.0-kb BamHI/BglII fragment from plg-2 into BamHI-digested YEpl4; pNH119–ligation of the 2.2-kb SphI fragment from plg-2 into BamHI-digested YEpl4; pNH121–ligation of the 4.0-kb BamHI/BglII fragment from plg-2 into BamHI-digested pib-1.

Mutant screen protocol: Strain 7859-10-2 was mutagenized at 25° with ethyl methanesulfonate (EMS) (SHERMAN, FINK and LAWRENCE 1979). The 25-min time point gave 21% viability. Cells from this time point were plated on YEpl4 at a density of 150–200/plate and grown at 30° for 3 days. The colonies were then replica-plated to YEpl4 plates carrying fresh replica-prints from a lawn of NH9-3/pNH96-1 to allow mating. [The plasmid pNH96-1 carries the null hopl-3 allele on it (HOLLINGSWORTH, GOETSCH and BYERS 1990). It was present for reasons unrelated to the screen for new hopl alleles and had no effect on these experiments.] After growth at 30° overnight, diploids were selected on  -ade, -ura, -leu plates at 30° overnight. Diploid colonies were patched onto -ade, -ura, -leu medium, grown for several hours at 30°, and sporulated by replica plating to SpO plates. The plates were incubated either at room temperature (approximately 23°) or at 30° for 3 days. Spore viability was monitored by selecting for Can' Cyh' spores as described in HOLLINGSWORTH and BYERS (1989).

Out of 5006 diploid tested, 55 initially gave the phenotype of no growth on -arg +can +cyh plates after sporulation. This phenotype, however, may arise in a number of ways in addition to homozygosity for HOP1. For example, ten diploids contained a spore viability defect which most likely occurs as the result of a dominant mutation in some other gene. Such mutants were detected by the lack of ascii when the sporulated cells were examined under the light microscope. Since hopl deletion diploids form spores, we did not pursue these strains, although we did not rule out the possibility that a novel allele of HOP1 was responsible. Secondly, diploidization of a mutagenized cell results in a colony which, when mated to the hopl-3:LEU2 haploid, gives rise to a triplid. Triplids generate inviable spores independently of HOP1. It was possible to determine whether the cells were triplid by assaying vegetatively growing patches for their ability to papillate to resistance to either canavanine or cycloheximide. Diploid cells arising from this cross are heterozygous for both canl and cyh2 and should papillate to either Can' or Cyh' at a frequency of 10%. However, triplid cells derived in the manner described above should carry two copies of the dominant sensitive Canl and Cyh2 genes. Papillation to resistance to either drug in this case now requires two independent mitotic recombination events and occurs in only 1 in 108 cells. Eight colonies were deemed to be triplid because they formed asc when sporulated, but the vegetative cells were unable to papillate to either Can' or Cyh'. A third way in which a false positive may be generated arises from the…
fact that the resistant cyh2 allele is due to a specific missense mutation which results in a ribosomal protein which is resistant to cycloheximide. If a second mutation creates a null allele of this gene, then only the CHY2 ribosomal protein will be present and the cells will be sensitive to cycloheximide. Such diploids should still form asc and papilate vegetatively to Can' but not to Cyh'. Using this criterion, seven null alleles of CHY2 were eliminated. Twenty-three diploids either did not retest or had inexplicable phenotypes. Five diploids papilated vegetatively both to Can' and Cyh', formed asc and the spores failed to grow on -arg +can +cyh medium. These diploids were shown to be homозygous for hopl by linkage analysis and plasmid complementation (see below).

**Linkage analysis of new hopl alleles:** To determine whether the mutant diploids, B642, B4088, B327, B4686 and B628, were homozygous for hopl, the degree of linkage was determined between the new mutations and the HOPI locus. Rare viable spores from each mutant diploid were selected on -arg +can +cyh medium and their genotypes determined by replicating to the appropriate selective media. Several spore clones from each diploid were patched onto YEPD and their genotypes determined by replicating to the appropriate selective media. Since the disruption allele of HOPI is marked with LEU2, spores containing new hopl alleles can easily be distinguished by their Leu- phenotype. Each spore was then backcrossed to hopl1 or hopl-1 testers of the appropriate mating type. If the mutant diploid found by the screen are homozygous for hopl, then only Hop- spores can be produced and 100% of the spores should give the hopl phenotype when backcrossed to the hopl-1 tester. If the mutations are recessive, then all the spores should exhibit Hop+ levels of spore viability when backcrossed to the hopl1 tester. If, however, the new mutations are unlinked to HOPI, then 50% of the Leu- spores should be wild type when backcrossed to hopl and 50% of the Leu+ spores should be mutant when backcrossed to HOPI (a phenomenon known as unlinked noncomplementation). All Leu- spores from B642, B4088, B327, B4686 and B628 were Hop- when backcrossed to hopl1 strains (14, 13, 11, 10 and 12 spores were tested, respectively), demonstrating that the new mutations are tightly linked to the HOPI locus.

**Rescuing the hopl-628 allele by gap repair:** The hopl-628 allele was transferred from the chromosome to a plasmid by gap repair (Hoffman and Szostak 1983). The HOPI coding sequence was contained in pNH3-2 which was digested with BamHI and SacI to create a 1.0-kb gap within the HOPI coding sequence. The gapped plasmid was gel-purified and transformed into NH33, a diploid homozygous for hopl. Seventy five of the Ura+ transformants were patched onto -ura medium, sporulated and assayed for spore viability on -ura plates and incubated at 30°C for 3 days. Spore viability was assayed by replicating the sporulated colonies to -arg +can +cyh plates and growing the spores for 2 days at 30°C. Positive colonies were patched from the -ura master plate onto -ura plates (17/14,300 transformants for pool A; 10/18,700 transformants for pool B). Plasmid DNA was recovered from yeast as described above and electroporated into E. coli. Two E. coli transformants from each positive yeast colony were examined by restriction enzyme digests for a 1.3-kb BamHI/HindIII fragment containing internal coding sequence of HOPI. Many (31/54) of the plasmids recovered were simply the vector YEP24. One pair of plasmids contained the diagnostic HOPI fragment. Its identity as HOPI was confirmed by more extensive restriction mapping (data not shown). Plasmids with inserts that did not contain HOPI were retransformed into NH33 to see if the suppression was reproducible. From the 27 transformants which originally scored as positive, two new plasmids were found which repeatedly exhibited suppression of NH33: plb-1 and plg-2.

**Sequence analysis of RED1 and REC104:** A number of different plasmids were constructed containing smaller pieces of the inserts present in either plb-1 and plg-2. These subclones were then tested for their ability to suppress hopl-628 by assaying colonies transformed with these plasmids for viable spore yield as described above (see Figure 3). Subcloning analysis indicated that a unique XhoI site in plb-1 resides in or very near the RED1 suppressor. To obtain the DNA sequence adjacent to this XhoI site, a 3.2-kb XhoI/SalI fragment from plb-1 was subcloned into pVZ1 such that the XhoI site is situated adjacent to the T3 promoter. Sequence extending from the promoter into the insert was generated using a T3 promoter primer on double-stranded DNA (Sanger, Nicklen, and Coulsen 1977). A search of the GenBank database indicated a 150/155-bp match between our sequence and that of RED1 (Thompson and Roeder 1989). Using the Thompson and Roeder sequence, an oligonucleotide was designed based on sequences around the RED1 ATG initiation codon (5'CAGTGAGGACCACAAAGGGAC3') provided by the UCSF Biomolecular Research Facility. Sequence from plb-1 using this oligonucleotide also corresponded to that of RED1 (151/152 match).

Sequence adjacent to the Sphl site in REC104 was obtained by subcloning a 2.0-kb Sphl fragment from pNH18 into Sphl-digested pVZ1 and sequencing with the T3 promoter primer. No match was found to sequences in the

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<th>High Copy Suppressors of hopl-628</th>
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High Copy Suppressors of hopl-628
Genbank database and so the entire gene was sequenced. Both strands of the entire open reading frame (ORF) were sequenced using a combination of Exonuclease III-generated deletions (HENIKOFF 1984) and synthesis of oligonucleotides for use as primers. The coding sequence we determined for REC104 is identical to that determined independently for the gene by A. GALBRAITH and R. E. MALONE (personal communication).

RESULTS

Isolation of new mutant alleles of HOPl: A goal of our work is the identification of genes encoding components of the synaptonemal complex in yeast. One approach is the isolation of genes capable of suppressing mutant alleles of HOPl, a gene we believe encodes an SC protein. Such suppressors may be due to new mutations in different genes or may result from overexpression of wild-type copies of other genes. In general whether such suppression approaches are successful or not seems to be highly dependent on the nature of the mutant allele which is chosen to be suppressed. For example bypass suppressors are often found when null alleles of the gene of interest are used. Because we are interested in identifying genes which encode proteins that physically interact with HOPl protein, we chose to isolate high copy suppressors of a temperature-sensitive allele of HOPl. We reasoned that overexpression of a component that normally associates with HOPl might prevent the temperature-sensitive unfolding of the mutant protein. Since the only hop1 alleles available at the time this study was initiated were nonconditional null alleles, it was necessary to generate conditional alleles of hop1.

hop1 diploids produce mainly inviable spores (HOLLINGSWORTH and BYERS 1989). This phenotype is due to the high levels of aneuploidy which result from nondisjunction of homologous chromosomes at the first meiotic division because of a failure of the chromosomes to synapse and cross over properly (HAWLEY 1987). New hop1 mutants were therefore isolated by mutagenizing a HOPl strain (7859-10-2) with EMS, crossing the mutagenized colonies to a haploid strain in which the HOPl gene has been disrupted with LEU2 (NH9-3), and testing the resulting diploids for spore inviability (see MATERIALS AND METHODS). Spore viability was monitored by the growth of Can + Cyh1 cells resulting from sporulation as described in HOLLINGSWORTH and BYERS (1989). Out of 5096 diploids tested, 53 gave the phenotype of reduced growth on -arg +can +cyh plates after sporulation. False positives resulting from mutations causing dominant sporulation defects, null alleles of cyh2 and triploidy were eliminated as described in MATERIALS AND METHODS.

Five diploids were candidates for being either homozygous for mutations at the HOPl locus or for containing unlinked mutations which exhibit noncomplementation with hop1 (for a discussion of noncomplementation see FULLER et al. 1989). Allelism tests with known hop1 strains demonstrated that all five mutations are tightly linked to the HOPl locus (MATERIALS AND METHODS). Furthermore, the spore inviability of all five diploids is complemented by HOPl when the gene is introduced on a plasmid, confirming the identity of the mutations as new recessive alleles of HOPl (complementation data for hop1-628, hop1-4088 and hop1-842 are presented in Table 3). Two of the five new alleles, hop1-628 and hop1-327, are temperature-sensitive for spore viability with the restrictive temperature being 30°. hop1-842 is cold sensitive, generating inviable spores at 25°. The remaining two alleles, hop1-4686 and hop1-4088 are nonconditional. To quantify the effects of some of the newly isolated hop1 alleles on sporulation and spore viability, liquid sporulation experiments were performed using diploids homozygous for either hop1-628 (NH33), hop1-4088 (NH43) or hop1-842 (NH41) (for construction, see MATERIALS AND METHODS). Random spore analysis was performed so that all ascii could be tested, including those with only one, two or three spores. The three diploids were transformed with either YEp24, a 2-μm high copy number yeast vector, or pNH83-2 (HOPl cloned into YEP24) so that both the Hop+ and Hop− phenotypes could be monitored in the same strain. At least two independent colonies were sporulated in SPM and the sporulated cells were plated onto -arg +can +cyh plates to select for viable spores. (It should be noted that it was not discovered until after these experiments were completed that NH33 is actually homozygous for can1; therefore the presence of canavanine in the medium had no effect

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Percent Ura+/cfu</th>
<th>Percent sporulation</th>
<th>Percent Can/Cyh+/cfu</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH33/plasmid</td>
<td>70.8</td>
<td>32.0</td>
<td>27.7</td>
</tr>
<tr>
<td>YEp24</td>
<td>67.2</td>
<td>35.5</td>
<td>27.7</td>
</tr>
<tr>
<td>pNH83-2(HOPl)</td>
<td>54.9</td>
<td>34.0</td>
<td>32.0</td>
</tr>
<tr>
<td>p1b-1(RED1)</td>
<td>56.5</td>
<td>34.0</td>
<td>32.0</td>
</tr>
<tr>
<td>p1g-2(REC104)</td>
<td>60.5</td>
<td>34.0</td>
<td>32.0</td>
</tr>
<tr>
<td>NH43/plasmid</td>
<td>59.9</td>
<td>34.0</td>
<td>32.0</td>
</tr>
</tbody>
</table>

a "cfu" stands for colony-forming unit.

b Measurements are the average of two independent colonies.

c NH33 is homozygous for hop1-628, NH43 is homozygous for hop1-4088 and NH41 is homozygous for hop1-842.

d The number in parentheses represents the level of spore viability expected for a complementing plasmid. The value was calculated by multiplying the spore viability at 23° by the percent of cells with plasmid.

e Measurements are the average of three independent colonies.
for NH33. Since NH33 is heterozygous for cyh2, a recessive mutation which confers resistance to cycloheximide, only $10^{-4}$ vegetative cells are resistant to the drug (homozygous cyh2 cells arise by mitotic recombination). Due to the haploidization of chromosomes which occurs during meiosis, one-half of spores are resistant. The negligible vegetative background makes growth of sporulated cultures on medium containing cycloheximide a good measure of viable spore yield. The percent of cells containing plasmid was measured immediately prior to sporulation. The degree of sporulation was determined by counting the number of ascis (one-, two-, three- and four-spored) in a field of 200 cells using phase contrast microscopy.

When carrying the vector YEp24, the homozygous hop1-628 diploid NH33 showed a drastic decrease in spore viability at the restrictive temperature of 30°C compared to 23°C (1.3–33.8%; Table 3). This decrease is not due to a defect in sporulation as the number of ascis remains approximately the same at both temperatures. The temperature-sensitive spore viability defect is complemented by the presence of HOPI in the cell (19.2% for NH33/pNH83-2 compared to 1.3% for NH33/YEp24 at 30°C; Table 3). The complementation by the HOPI gene on a plasmid is complete if the fact that only 67.2% of the sporulated cells contained the plasmid is taken into consideration. The phenotype of hop1-842 is cold-sensitive when NH41 is sporulated at 23°C; only 0.2% of the colony forming units are resistant to canavanine and cycloheximide, while at 30°C a marked increase is seen (9.5%, Table 3). The presence of HOPI on a plasmid (pNH83-2) improves the spore viability at both temperatures, demonstrating that the hop1-842 defect is complemented by HOPI. This complementation is also found for NH43 which carries the nonconditional hop1-4088 allele (Table 3).

The hop1-628 allele results from a single missense mutation: The nature of the mutation present in hop1-628 was determined after first transferring the allele from the chromosome to a HOPI gene carried on a plasmid by gap repair as described in MATERIALS AND METHODS. Plasmids carrying the hop1-628 mutation are capable of conferring temperature-sensitive spore viability when transformed into a diploid carrying nonconditional alleles of HOPI (data not shown). The high frequency of recovered plasmids that were wild type (64%) indicated that the mutation does not reside within the gap. To rule out the possibility that multiple mutations are responsible for the hop1-628 temperature-sensitive phenotype, the entire sequence of the hop1-628 allele was determined and compared to that of the HOPI gene carried on pNH33-2. Several errors were detected between pNH33-2 and the published HOPI sequence (see MATERIALS AND METHODS). Only one difference between the two alleles was detected—a G to A transition located at bp 1993 which changes the codon for amino acid 595 from serine to asparagine (HOPI protein contains 605 amino acids). We therefore conclude that a single amino acid substitution creates the conditional hop1-628 allele.

Two genes other than HOPI can partially suppress the temperature-sensitive spore viability defect of hop1-628 when present in high copy number: As a means of identifying proteins that interact with HOPI, high copy suppressors of hop1-628 were isolated. NH33 was transformed with a yeast genomic library carried in the URA3 2-kb vector, YEp24 (CARLSON and BOTSTEIN 1982). Approximately 33,000 transformants were sporulated at 30°C for three days, followed by replica-plating to −arg +can +cyh plates. pNH119 carries a LEU2 insertion in the unique XhoI site of p1b-1.

Plasmid DNA was isolated from each apparently suppressed transformant and electroporated into E. coli as described in MATERIALS AND METHODS. Two plasmids from each of the original yeast transformants were analyzed by restriction enzyme digestion for a 1.3-kb BamHI/HindIII fragment diagnostic of the HOPI gene. One pair of plasmids contained the diagnostic HOPI fragment and further restriction mapping confirmed their identity as HOPI. Plasmids with inserts that did not contain HOPI were retransformed into NH33 and tested again for suppression of the spore viability defect at 30°C. Two of these plasmids, designated p1b-1 and p1g-2, gave reproducible levels of suppression (see Figure 1 for picture of p1b-1 suppression). The degree of suppression was quantitated by liquid sporulation experiments as described above. For both p1b-1 and p1g-2 the suppression of

![Figure 1.-Suppression of hop1-628 by p1b-1 (RED1). Three transformants of the homozygous hop1-628 diploid, NH33, carrying either YEp24, pNH83-2, p1b-1 or pNH119, were patched and grown on −ura medium, replica-plated to Spor plates and sporulated for 3 days at either 23°C or 30°C. Viable spore yield was assayed by replica printing the sporulated colonies onto −arg +can +cyh plates. pNH119 carries a LEU2 insertion in the unique XhoI site of p1b-1.](image-url)
Suppression of hop1-628 by plb-1 (RED1) and plg-2 (REC104) is confirmed by tetrad analysis

<table>
<thead>
<tr>
<th>NH35/plasmid</th>
<th>Viable:inviable spores</th>
<th>Percent spore viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 23°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YEp24</td>
<td>24</td>
<td>5 3 2 5</td>
</tr>
<tr>
<td>pNH83-2(HOP1)</td>
<td>5</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>plb-1(REDI)</td>
<td>12</td>
<td>1 1 0 3</td>
</tr>
<tr>
<td>plg-2(REC104)</td>
<td>11</td>
<td>2 0 0 2</td>
</tr>
<tr>
<td>At 30°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YEp24</td>
<td>9</td>
<td>0 6 4 33</td>
</tr>
<tr>
<td>pNH83-2(HOP1)</td>
<td>28</td>
<td>8 1 2 9</td>
</tr>
<tr>
<td>plb-1(REDI)</td>
<td>24</td>
<td>5 7 3 13</td>
</tr>
<tr>
<td>plg-2(REC104)</td>
<td>26</td>
<td>2 7 2 18</td>
</tr>
</tbody>
</table>

* NH35 transformants containing plasmids were patched onto -ura medium and directly replica-plated to Spo plates to maintain selection for the plasmid until sporulation.

hop1-628 was partial. The presence of plb-1 resulted in levels of spore viability that were 65% of the HOP1 level while plg-2 suppressed more weakly, producing viable spores equal to only 29% of the HOP1 level (Table 3).

The suppression of hop1-628 by plb-1 and plg-2 detected by random spore analysis was confirmed by tetrad dissection. The homozygous hop1-628 diploid NH33 was sporulated either at 23°C or 30°C and the resulting tetrads dissected. Consistent with the random spore data, spore viability is decreased at 30°C (76.3% to 25.0%; Table 4). The pattern of complementation and suppression is the same as that observed by random spore analysis with HOP1 (pNH83-2) exhibiting the greatest improvement in spore viability, followed by plb-1 and plg-2, respectively (Table 3). The degree of suppression is not as great by tetrad dissection as was observed by random spore analysis. This is most likely due to the bias introduced in the tetrad dissection data by the fact that only four-spored asci were examined. The distribution of viable spores clearly shows that spore death is nonrandom at the restrictive temperature—i.e., there is a preponderance of four and two-spored viable asci in NH33/ YEp24 (Table 3). This suggests that hop1-628 protein is limiting and that some cells by chance accumulate enough of the protein to be able to go through a normal meiosis to produce asci with four spores in which all the spores are viable.

Suppression by plb-1 and plg-2 is specific for the hop1-628 allele: High copy suppression of a mutant phenotype could, in principle, occur by bypassing the need for the mutant gene by expressing a redundant function. Such bypass suppressors should also suppress null alleles. Alternatively, suppression could be the result of the overexpressed protein physically associating either directly with the mutant gene product or indirectly through a protein complex to stabilize it. This latter type of suppression should not work for null mutations. The specificity of suppression for plb-1 and plg-2 was tested by transforming the diploid NH33 with either of the two plasmids and assaying for spore viability. NH35 is heteroallelic for two nonconditional null alleles of HOP1: hop1-3::LEU2 (Hollingsworth and Byers 1989) and hop1-4686. While HOP1 is capable of complementing the spore viability defect in this diploid, neither plb-1 nor plg-2 exhibited any suppression in this strain at either 23°C or 30°C (Figure 2). In addition plb-1 and plg-2 were unable to increase the number of viable spores in diploids homozygous for either hop1-4088 or the cold-sensitive allele, hop1-842 (data not shown). The allele specificity demonstrated by plb-1 and plg-2 indicates that they are not capable of substituting for HOP1 even when present in high copy number.

Subcloning experiments localize the regions responsible for suppression in plb-1 and plg-2: plb-1 and plg-2 contain inserts of genomic yeast DNA in YEp24 of 6.9 and 8.4 kb, respectively (Figure 3). To localize the genes responsible for suppression of hop1-628 (designated RED1 and REC104), various subclones of plb-1 and plg-2 were tested for their ability to suppress the spore inviability of NH33. When the insert in plb-1 is cut approximately in half with XhoI, neither half is capable of suppression (Figure 3, pNH112 and pNH116). This strongly suggests that the RED1 gene is located at or very near the XhoI site. This was confirmed by inserting a 2.2-kb fragment containing the LEU2 gene into this XhoI site: this insertion inactivates suppression (pNH119; Figure 1).

The REC104 suppressor was first localized to a 4.0-kb BamHI/BglII fragment. This fragment can be biallelically categorized by SpH1. As was the case for plb-1, neither half of the bisected fragment confers suppression of hop1-628 (Figure 3). We therefore conclude that REC104 is located at or very close to the SpH1 site.

Disruption of RED1 and REC104 results in recessive spore inviability: Overexpression of the wild-
High Copy Suppressors of hop1-628

A. Subcloning analysis of plb-1 (RED1)

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Suppression of hop1-628</th>
</tr>
</thead>
<tbody>
<tr>
<td>plb-1</td>
<td>+</td>
</tr>
<tr>
<td>pNH112</td>
<td>-</td>
</tr>
<tr>
<td>pNH116</td>
<td>-</td>
</tr>
<tr>
<td>pNH119</td>
<td>-</td>
</tr>
</tbody>
</table>

B. Subcloning analysis of plg-2 (REC104)

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Suppression of hop1-628</th>
</tr>
</thead>
<tbody>
<tr>
<td>plg-2</td>
<td>+</td>
</tr>
<tr>
<td>pNH113</td>
<td>+</td>
</tr>
<tr>
<td>pNH114</td>
<td>-</td>
</tr>
<tr>
<td>pNH117</td>
<td>-</td>
</tr>
<tr>
<td>pNH118</td>
<td>-</td>
</tr>
<tr>
<td>pNH121</td>
<td>-</td>
</tr>
<tr>
<td>pNH123</td>
<td>-</td>
</tr>
</tbody>
</table>

Type alleles of RED1 and REC104 are capable of specifically suppressing a temperature-sensitive allele of HOPI. One possibility is that this suppression is due to an interaction between the HOPI gene product and RED1 or REC104 protein, either directly or through other components of the synaptonemal complex. If these two genes encode proteins essential for the formation of the SC, then one might predict that mutations in these genes would result in spore inviability. A 4.9-kb BglII/PstI fragment from pNH119 containing the LEU2-disrupted RED1 suppressor was substituted for the wild-type copy on the chromosome in two different HOPI haploids, 7859-10-2 and MR1-51 by the one-step gene transplacement procedure of Rothstein (1983). No obvious mitotic growth defects were seen in the transformants from either haploid. Diploid strains homozygous for either the wild-type copy on the chromosome in two different HOPI haploids, 7859-10-2 and MR1-51 by the one-step gene transplacement procedure of Rothstein (1983). No obvious mitotic growth defects were seen in the transformants from either haploid.

Figure 3.—Subcloning analysis of plb-1 (RED1) and plg-2 (REC104). Bars indicate the inserts present in the vector YEp24. Arrows represent the direction of transcription for RED1 and for REC104. Blackened areas define the ORFs for RED1 and REC104. The stippled bar represents the region deleted to make plasmid pNH13. Viable spore yield was assayed as in Figure 1. Restriction enzyme sites are abbreviated as follows: Ba, BamHI; B, BglII; C, ClaI; V, EcoRV; P, PstI; S, ScaI; Sp, SphI; X, XbaI; XhoI.

Figure 4.—Disruption of RED1 with LEU2 causes a recessive reduction in viable spore yield. The 1b-l::LEU2 (red1::LEU2) insertion allele was substituted for the wild-type copy of RED1 in the genomes of two haploid strains, 7859-10-2 and MR1-51. The wild-type homozygote was constructed by crossing the two untransformed strains together. The 1b-l::LEU2 (red1::LEU2) homozygote was generated by crossing the two haploids after replacement of RED1 with 1b-l::LEU2 (red1::LEU2). The colonies were patched first onto YEPD, replica-plated to Spo plates, incubated for 3 days at 30°C and then assayed for viable spore yield on -arg +can +cyh plates.

plays an important role in meiosis independently of HOPI.
in \textit{REC104} is located in or near the suppressor gene. The DNA surrounding this site was deleted by substituting a 1.7-kb \textit{ClaI}/\textit{EcoRV} fragment from \textit{pNH128} with \textit{LEU2} as described in MATERIALS AND METHODS. Haploids carrying the \textit{rec104::LEU2} deletion allele were mated, sporulated and tetrads dissected. While the isogenic wild-type parent generated 89.8\% viable spores (115/148), the \textit{rec104::LEU2} homozygote produced no viable spores (<0.7\%; 0/148). The \textit{rec104::LEU2} deletion is recessive as shown by the ability of heterozygous diploids to produce viable spores by the random spore assay (data not shown).

\textbf{Sequence analysis of 1b-1 reveals that it is \textit{RED1}}: Having demonstrated that the \textit{Xhol} site in p1b-1 must reside either within or very close to the \textit{RED1} coding sequence, the DNA sequence adjacent to this site was obtained. When 155bp of sequence to the right of the p1b-1 \textit{Xhol} site as depicted in Figure 3 was compared with sequences in the GenBank database, a 150/155 match was found with the meiosis-specific gene, \textit{RED1} (data not shown; \textsc{Thompson and Roeder} 1989). To further test the sequence identity a primer was generated based on a different part of the \textit{RED1} sequence of \textsc{Thompson and Roeder} (1989) and used to sequence our suppressor. Once again a strong match was observed (151/152). Comparison of restriction maps of the regions flanking the suppressor and \textit{RED1} also proved to be the same (Figure 3; \textsc{Thompson and Roeder} 1989). Furthermore null alleles of \textit{RED1} have a spore inviability phenotype similar to that observed for \textit{1b-1::LEU2} (\textit{red1::LEU2}) (\textsc{Rockmill and Roeder} 1990). We therefore conclude that overexpression of \textit{RED1} specifically allows partial suppression of \textit{hop1-628}.

\textbf{Mapping and sequence analysis of 1g-2 reveals that it is \textit{REC104}}: Subcloning analysis of p1g-2 indicated that the \textit{SphI} site within the insert is present within or near the coding sequence of the \textit{REC104} suppressor. The DNA sequence surrounding this site was determined, and an ORF of 182 amino acids spanned this site (Figure 5). The protein has a calculated molecular mass of 21,000 daltons and a net charge of -6. The insert containing \textit{REC104} was placed on the physical map at a region on chromosome \textit{VIII} approximately 75 kb distal to \textit{CDC12} by hybridization of \textit{pNH128} to a bank of \textsc{lambda} clones containing overlapping fragments of the yeast genome (L. \textsc{Riles} and M. V. \textsc{Olson}, personal communication). Tetrad analysis demonstrated genetic linkage between \textit{CDC12} and \textit{REC104} of 29 cM (28 P:\textsc{D}:0 NPD:39 T; the \textit{len2 cdc12} strain was generously supplied by J. \textsc{Chant}). This map position coincides with that of \textit{REC104} (A. \textsc{Galbraith} and R. E. \textsc{Malone}, personal communication). Subsequent comparison of the DNA sequences from our suppressor and \textit{REC104} confirmed that they are the same gene (A. \textsc{Galbraith} and R. E. \textsc{Malone}, personal communication).

\textbf{DISCUSSION}

The synaptonemal complex is the structure formed when homologous chromosomes synapse during meiotic prophase (reviewed in \textsc{Von Wettstein, Rasmus and Holm} 1984). The function of the SC in meiosis has been a source of great speculation. One way of determining the function of the SC in meiosis is through the identification of genes that encode components of the SC and the determination of which processes are affected when these genes are mutated or absent.

Our approach to identifying genes involved in SC formation has been to isolate high copy suppressors to conditional alleles of \textit{HOPl}, a gene believed to encode an SC component in \textit{S. cerevisiae}. Five new \textit{HOPl} alleles were isolated by mating EMS-mutanized cells to a \textit{hop1} haploid and assaying spore inviability. Three of the new \textit{hoa} alleles are conditional for spore viability; two are temperature-sensitive (\textit{hoa}1-628 and \textit{hoa}1-327) and one is cold-sensitive (\textit{hoa}1-842). The other two (\textit{hoa}1-4088 and \textit{hoa}1-4686) are defective for \textit{HOPl} function at both 23° and 30°. The temperature sensitive \textit{hoa}1-628 allele was used in a screen to isolate high copy suppressors. From this screen two previously identified meiosis-specific genes, \textit{RED1} and \textit{REC104}, were isolated.

That \textit{RED1} can suppress a conditional allele of \textit{HOPl} is especially interesting given that \textit{HOPl} and \textit{RED1} form a distinct class of meiosis-specific genes. Mutants in \textit{RED1} and \textit{HOPl}, as well as \textit{Merk1} and \textit{MEK1}, display residual levels of recombination between chromosomes (\textsc{Engbrecht and Roeder} 1989; \textsc{Rockmill and Roeder} 1991). In contrast, other meiotic genes such as \textit{MEH4, REC102, SPO11, MER2} and \textit{RAD50} are completely recombination defective (\textsc{Menees and Roeder} 1989; \textsc{Bhargava, Engbrecht and Roeder} 1992; \textsc{Klapholz, Waddell and Esposito} 1985; \textsc{Engbrecht, Hirsch and Roeder} 1990; \textsc{Malone and Esposito} 1981). Epistasis experiments have suggested that \textit{HOPl}, \textit{RED1} and \textit{MEK1} work through one pathway and \textit{MER1} through another (\textsc{Engbrecht and Roeder} 1989; \textsc{Rockmill and Roeder} 1991). A further distinction between \textit{HOPl/RED1/MEK1 and MER1} is that the former are not required for intrachromosomal recombination of an 11.4kb duplication while \textit{MER1} is necessary (\textsc{Hollingsworth and Byers} 1989; \textit{RED1} (\textsc{Rockmill and Roeder} 1990); \textit{MEK1} (B. \textsc{Rockmill}, personal communication); \textit{MER1} (\textsc{Engbrecht and Roeder} 1989)). Although \textit{HOPl, RED1} and \textit{MEK1} share many genetic characteristics and are placed in the same epistasis group, \textit{HOPl} and \textit{RED1} are distinct from \textit{MEK1} in that no SC is present in \textit{hop1} and \textit{red1} diploids (\textsc{Hollingsworth and Byers} 1989; \textsc{Rockmill and Roeder} 1990) while short stretches of SC are observed in the absence of \textit{MEK1} (\textsc{Rockmill and Roeder} 1991).
High Copy Suppressors of hop1-628

Given the similarities in phenotypes between red1 and hop1 mutants, as well as the epistasis data, it is reasonable to propose that both genes are involved in the same process—in this case as part of the structure of the SC. The suppression of the hop1-628 allele by RED1 overexpression suggests that RED1 protein may act to stabilize the mutant HOP1 protein directly by interacting with it, or, alternatively, overproduction of RED1 gene product may result in formation of a slightly different SC structure which is now capable of stabilizing the hop1-628 protein. Additional data suggest that the hop1-628 defect is specific for chromosome segregation—i.e., hop1-628 diploids recombine proficiently at the temperature restrictive for spore viability (data not shown). This suggests that HOP1 may have an active role not only in synapsis but...
in chromosome segregation as well. Overexpression of \textit{RED1} may be able to bypass the segregation function which is specifically lacking in \textit{hop1-628} while not being able to overcome the synopsis defect present in \textit{hop1} null mutants. It is also possible that \textit{RED1} acts in some way to modify the \textit{HOP1} protein. Experiments are currently underway to test whether \textit{HOP1} and \textit{RED1} physically interact.

Like \textit{RED1} and \textit{HOP1}, the \textit{REC104} suppressor is required for viable spore formation. The DNA sequence of \textit{REC104} predicts an acidic protein of 182 amino acids (Figure 5). Upstream of the initiation codon are two sites required for the proper regulation and expression of two meiosis-specific genes, \textit{HOP1} and \textit{SP013}. Mutation of the \textit{URS1} element (GGCGGCT) upstream of either of these genes results in a derepression of transcription in vegetative cells (Buckingham et al. 1990; Vershon, Hollingsworth and Johnson 1992). In addition \textit{REC104} contains a good match to the \textit{HOP1} activator sequence UAS\textsubscript{H} (GTAGTG). This site is required both for full activation of \textit{HOP1} gene expression in sporulating cells and for the vegetative derepression observed in \textit{URS1} mutants (Vershon, Hollingsworth and Johnson 1992). The presence of these sites suggests that \textit{REC104} is a meiotically regulated gene and this turns out indeed to be the case (see below).

Mapping and sequence data demonstrated that the \textit{lg2} suppressor is \textit{REC104}, a gene previously identified by Malone et al. (1991) using a screen for mutants capable of rescuing the spore lethality of \textit{rad52 spo13} diploids. \textit{REC104} is transcribed exclusively during the early part of meiotic prophase, consistent with the presence of the meiotic regulatory sites (A. Galbraith and R. E. Malone, personal communication). Diploids homozygous for \textit{rec104} make inviable spores (Malone et al., 1991), a result similar to that observed for our \textit{rec104::LEU2} disruption (see RESULTS). A drastic decrease is observed in recombination between homologs in \textit{rec104} strains (Malone et al. 1991; A. Galbraith and R. E. Malone, personal communication). This fact, combined with the ability of the mutant to rescue the spore lethality of \textit{rad52 spo13} diploids led Malone et al. (1991) to speculate that \textit{REC104} is involved early in meiosis, perhaps in the initiation of meiotic recombination. How can we reconcile the fact that a gene which apparently is required very early in meiosis is capable, when overproduced, of suppressing a defect in \textit{hop1-628}? This question is especially puzzling given that the recombination proficiency of \textit{hop1-628} leads us to suspect that the defect in this case is occurring after synopsis. One possibility is that \textit{REC104} encodes a transcriptional regulator that is required to activate expression of a number of early meiotic genes, including \textit{HOP1}. Overexpression of such an activator could result in an increase in the amount of \textit{hop1-628} protein in the cell. In fact the \textit{hop1-628} mutant phenotype is eliminated if the allele is transferred to a high copy number plasmid (data not shown). This idea is ruled out, however, by the observation that \textit{HOP1} gene expression is fully induced when \textit{rec104} diploids are sporulated (a similar result was also obtained for \textit{red1}; data not shown). An alternative model is that \textit{REC104} is actively required for chromosome condensation. In the absence of this function the chromosomes are unable to condense or condense abnormally such that they cannot be recognized by the recombination machinery. Overexpression of such a function may cause hypercondensation of the chromosomes such that the temperature-sensitive \textit{hop1-628} protein is stabilized, albeit weakly.

We thank Barbara Garvie, Lee Hartwell, Michael Redd and John Gant for supplying strains. Joachim Li generously provided us with the YEp24 library and the filters for the physical mapping experiment. We are grateful to Anne Galbraith and Robert Malone for sharing unpublished results with us and to Linda Riles for her help in the physical mapping of \textit{REC104}. We wish especially to thank Aaron Neiman, Beth Rockmill, Shireen Roeder and Breck Byers for constructive comments on the manuscript and Aaron Neiman and Breck Byers for invaluable insights during the course of these experiments. This work was supported by National Institute of Health grant GM44532-01A1. N.M.H. was supported in part by a Damon Runyan-Walter Winchell Cancer Research Fund Fellowship, DRG-965, and in part by the American Cancer Society, California Division Fellowship, S-29-91.

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Communicating editor: G. R. Smith