

Splicing of the Meiosis-Specific *HOP2* Transcript Utilizes a Unique 5' Splice Site

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The *Saccharomyces cerevisiae* *HOP2* gene is required to prevent formation of synaptonemal complex between nonhomologous chromosomes during meiosis. The *HOP2* gene is expressed specifically in meiotic cells, with the transcript reaching maximum abundance early in meiotic prophase. The *HOP2* coding region is interrupted by an intron located near the 5' end of the gene. This intron contains a nonconsensus 5' splice site (GUUAAGU) that differs from the consensus 5' splice signal (GUAPyGU) by the insertion of a nucleotide and by a single nucleotide substitution. Bases flanking the *HOP2* 5' splice site have the potential to pair with sequences in U1 small nuclear RNA, and mutations disrupting this pairing reduce splicing efficiency. *HOP2* pre-mRNA is spliced efficiently in the absence of the Mer1 and Nam8 proteins, which are required for splicing the transcripts of two other meiosis-specific genes.

RNA splicing is required to generate an mRNA containing a functional open reading frame (ORF) from a pre-mRNA containing one or more introns. Splicing is a two-step reaction (54). During the first step, the 5' end of the intron is cleaved and joined to a specific nucleotide within the intron, resulting in formation of a lariat. In the second reaction, the two exons are ligated together and the intron is released.

RNA splicing is catalyzed by a protein-RNA complex called the spliceosome, which assembles in a highly ordered manner. Many of the steps in spliceosome assembly involve base pairing between conserved pre-mRNA sequences and the small nuclear RNA (snRNA) components of the spliceosome (46, 54). In *Saccharomyces cerevisiae*, most introns contain three highly conserved splicing signals (53): the 5' splice site, GUAPyGU; the 3' splice site, PyAG; and the UACUAAC sequence that lies upstream of the 3' splice site and functions as the branch point for lariat formation. Previous studies have demonstrated that the 5' splice site pairs sequentially with sequences in U1 and U6 snRNAs. Exon sequences immediately adjacent to the 5' and 3' splice sites pair with U5 snRNA, while the branch point sequence pairs with U2 snRNA. Proper pairing between splicing signal sequences and snRNAs is important for the efficiency and accuracy of splicing (54).

In some cases, splicing serves to regulate gene expression (28, 52). Regulated splicing often requires specialized factors, in addition to components of the general splicing machinery (28, 59). The *MER2* gene provides one of few examples of regulated splicing in budding yeast. The *MER2* gene has a nonconsensus 5' splice site (16), and splicing of *MER2* pre-mRNA requires two proteins, Mer1 and Nam8 (also known as Mre2), that are not required for general splicing (11, 16, 32, 38). *MER2* pre-mRNA is present in both mitotic and meiotic cells (16), but the transcript is spliced efficiently only in meiotic cells because the Mer1 protein is produced only during meiosis (14). Compared to the consensus 5' splice site in yeast, the *MER2* 5' splice site has one less potential base pair with U1

snRNA (16). If pairing between the *MER2* 5' splice site and U1 snRNA is increased by mutating nucleotides in either the *MER2* intron or U1 snRNA, the requirement for Mer1 is alleviated (34). These observations suggest that the function of Mer1 is to promote or stabilize the interaction between the *MER2* 5' splice site and U1 snRNA. Like *MER2*, the *MER3* gene contains an intron with a nonconsensus 5' splice site and requires the Mer1 and Nam8 proteins for splicing. However, no obvious regulatory role can be ascribed to *MER3* splicing because the *MER3* gene is transcribed only in meiotic cells (33).

Meiosis is a special form of cell division that produces haploid gametes from diploid parental cells. During meiotic prophase, homologous chromosomes pair with each other, undergo genetic recombination and engage in synaptonemal complex formation (44). These interactions between homologous chromosomes are essential for proper chromosome segregation at the first meiotic division. In *S. cerevisiae*, the meiosis-specific *HOP2* gene plays an important role in promoting interhomolog interactions (26). In the *hop2* mutant, chromosomes engage in nearly wild-type amounts of synaptonemal complex formation, but most synapsis involves nonhomologous chromosomes. In addition, the *hop2* mutant sustains approximately the wild-type number of meiotic double-strand breaks (the initiators of meiotic recombination), but these breaks remain unrepaired. *hop2* cells arrest at pachytene due to a checkpoint triggered by the failure to complete recombination and/or by the aberration in synaptonemal complex assembly.

In this study, we have identified the *HOP2* ORF and an intron in the *HOP2* coding region. This intron contains an unusual 5' splice site that differs from the consensus sequence by the insertion of an extra nucleotide and by a single nucleotide substitution. Nonetheless, splicing of *HOP2* transcripts does not require the Nam8 protein, nor does it require Mer1 or any other meiosis-specific factors. There is potential for extended base pairing between U1 snRNA and sequences flanking the *HOP2* 5' splice site; these flanking sequences are important for efficient splicing of *HOP2* pre-mRNA.

MATERIALS AND METHODS

Strains and genetic procedures. Yeast strain genotypes are listed in Table 1. Yeast manipulations were performed and media were prepared as specified by

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TABLE 1. Yeast strains used

Strain	Genotype ^a
BR2171-7B	<i>MATα leu2 ura3-1 HO trp1-1 arg4-8 thr1-4 ura3-1 ade2-1</i>
YAB36	<i>MATα leu2 ura3-1 HO trp1-1 arg4-8 thr1-4 ura3-1 ade2-1</i>
YAB27	<i>MATα leu2-27 his4-280 ura3-1 trp1-1 spo13::ura3-1 arg4-9 thr1-4 cyh10 LYS2 ade2-1</i>
YAB97	<i>MATα leu2-3,112 his4-260 ura3-1 trp1-289 SPO13 arg4-8 thr1-1 CHY10 lys2-1 ade2-1</i>
YAB98	YAB36 but homozygous <i>HOP2-H2</i>
YAB99	YAB36 but homozygous <i>hop2-M3</i>
YAB100	YAB36 but homozygous <i>hop2-M4</i>
YAB103	YAB36 but homozygous <i>hop2-H3</i>
YAB274	YAB36 but <i>hop2-H2::lacZ</i>
YAB275	YAB36 but <i>HOP2</i>
YAB265	YAB27 + pL44 (<i>HOP2</i>)
YAB266	YAB27 + pL45 (<i>HOP2-H2</i>)
YAB71	YAB36 but homozygous <i>mer1::ADE2</i>
YAB276	YAB71 but <i>hop2-H2::lacZ</i>
YAB277	YAB71 but <i>HOP2</i>
YAB68	YAB36 but homozygous <i>hop2::URA3 mer1::ADE2</i>
YAB262	YAB68 + pL44 (<i>HOP2</i>)
YAB263	YAB68 + pL45 (<i>HOP2-H2</i>)
YAB230	YAB36 but homozygous <i>nam8::TRP1</i>
YAB278	YAB230 but <i>hop2-H2::lacZ</i>
YAB279	YAB230 but <i>HOP2</i>
YAB231	YAB36 but homozygous <i>hop2::URA3 nam8::TRP1</i>
YAB267	YAB231 + pL44 (<i>HOP2</i>)
YAB268	YAB231 + pL45 (<i>HOP2-H2</i>)
YAB270	YAB36 + pL52 (<i>ADH1::hop2::lacZ</i>)
YAB269	YAB36 + pL53 (<i>ADH1::hop2-H2::lacZ</i>)
YAB280	YAB27 + pL62 (<i>hop2-M2</i>)
YAB281	YAB27 + pL66 (<i>hop2-M5</i>)
YAB248	YAB27 + pL81 (<i>hop2-5P</i>)
YAB247	YAB27 + pL82 (<i>hop2-3P</i>)
YAB249	YAB27 + pL84 (<i>hop2-35P</i>)
S168	<i>MATα ura3 lys2 ho::LYS2 arg4-bgl</i>
NKY291	<i>MATα leu2::his4G ura3 lys2 ho::LYS2</i>
AS4	<i>MATα trp1 arg4 tyr7 ade6 ura3 MAL2</i>
Y260	<i>MATα his4-580 leu2-3,112 ura3-52 pep4-3</i>
BR1373-6D	<i>MATα leu2-27 his4-280 ura3-1 spo13::ura3-1 arg4-8 thr1-1 trp1-1 ade2-2 cyh 10</i>

^a The relevant *HOP2* alleles present on replicating plasmids are indicated in parentheses following the plasmid designations.

Sherman et al. (49). Substitutive and integrative transformations (45) were carried out by the lithium acetate procedure (23). In diploid strains carrying the *HOP2-H2*, *hop2-M*, *hop2-H3*, *hop2-M3*, and *hop2-M4* mutations, both copies of the chromosomal *HOP2* gene were replaced with the indicated *hop2* mutant allele by the two-step transplacement method (45). The *nam8::TRP1* disruption was generated by the method of Baudin et al. (3). Oligonucleotides with homology to the *NAM8* gene were used to amplify *TRP1* from pR314 (50); the resulting PCR product, containing a *TRP1* gene flanked by 50 bp of sequences upstream and downstream of the *NAM8* coding region, was then transformed into yeast.

In sporulation assays, diploid cells were grown to saturation in YPAD, or synthetic medium lacking leucine or uracil (to select for plasmids), and then diluted two- to fourfold into YPAD and grown for 12 h. Cells were then washed, diluted fivefold into 2% potassium acetate, and incubated at 30°C with vigorous shaking.

Escherichia coli XL1-Blue (Stratagene) was used for plasmid constructions. Bacterial strains used for transposon mutagenesis have been described by Hoekstra et al. (22). pL14 (25) was subjected to transposon mutagenesis in *E. coli*, using derivatives of the Tn3 transposon containing the bacterial *lacZ* coding region and the yeast *LEU2* or *URA3* gene (22). The transposon insertions were mapped by sequence analysis and introduced into a homothallic strain (BR2171-7B [42]) after digestion with *NotI*. The resulting transformants (heterozygous for the transposon) were sporulated, and tetrads were dissected; cells from Leu⁺ or Ura⁺ (depending on the transposon) diploid spore colonies were then tested for the ability to sporulate.

Plasmids. Plasmids were constructed by standard methods (47). Gene disruptions were introduced into yeast by using pME39 for *mer1::ADE2* (16) and pL21 for *hop2::URA3* (25).

Plasmids carrying mutant *HOP2* genes were constructed as follows. To generate pL68, the 2.2-kb *SphI-EcoRI* fragment containing the wild-type *HOP2* gene from pL14 (25) was subcloned into pUN50 (12); the 2.2-kb *HOP2*-containing *HindIII* fragment from the pUN50 derivative was then subcloned into the integrating vector, pRS306 (50). The *hop2-M*, *hop2-H3*, *hop2-M3*, and *hop2-M4* mutants were engineered by recombinational PCR (21). The PCR fragments were digested with *NheI* and *BglII* and substituted for the wild-type *NheI-BglII* fragment in pL68 to make the integration plasmids pL69 (*hop2-M*), pL73 (*hop2-H3*), pL70 (*hop2-M3*), and pL71 (*hop2-M4*). The *HOP2-H2* integration plasmid, pL72, was made by replacing the wild-type *NheI-BglII* fragment of pL68 with the cDNA fragment obtained by reverse transcription-PCR (RT-PCR). All integration plasmids described above were cut with *NheI* before transformation into yeast. pL36 was derived from pL13 (containing *CEN4*, *ARS1*, *LEU2*, and the wild-type *HOP2* gene) (25) by deleting the 0.5-kb *SpeI-HindIII* fragment of *HOP2*-adjacent DNA and the *EcoRI-SalI* segment of the polylinker. The *hop2-M2*, *hop2-M5*, *hop2-3P*, *hop2-5P*, and *hop2-35P* mutations were engineered by recombinational PCR. The PCR fragments were digested with *BsiWI* and *BamHI* and substituted for the wild-type *BsiWI-BamHI* fragment in pL36 to make pL62 (*hop2-M2*), pL66 (*hop2-M5*), pL82 (*hop2-3P*), pL81 (*hop2-5P*), and pL84 (*hop2-35P*).

Plasmids used to generate probes for RNase protection assays were con-

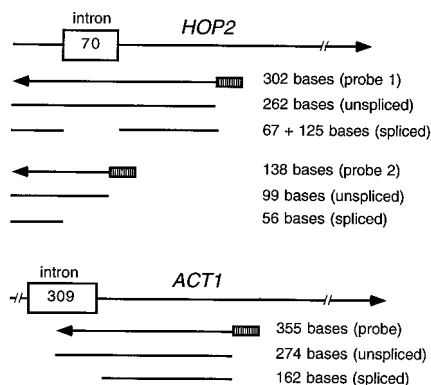


FIG. 1. *HOP2* and *ACT1* probes used for RNase protection experiments. The *HOP2* and *ACT1* primary transcripts are represented diagrammatically; indicated below each diagram are the probes used for RNase protection and the protection products expected from spliced and unspliced RNA. The hatched boxes indicate the promoter and polylinker regions of the vector.

structured as follows. To create pL59 (which was used to generate wild-type *HOP2* probe 1 [Fig. 1]), a 0.7-kb PCR product was amplified by using primers P10 (5'-GAACGACCAAGCTTTATTGAAAGATATG-3', corresponding to bases -27 to +3) and P5 (5'-GGTCTCGAAAAAGCTTCAAATACATACC A-3', corresponding to bases +717 to +688). (Nucleotides are numbered as in Fig. 2C, where +1 indicates the first nucleotide in the *HOP2* ORF.) The resulting PCR product was digested with *Hind*III and *Hinc*II and inserted between the *Hind*III and *Hinc*II sites of pBS M13+ (Stratagene). To generate *HOP2* probe 2 for wild-type and mutant genes, 134-bp PCR products were amplified from pL68, pL73, pL70, pL82, pL81, and pL84 by using primers P20 (5'-GCTCTATTTGA ATTCTATGGCACC-3', corresponding to bases -16 to +9) and P11 (5'-AAT ACCCGGGGTACAATTACTAGTAATGTC-3', corresponding to bases +118 to +89). The PCR fragments were then digested with *Eco*RI and *Spe*I and inserted between the *Eco*RI and *Xba*I sites of pBS M13+ to generate pL107 (*HOP2*), pL108 (*hop2-H3*), pL109 (*hop2-M3*), pL110 (*hop2-3P*), pL111 (*hop2-5P*), and pL118 (*hop2-35P*).

Plasmids containing *HOP2::lacZ* fusion genes were engineered as follows. pL105 and pL106 were constructed by subcloning the 3.2-kb *Bam*HI-*Bgl*II fragment containing *lacZ* from RP370 (60) into the *Bgl*II sites of pL72 and pL68. pL105 and pL106 were cut with *Nhe*I and integrated into the yeast genome to generate *hop2-H2::lacZ* and *hop2::lacZ* strains, respectively, for β -galactosidase assays.

Plasmids in which *HOP2::lacZ* genes are fused to the *ADH1* promoter were constructed as follows. First, a *Hind*III site was introduced just before the *HOP2* ORF by recombinational PCR. The PCR fragment was then digested with *Bam*HI and *Nhe*I and substituted for the wild-type *Bam*HI-*Xba*I fragment in pL36 to generate pL44. pL45 was constructed in a similar manner except that the intronless *HOP2* cDNA was used as the template for amplification by PCR. The 1.5-kb *Bam*HI-*Hind*III fragment containing the *ADH1* promoter from pAAH5 (2) was inserted between the *Hind*III site and the *Bam*HI site upstream of *HOP2* in pL44 and pL45 to generate pL50 and pL51, respectively. The 2.1- and 2.0-kb *Bam*HI fragments from pL50 and pL51 were then cloned into RP370, which contains *lacZ*, *URA3*, and the 2 μ m circle origin of DNA replication (60), to generate pL52 (*hop2::lacZ*) and pL53 (*hop2-H2::lacZ*), respectively.

To clone the *HOP2* gene from different strains, total yeast genomic DNA was prepared from BR1373-6D (41), AS4 (10), S168 (20), NKY291 (1), and Y260 (obtained from Michael Snyder), and the *HOP2* ORF was then amplified by PCR using primers P10 and P5. The products of PCR were purified and subcloned into pBS M13+ prior to sequencing.

RNase protection analysis and PCR amplification of *HOP2* cDNA. RNA isolation was carried out as described by Engebrecht et al. (16). RNase protection assays were carried out by using an RPAII kit from Ambion, Inc. (Austin, Tex.) as described by Engebrecht et al. (16). The antisense *ACT1* probe was generated by cutting pL85 (26) with *Ssp*I and transcribing with T7 RNA polymerase (Fig. 1). The antisense *HOP2* probe 1 (Fig. 1) was synthesized by in vitro transcription (24) of pL59 linearized with *Hind*III, using T7 RNA polymerase. The antisense *HOP2* probe 2 (Fig. 1) for wild-type and mutant *HOP2* genes was generated by cutting pL107, pL108, pL109, pL110, pL111, and pL112 with *Eco*RI and transcribing with T3 RNA polymerase. The levels of spliced and unspliced RNAs were quantitated using Multi-Analyst software (Bio-Rad) to scan autoradiograms and analyze the resulting data.

PCR analysis was performed from RNA templates with a GeneAmp Thermo-stable rTth reverse transcriptase RNA PCR kit (Perkin-Elmer Cetus) as instructed by the supplier. The upstream primer was P20, and the downstream primer was P17 (5'-CATTCTCAGTTGCAATACAG-3', corresponding to bases +383 to +363).

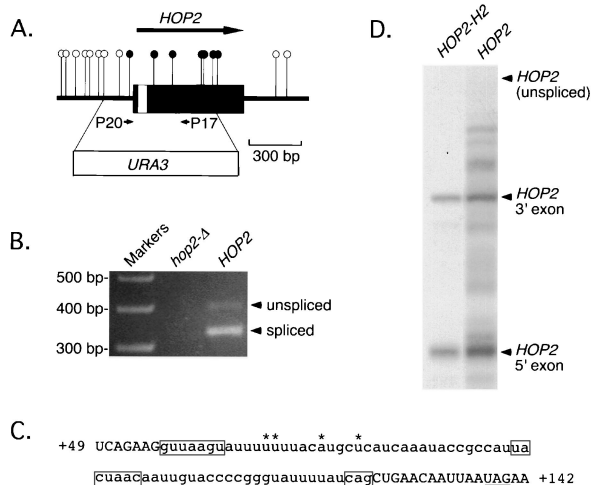


FIG. 2. Identification and sequence of the *HOP2* intron. (A) Diagram of the *HOP2* gene, with exon sequences indicated in black and the intron indicated in white. The arrow depicts the direction and extent of the *HOP2* coding region. Closed circles represent transposon insertions that disrupt *HOP2* function, while open circles represent those that do not disrupt gene function. P20 and P17 are the primers that were used for RT-PCR. Indicated below the *HOP2* diagram are the endpoints of the *hop2::URA3* deletion/disruption mutation used for panel B. (B) Products of RT-PCR derived from wild-type (YAB36) and *hop2::URA3* (YAB27) cells. Molecular weight markers are indicated on the left. (C) RNA sequence of the *HOP2* intron (lowercase) and flanking regions (uppercase). Numbers refer to the RNA sequence, with +1 indicating the first base of the *HOP2* initiation codon. The boxed regions represent the splicing signal sequences. An alternative 3' splice site that may be used in the *hop2-M* mutant is underlined. The nucleotides that vary among yeast strains are indicated by asterisks. (D) RNase protection of RNA from strains carrying the wild-type *HOP2* gene (YAB36) or the *HOP2-H2* cDNA allele (YAB97). RNA isolated from diploid cells harvested after 14 h in sporulation medium was subjected to RNase protection using the antisense *HOP2* probe 1 (Fig. 1). Unspliced *HOP2* RNA is detectable only after prolonged exposure.

β -Galactosidase assays. β -Galactosidase assays were performed as described by Chua and Roeder (6). β -Galactosidase activity units are defined as nanomoles of *o*-nitrophenyl- β -D-galactopyranoside cleaved per minute per milligram of protein.

RESULTS

The *HOP2* intron contains a unique 5' splice site. The wild-type *HOP2* gene was cloned from a yeast genomic library based on complementation of the *hop2-1* sporulation defect (25). Complementation activity was localized to a region of 0.8 kbp by subcloning followed by transposon mutagenesis (Fig. 2A). Sequence analysis revealed that the 0.8-kb fragment does not contain an obvious ORF but does include a long coding region that lacks an in-frame initiation codon. These observations suggested that the *HOP2* transcript might be spliced. A potential branch point sequence (UACUAAc) is found near the 5' end of the 0.8-kbp fragment, and several consensus 3' splice sites are located downstream of the UACUAAc box. However, no consensus 5' splice site sequence is located upstream of the UACUAAc box.

To determine whether the *HOP2* transcript is spliced and, if it is, to define the ends of the intron, *HOP2* RNA from meiotic cells was reverse transcribed and the resulting cDNA was then amplified by PCR. Two PCR products that differ in size by less than 100 bp were amplified from total RNA of wild-type cells but not from cells carrying a *HOP2* deletion mutation (Fig. 2B). Cloning and sequencing of the smaller RT-PCR product revealed that this fragment lacks 70 bp present in the genomic sequence. The UACUAAc box is located within the putative

intron (nucleotides [nt] 98 to 105 [Fig. 2C]), and the 3' end of the intron is defined by a consensus 3' splice site (nt 123 to 135 [Fig. 2C]). However, the sequence present at the 5' end of the intron (GUUAAGU, nt 56 to 62 [Fig. 2C]) differs from the consensus 5' splice site (GUAPyGU) by the insertion of a U residue adjacent to the first U in the consensus to create a UU dinucleotide. In addition, the pyrimidine residue present at the fourth position of the consensus sequence is a purine (at the fifth position) in the *HOP2* 5' splice site.

Diploids homozygous for the intronless version of *HOP2* recovered by RT-PCR sporulate efficiently and make viable spores (Fig. 3C), indicating that this version of the gene encodes a functional Hop2 protein. To determine whether the *HOP2* mRNA amplified by RT-PCR is the major product of *HOP2* splicing, total RNA isolated from meiotic cells was analyzed by RNase protection assays using a probe spanning the intron (Fig. 1). Diploids in which both copies of the wild-type *HOP2* gene have been replaced by the intronless *HOP2* gene (*HOP2-H2*) recovered by RT-PCR generate two RNase protection products (Fig. 2D). One fragment is the size expected for sequences upstream of the putative intron, while the other corresponds to sequences downstream of the intron. The two predominant RNase protection products derived from wild type are the same size as those derived from the *HOP2-H2* gene (Fig. 2D), indicating that the spliced product recovered by RT-PCR is indeed the predominant spliced mRNA generated from the wild-type gene. In addition to the two major products, we detected a number of other fragments that may represent minor splicing products or RNAs that have been degraded. Little or no unspliced *HOP2* pre-mRNA was detected in wild-type meiotic cells.

The sequence of the spliced *HOP2* transcript contains an ORF that is 203 codons in length, with 55 nt of coding sequence located upstream of the intron and 554 nt of coding sequence downstream (25). To confirm that the *HOP2* ORF has been correctly identified, sequences near the putative ATG initiation codon were mutated and examined for their effects on *HOP2* gene function by monitoring sporulation efficiencies. Insertion of a nucleotide immediately 5' of the initiation codon will not change the ORF and therefore should not affect gene function. In contrast, insertion of a single nucleotide immediately 3' of the initiation codon will create a frameshift mutation and thus should result in a failure to make functional protein. In the *hop2-M5* mutant, a T residue was inserted before the predicted initiation codon; this mutant sporulates as efficiently as the wild type (65% sporulation in both mutant [YAB281] and wild type [YAB267]) and displays the wild-type level of spore viability type (92% in the mutant and 93% in the wild type). In the *hop2-M2* mutant strain YAB280, a G residue was inserted after the initiation codon; this mutant fails to sporulate (0% spore formation). These data indicate that translation of the Hop2 protein does indeed initiate at the predicted ATG codon.

Mutational analysis of *HOP2* splice site signals. To confirm that splicing is required for *HOP2* gene expression and to identify the signal sequences required for splicing, a series of mutations were constructed by site-directed mutagenesis and then examined for their effects on splicing and sporulation. In the *hop2-M4* mutant, the UACUAAC box is changed to UACUAGU (Fig. 3A), which should prevent the first step in splicing (54). *hop2-M4* cells make very little spliced *HOP2* mRNA; some unspliced transcript is detected, as well as a number of other RNAs that may be degradation products (Fig. 3B). Diploids homozygous for the *hop2-M4* mutation fail to sporulate (Fig. 3C), as expected if proper splicing is required for *HOP2* gene expression.

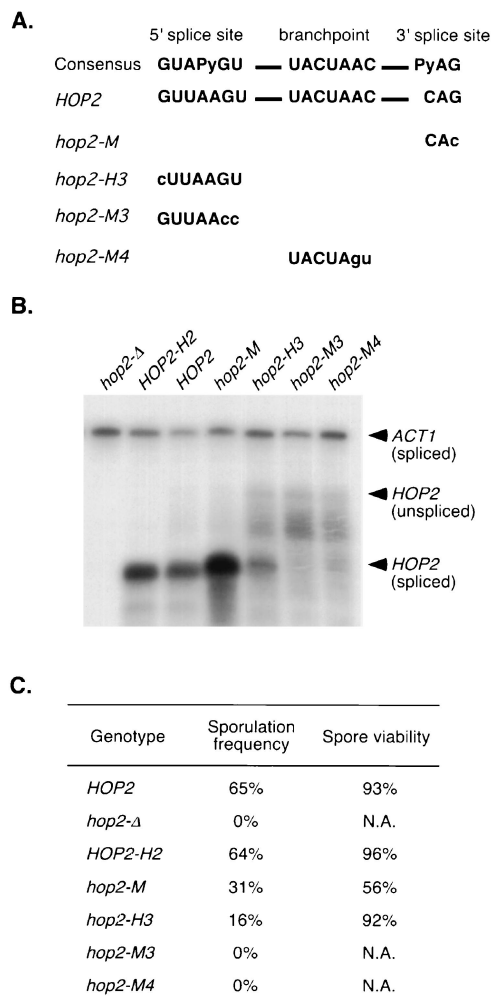


FIG. 3. Mutational analysis of *HOP2* splicing signal sequences. (A) Sequences of *HOP2* mutations in the branch point sequence, 5' splice site, and 3' splice site. The nucleotides altered by mutation are presented in lowercase. (B) RNase protection of RNA from the wild type and *hop2* mutants. RNAs isolated from diploid cells harvested after 14 h in sporulation medium were assayed by RNase protection using the antisense *HOP2* probe 2 (Fig. 1). RNAs derived from strains YAB27 (*hop2::URA3*), YAB36 (*HOP2*), YAB97 (*HOP2-H2*), YAB98 (*hop2-M*), and YAB100 (*hop2-M4*) were hybridized with probe derived from pL107. RNAs from strains YAB103 (*hop2-H3*) and YAB99 (*hop2-M3*) were hybridized with probes derived from pL108 and pL109, respectively. All *HOP2* probes are similar except that 1 or 2 nt have been changed, such that each probe is complementary to the corresponding mutant transcript. Transcription of the *ACT1* gene is constitutive; therefore, *ACT1* mRNA serves as a loading control. Use of the alternative 3' splice site postulated for the *hop2-M* mutant (see Results) would not change the size of the protection product obtained with *HOP2* probe 2 (Fig. 1), because this probe does not extend to the 3' end of the intron. (C) Sporulation efficiency and spore viability of the wild type and *hop2* mutants. Spore viability was determined from dissected tetrads; at least 200 spores were scored for each strain.

In the *hop2-M3* mutant, the *HOP2* 5' splice site GUUAAGU is changed to GUUAACC (Fig. 3A). The *hop2-M3* mutant does not sporulate and makes little or no spliced RNA of the appropriate size (Fig. 3B and C), suggesting that the 5' splice site identified by RT-PCR is the only 5' splice site used to make functional *HOP2* mRNA. Alternative (potential) 5' splice sites are not utilized and/or lead to the production of mRNAs that cannot be translated to generate functional protein. (For example, the potential 5' splice site [GUAUUU] at

positions 61 to 66 [Fig. 2C] could not be used to make protein since there is a stop codon [UAA, nt 56 to 58 [Fig. 2C] in frame with the *HOP2* coding region immediately upstream of this potential splice site.) In the *hop2-H3* mutant, the first nucleotide of the 5' splice site is changed from a G to a C. This mutation reduces both the efficiency of splicing and the level of sporulation, but not as dramatically as the *hop2-M3* mutation (Fig. 3B and C). Thus, the presence of a G residue at the first position in the *HOP2* 5' splice site appears to be important for splicing, but it is not absolutely essential.

In the *hop2-M* mutant, the *HOP2* 3' splice site (bases 123 to 125 [Fig. 2C]) is changed from CAG to CAC, but the resulting *HOP2* pre-mRNA appears nevertheless to be spliced efficiently (Fig. 3B). Given the unusual *HOP2* 5' splice site and known interactions between the 5' and 3' splice site, it is possible that the mutant 3' splice site can still be utilized. Alternatively, it is possible that splicing of *hop2-M* mutant pre-mRNA depends on a consensus 3' splice site (UAG, corresponding to bases 138 to 140 in Fig. 2C) located 15 bp downstream of the 3' splice site identified by RT-PCR. Use of the normally cryptic 3' splice site would result in an mRNA that is 15 bases smaller than its wild-type counterpart and a protein that differs from the wild-type Hop2 protein by the deletion of five amino acids. In the *hop2-M* mutant, both sporulation efficiency and spore viability are decreased about twofold. If the mutant 3' splice site (CAC) is used, then this meiotic defect suggests a reduction in efficiency of the second step in splicing (the RNase protection assay in Fig. 3B monitors only the first step in splicing). If the cryptic 3' splice site (UAG) is used, then the meiotic phenotype suggests that the five amino acids deleted from the mutant protein influence the function or stability of the Hop2 protein.

Sequences flanking the *HOP2* 5' splice site are important for splicing. Alignment of the *HOP2* 5' splice site with sequences in U1 snRNA indicates that the potential for base pairing between U1 snRNA and *HOP2* pre-mRNA extends outside the 5' splice site, to include 3 nt upstream of the 5' splice site and 2 nt downstream of the splice site (Fig. 4A). In yeast introns, sequences flanking the 5' splice site per se are not very conserved (27), suggesting that pairing between the 6-nt 5' splice site and the complementary sequence in U1 snRNA is normally sufficient to stabilize the interaction between these RNA molecules. However, since pairing between U1 snRNA and the 7-nt *HOP2* 5' splice site is predicted to be less stable due to the extra nucleotide, pairing between snRNA and nucleotides flanking the 5' splice site proper may be important for a stable interaction. To investigate this possibility, mutants that reduce or eliminate the potential for pairing of U1 snRNA with *HOP2* nucleotides outside the 5' splice site were constructed and analyzed.

In the *hop2-3P* mutant, the potential for base pairing between U1 snRNA and sequences downstream of the *HOP2* 5' splice site was destroyed by changing the eighth and ninth nucleotides (bases 63 and 64) of the intron from AU to UA (Fig. 4A). The efficiency of splicing of the *hop2-3P* transcript is reduced to 35% of the wild-type level (Fig. 4B), and the sporulation frequency of *hop2-3P* cells is also slightly decreased (Fig. 4C). In the *hop2-5P* mutant, the last 3 nt of the upstream exon (bases 53 to 55) were changed to eliminate the potential for pairing between U1 snRNA and sequences upstream of the 5' splice site (Fig. 4A). Splicing of the *hop2-5P* transcript is reduced severely (Fig. 4B). Although 27% of *hop2-5P* cells eventually form asci (Fig. 4C), sporulation is delayed by about 24 h and many asci contain fewer than four spores. (This defect in sporulation may be due in part to the two amino acid substitutions effected by the *hop2-5P* mutation, E18V and A19P.) In the *hop2-35P* mutant, sequences both upstream and down-

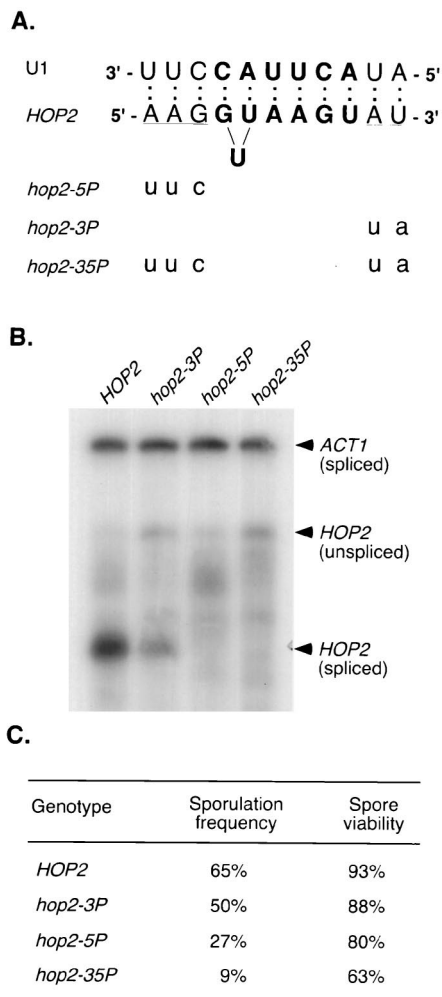


FIG. 4. Mutational analysis of sequences flanking the *HOP2* 5' splice site. (A) Mutations in sequences flanking the 5' splice site. The sequences flanking the wild-type 5' splice site (indicated in bold) are underlined, and potential base pairing between U1 snRNA and *HOP2* RNA is indicated by colons. The exact nature of pairing between the *HOP2* 5' splice site and the U1 snRNA is uncertain, since the nucleotide that fails to pair could be either one of the two adjacent U residues in the splice site. Nucleotides altered by mutation are presented in lowercase. (B) RNase protection assays of RNA from the wild type and *hop2* mutants. RNA isolated from diploid cells after 14 h in sporulation medium was assayed by RNase protection using antisense *HOP2* probe 2 and the *ACT1* probe (Fig. 1). RNAs from strains YAB265 (*HOP2*), YAB247 (*hop2-3P*), YAB248 (*hop2-5P*), and YAB249 (*hop2-35P*) were hybridized with *HOP2* probes derived from pL107, pL110, pL111, and pL112, respectively. All *HOP2* probes are similar except that 2 to 5 nt have been changed to match the mutant transcripts. (C) Sporulation efficiency and spore viability of the wild type (YAB265) and *hop2* mutants (YAB247, YAB248, and YAB249). Spore viability was determined from dissected tetrads; at least 200 spores were scored for each strain.

stream of the 5' splice site were mutated to eliminate the potential for pairing with U1 snRNA (Fig. 4A). In this mutant, sporulation efficiency and spore viability are even lower than those of the *hop2-3P* and *hop2-5P* mutants (Fig. 4B and C). Furthermore, no spliced product is detected (Fig. 5B), indicating that *hop2-3P* and *hop2-5P* have additive or synergistic effects on splicing. These results indicate that nucleotides flanking the *HOP2* 5' splice site are important for splicing, presumably because they pair with nucleotides in U1 snRNA.

Transcription of the *HOP2* gene is induced in meiotic prophase. Previous studies have shown that the abundance of the Hop2 protein increases during meiosis (25). In principle,

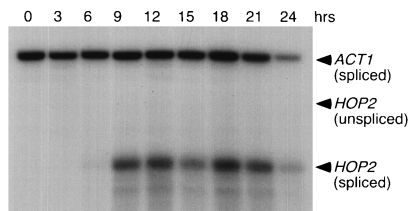


FIG. 5. Transcription of the *HOP2* gene is induced during meiotic prophase. RNA prepared from wild-type cells (YAB36) at various times after transfer to sporulation medium was subjected to RNase protection using *HOP2* probe 2 and the *ACT1* probe (Fig. 1). Unspliced *HOP2* RNA is detectable only after prolonged exposure.

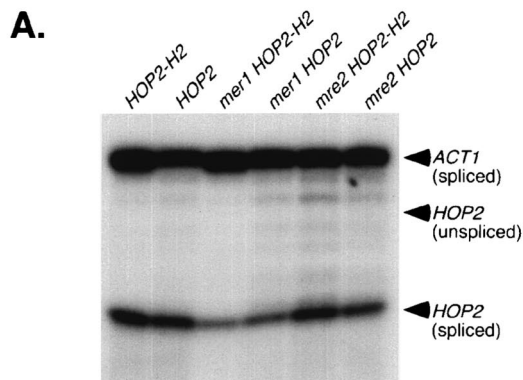
the elevated level of Hop2 protein might be achieved through either transcriptional or posttranscriptional regulation. Since RNA splicing is required for *HOP2* gene expression, we considered the possibility that the *HOP2* gene is transcribed in both vegetative and meiotic cells, but the transcript is spliced efficiently only in meiotic cells (as found for *MER2*).

To examine the regulation of *HOP2* gene expression, RNase protection assays were used to analyze total RNA isolated from wild-type cells at various times after the introduction into sporulation medium (Fig. 5). The *HOP2* transcript was first detected after 6 h in sporulation medium; a maximum level of transcript was reached by 9 h and then maintained for several hours thereafter. Unspliced *HOP2* RNA was barely detectable in meiotic cells, indicating that the *HOP2* transcript is efficiently spliced. Neither spliced nor unspliced *HOP2* RNA was detected in vegetative cells, suggesting that the *HOP2* gene is transcribed only during meiosis. Consistent with this result, a sequence (TCGGCGGCTA) that matches the consensus URS1 sequence (YCGGCGGCTA) is located at bases -130 to -121 in the *HOP2* upstream region. The URS1 sequence is responsible for both repressing transcription in vegetative cells and inducing transcription in meiotic cells (5).

In the strain used for this analysis, most cells reach the pachytene stage of meiotic prophase (when chromosomes are fully engaged in synaptonemal complex formation) after 15 to 16 h in sporulation medium. Thus, the timing of *HOP2* mRNA accumulation is consistent with the requirement for Hop2 specifically for meiotic interhomolog interactions and with the observed localization of the Hop2 protein to chromosomes prior to and during the pachytene stage (25).

***HOP2* splicing does not require Nam8 or meiosis-specific factors.** The meiosis-specific Mer1 protein is required for efficient splicing of the *MER2* and *MER3* transcripts, both of which have nonconsensus 5' splice sites (16, 33). To determine whether Mer1 is required for splicing of *HOP2* pre-mRNA, RNase protection assays were used to analyze total RNA from meiotic cells of *mer1* mutant strains carrying either the wild-type *HOP2* gene or the intronless *HOP2-H2* gene. Similar levels of spliced products were observed in both strains (Fig. 6A), indicating that the Mer1 protein is not essential for splicing of *HOP2* transcripts. To assay the levels of Hop2 protein produced by the wild-type and intronless *HOP2* genes in the absence of Mer1, a *lacZ* coding domain was fused in frame to sequences in the second exon and β -galactosidase activity was measured in strains carrying these constructs. *mer1* mutants carrying the *hop2::lacZ* or the *hop2-H2::lacZ* translational fusion gene exhibit similar levels of β -galactosidase activity (Fig. 6B), indicating that the *HOP2* gene is expressed normally in the absence of the Mer1 protein.

To determine whether splicing of the *HOP2* transcript requires any other meiosis-specific factor(s), the *hop2::lacZ* and



B.

Relevant genotype	β -Galactosidase activity	
	Vegetative	Meiotic
<i>ADH1::hop2::lacZ</i>	105.09	82.49
<i>ADH1::hop2-H2::lacZ</i>	171.76	128.82
<i>hop2::lacZ</i>	0.35	3.02
<i>hop2-H2::lacZ</i>	0.37	3.59
<i>hop2::lacZ mer1</i>	0.31	2.16
<i>hop2-H2::lacZ mer1</i>	0.33	2.06
<i>hop2::lacZ mre2</i>	0.35	2.35
<i>hop2-H2::lacZ mre2</i>	0.33	2.04

FIG. 6. Analysis of *HOP2* splicing in the wild type and *mer1* and *nam8* mutants. (A) RNase protection of RNA from wild-type, *mer1*, or *nam8* cells carrying either the wild-type or intronless version of the *HOP2* gene. RNA isolated from cells after 13 h in sporulation medium was assayed by RNase protection using the antisense *HOP2* probe 2 and the *ACT1* probe (Fig. 1). Unspliced *HOP2* RNA is detectable only after prolonged exposure. The level of *HOP2* mRNA is lower in *mer1* cells than wild-type cells because *mer1* cells sporulate poorly (15). Strains analyzed are (from left to right) YAB266, YAB265, YAB263, YAB262, YAB268, and YAB267. (B) β -Galactosidase assays. Production of the Hop2- β -galactosidase fusion protein was monitored in strains carrying either the *hop2::lacZ* or the intronless *hop2-H2::lacZ* gene. Strains analyzed were (from top to bottom) YAB270, YAB269, YAB275, YAB274, YAB277, YAB276, YAB279, and YAB278. All fusion genes are controlled by the original *HOP2* promoter except in strains YAB270 (*ADH1::hop2::lacZ*) and YAB269 (*ADH1::hop2-H2::lacZ*), in which *HOP2* is fused to the constitutive *ADH1* promoter. β -Galactosidase assays were performed on vegetative cells (0 h of sporulation) and meiotic cells (15 h of sporulation). Values given are the averages of three independent cultures for each strain.

hop2-H2::lacZ genes were fused to the *ADH1* promoter such that the fusion genes are expressed in both vegetative and meiotic cells (2). The wild-type *ADH1::hop2::lacZ* and intronless *ADH1::hop2-H2::lacZ* fusion genes were introduced into wild-type cells, and β -galactosidase activity was measured both prior to the introduction into sporulation medium and after 15 h of sporulation. In both vegetative and meiotic cells, β -galactosidase activity was somewhat higher in the strain carrying

the intronless *ADH1::hop2-H2::lacZ* fusion gene than in the strain carrying the intron-containing fusion gene (Fig. 6B), raising the possibility that the *HOP2* transcript is not correctly spliced with 100% efficiency when the gene is overexpressed. However, the levels of β -galactosidase activity in meiotic cells relative to vegetative cells are similar for the two constructs (*ADH1::hop2::lacZ* and *ADH1::hop2-H2::lacZ*), indicating that none of the factors required for *HOP2* splicing is specific to meiosis.

The Nam8 protein also is required for splicing of the *MER2* and *MER3* transcripts; however, unlike Mer1, the Nam8 protein is present in both vegetative and meiotic cells (11, 32, 33). To determine whether Nam8 is required for *HOP2* splicing, RNase protection and β -galactosidase assays were performed in the *nam8* mutant. The *hop2::lacZ* and *hop2-H2::lacZ* constructs produce equivalent levels of β -galactosidase activity both in the wild type and in the *nam8* mutant, indicating that the Nam8 protein is not necessary for splicing of *HOP2* pre-mRNA (Fig. 6).

Sequences in the *HOP2* intron are less conserved than exon sequences. The *HOP2* gene was cloned from five different yeast strains, and the sequences of the cloned genes were compared to that of the original *hop2*-complementing clone (25). The sequences derived from BR1373-6D (41) and AS4 (10) are identical to that of the original clone. In the Y260 strain (obtained from Michael Snyder), the nucleotide at position 73 (in the intron) is changed from an A to a G. In the SK1 strain, NKY291 (1), the intron nucleotides at positions 67 and 68 are changed from TT to CC, and the nucleotide at position 221 in the 3' exon is changed from an A to a T. The exon mutation changes a threonine residue to a similar amino acid (serine) and thus might not affect the function of the Hop2 protein. Another SK1-related strain, S168 (20), carries the same three mutations as NKY291, but the nucleotide at position 77 in the intron is also changed from a T to a C. The *HOP2* sequence present in the *Saccharomyces* genome database (46a) differs from that of the original *hop2*-complementing clone (25) by the deletion of an A residue at position +634. The sequences downstream of the site of the mutation encode 31 amino acids in the case of the database sequence and 16 (different) amino acids in the case of the published sequence (25).

Overall, this comparative analysis revealed variation at a total of 6 nucleotide positions, including 4 (out of 70) in the intron (Fig. 2C) and 2 (out of 609) in exon sequences. These results suggest, as expected, that there is greater selective pressure for maintenance of *HOP2* exon sequences than there is for intron sequences.

DISCUSSION

In the *Saccharomyces* genome database (46a) there is a hypothetical ORF called YGL033w (57) that overlaps extensively with the *HOP2* ORF (Fig. 7). Expression of YGL033w requires the removal of an intron that contains a consensus 5' splice (Fig. 7). However, several observations indicate that YGL033w, as annotated, is not the *HOP2* gene. First, the 5' exon of YGL033w is positioned at bases -374 to -351 relative to the *HOP2* start codon, but the results of our transposon mutagenesis demonstrate that insertions in the putative exon or a few hundred base pairs downstream do not disrupt *hop2*-complementing activity. Second, the results of site-specific mutagenesis of splice site signals indicate that the *HOP2-H2* mRNA is the only spliced product that encodes a fully functional Hop2 protein. Third, a cDNA corresponding to YGL033w was not detected by RT-PCR using a pair of primers that ought to have amplified both spliced and unspliced RNA corresponding to

this ORF. This pair of primers did generate small amounts of a product in which the 5' exon of YGL033w is fused to the 3' exon of *HOP2-H2* (data not shown). However, because the coding regions in the two exons are not fused in frame, this apparent cDNA has the capacity to encode only a very short peptide. Furthermore, when this intronless cDNA is introduced back into yeast (with appropriate 5' and 3' flanking sequences restored), it does not complement the *hop2* defect. Finally, although a conserved 5' splice site is used to generate the hypothetical spliced product encoding YGL033w, splicing depends on a 3' splice site (bases 191 to 193) that is far from the branch point sequence. The distance between the branch point and the 3' splice site is 90 nt, compared to 20 to 50 nt in most yeast introns. In addition, the 3' splice site predicted to result in expression of YGL033w would need to compete with three other 3' splice signals located further upstream. Based on these observations, it seems unlikely that the ORF designated as YGL033w is expressed *in vivo*.

The *HOP2* gene utilizes a unique 5' splice site (GUUAAG U). In fact, it is the only known 5' splice site in yeast in which the region of base pairing with U1 snRNA is interrupted by an extra nucleotide. Two factors may compensate for this interruption in pairing. First, the *HOP2* 5' splice site, unlike the consensus, contains an A residue at the nucleotide corresponding to position 4 in the consensus; this A residue can pair with the appropriately positioned U residue in U1 snRNA. In contrast, the nucleotide present at the fourth position in the consensus 5' splice site (U or C) cannot pair with the U residue in U1 snRNA. In addition, there are 3 nt immediately upstream of the *HOP2* 5' splice site and 2 nt downstream of the splice site that are capable of base pairing with U1 snRNA. Mutational analysis demonstrates that these flanking sequences are indeed important for efficient splicing of *HOP2* pre-mRNA. In the case of the *hop-3P* mutation, the reduction in splicing could result from reduced base pairing with U1 and/or U6 snRNA. The wild-type *HOP2* intron has the potential to form 5 bp with U6 snRNA; 5'-GUAUU-3' (positions 61 to 65 [Fig. 2C]) can pair with 3'-CAUAA-5' in U6. Two of these base pairs are destroyed by the *hop3-3P* mutation. Similarly, the *hop2-5P* mutation may affect splicing due to its effect on pairing with U1 and/or U5 snRNAs (27, 37). In the wild-type *HOP2* gene, nucleotides at the second and third positions upstream of the intron (nt 53 and 54 [Fig. 2C]) have the ability to base pair with nucleotides in U5; both of these base pairs are destroyed by the *hop2-5P* mutation.

Recently, Staley and Guthrie (55) have reported that increasing base pairing between U1 snRNA and the *ACT1* 5' splice site (from 6 bp interrupted by one mismatch to 10 or 12 continuous bp) decreases splicing efficiency. A number of observations indicate that this effect is due to hyperstabilization of the interaction between U1 snRNA and the 5' splice site. Thus, it is perhaps surprising that the *HOP2* transcript is spliced with nearly 100% efficiency, despite an extensive interaction between U1 and the 5' splice site (11 bp interrupted by one unpaired base). However, the interaction of U1 with the *HOP2* 5' splice site is not predicted to be as strong as its interactions with the extended *ACT1* 5' splice sites. The free energy of the U1 interaction with the *HOP2* 5' splice site is -9.8 kcal/mol, compared to -12.3 kcal/mol for the 10-bp *ACT1* site and -13.8 kcal/mol for the 12-bp *ACT1* site (48). Also, it is important to note that the extended *ACT1* 5' splice sites have significant effects only at low temperature. For example, the 10-bp *ACT1* 5' splice site reduced splicing efficiency 16-fold when cells were grown at 16°C but only 3-fold when cells were grown at 30°C (the temperature used to study *HOP2*). Also, Staley and Guthrie (55) showed that the defi-

-419
ACAATCTGTTAATTTCCATATAAAATAACTTGTAGTCGGATCGTACAATGGCTCTATATAG

-359
GTTTGGACCG**STATGT**AACTAATATGGCTGACTCAAACCTGCTAGATAGTTATCCAAG

-299
TGAATTTGAGTCTCGTATCCTTTATCACATTTTTACAATAGTGGCTAGCTATCTTTTTTCA

-239
TGTTAGGCTCACCCGGTATTCATGCAAGATCAAACAAAAATGGTAAAGTTTTGGACT

-179
TTACATTGCTTACAGTTGCTTTTTTATGAAGATTCAAAGATTATGCCTCGGCGGCTAA

-119
ATTTTCCATTAATAATATTATCCTTTTACGCGACCGAAAAGAGAAGCGTTTTAAGAGTT

-59
GTTTCTAACAAATATTAATAATAAAGCTAACGTGAACTGACCAGGCTCTATTTGAAAGAT

1
ATGGCACCGAAAAAGAAATCAAACGATAGAGCCATTCAGGCAAAGGCTCAGAAG**GTTAA**

61
GTATTTTTTTACATGCTCATCAAATACCGCCAT**TACTAAC**AATTGTACCCCGGTATTTT

121
AT**CAG**CTGAACAATTAATAGAAGATTACTTGGTGTCCACAATATAAACCATTTTCTGTAAA

181
TGATATAGTT**CAG**AATTTGCACAATAAAGTGACAAAAACAACAGCAACCAAAGCATTAGA

241
AAATCTCGTTAACGAGAAACGCATAGTGTCTAAAACCTTCGCAAAGATTATAATTTACTC

301
TTGTAATGAACAAGATACAGCATTACCAAGCAATATTGATCCTTCTCAGTTTGATTTTGA

361
AACTGTATTGCAACTGAGAAATGATTTAATAGAACTAGAGAGAGATAAATCAACGGCCAA

421
AGACGCTCTTGACTCTGTAACATAAAGAACCTGAAAATGAAGATCTCCTAACAAATCATTGA

481
AAACGAAGAAAACGAGTTGAAAAAGATTGAATCAAATTACAAAGTCTACAGGATGATTG

541
GGATCCAGCAAATGATGAGATTGTCAAGCGGATAATGTCTGAAGATACGCTGCTACAAA

601
AGAAATCACGAAGAGATCAAAGATTTGCAAAAAACCTAATTGCTACAATAAAGGACTCAG

661
TGTGCCCGAAAAATATGAATGAATTTTTGGTATGTATTTGAAATTTTTTCGAGACCTTT

721
TCTTTTGAATATACTAACGAAATCTTTACAGGAAGAAATGGATTGAGGACATTTGATG

FIG. 7. Comparison of the *HOP2* and YGL033w ORFs. Protein-coding sequences in the *HOP2* gene are indicated by the gray bars directly below the nucleotide sequence. Coding sequences for YGL033w are indicated by the black bars. The splice site signals for both *HOP2* and YGL033w are boxed; the two introns utilize the same branch point sequence but different 5' and 3' splice sites. The sequence present in the *Saccharomyces* genome database differs from that of the *hop2*-complementing clone (presented in the figure) by the deletion of an A residue from the stretch of A residues present at positions 629 to 634.

ciency in splicing conferred by hyperstabilization of the interaction between U1 and the 5' splice site reflects a competition between U1 and U6 snRNAs for base pairing with the 5' splice site. The defect in splicing conferred by hyperstabilization of the interaction between U1 and the 5' splice site can be suppressed by increasing the number of base pairs between U6 and the 5' splice site. In fact, as noted above, sequences in or near the *HOP2* 5' splice site have the potential to form 5 bp with U6 snRNA, compared to 3 bp between U6 and the consensus 5' splice site. Thus, hyperstabilization of the interaction between U1 and the 5' splice site might be compensated for by an increase in the strength of the interaction between the splice site and U6 snRNA.

The meiosis-specific *MER2* and *MER3* genes also contain nonconsensus 5' splice sites. The *MER2* 5' splice site (GUUC GU) differs from the consensus sequence by a single base substitution (A to U at the third position), while the *MER3* 5' splice site (GUA GU) is missing the fourth nucleotide of the consensus sequence (16, 33). The *MER2* intron is also unusual in terms of its position within the gene; the intron starts at position +319 of the coding region, whereas the vast majority of yeast introns are located within a few codons of the ATG start codon (46). In addition, the *MER2* and *MER3* introns are unusual with respect to the distance between the branch point sequence and the 3' splice site. This distance is 20 to 50 nt in most yeast introns (46), but it is 78 nt in the case of *MER3* and only 10 nt in the case of *MER2* (16, 33).

In contrast to the situation in the *HOP2* gene, the presumed defect in the interaction between U1 snRNA and the *MER2* and *MER3* 5' splice sites is not compensated for by pairing in regions flanking the 5' splice site. In both cases, the potential for pairing outside the splice site is limited to a single residue; an A residue located immediately downstream of the splice site in both introns could pair with the opposing U residue in U1 snRNA. Instead, in the case of *MER2* and *MER3*, the defect in splicing is compensated for by the Mer1 and Nam8 proteins, which apparently promote or stabilize the interaction between U1 snRNA and the impaired 5' splice site signals. The molecular basis for this enhancement of pairing is poorly understood. The Mer1 protein contains the K-protein-homologous motif characteristic of some RNA-binding proteins (51), and this protein has been demonstrated to bind specifically to sequences in and near the *MER2* intron (35). The Nam8 protein contains three copies of an RNA-binding domain and is a component of the U1 snRNP (19). Both in vivo and in vitro, the Nam8 protein is required for efficient 5' splice site recognition only when this process is impaired due to a nonconsensus 5' splice site or the absence of a methylated cap at the 5' end of the transcript (40).

Despite the fact that the *HOP2* transcript, like the *MER2* and *MER3* transcripts, contains a nonconsensus 5' splice site, the Mer1 and Nam8 proteins are not necessary for *HOP2* gene expression. Our data also indicate that no meiosis-specific factors are required for *HOP2* splicing. However, the possibility cannot be excluded that splicing of *HOP2* transcripts depends on specialized splicing factors (present in both vegetative and meiotic cells) that have not yet been identified. When the *HOP2* gene is fused to the *ADH1* promoter and therefore overexpressed, the intronless *hop2-H2::lacZ* fusion gene generates a higher level of β -galactosidase activity than the wild-type *HOP2* gene fused to *lacZ*. It is possible that the lower level of activity produced by the wild-type gene is due to the limited abundance or activity of a specialized splicing factor. Another possibility is that splicing of *HOP2* transcripts is negatively regulated by the Hop2 protein, leading to inhibition of splicing in the presence of excess Hop2. Such a regulatory mechanism

has been demonstrated to operate in the yeast *RPL32* gene, which also contains a nonconsensus 5' splice site (7, 13).

Unexpectedly, we found that mutation of the first nucleotide in the *HOP2* 5' splice site from a G to a C reduces splicing, but it does not completely prevent splicing. This result is surprising since the same mutation completely abolishes splicing in the *ACT1* gene (18, 58). Analysis of this mutation and others at the first position in the intron has led to the conclusion that the 5' splice site sequence performs two distinct and important functions (18, 36, 58). First, it is involved in defining the 5' splice site. Second, if the first step in splicing is executed and a lariat is formed, then the presence of a G at the branch junction is necessary for the next step in splicing (i.e., cutting at the 3' splice site and exon ligation). Perhaps the less stringent requirement for a G at the first position in the *HOP2* intron (compared to other introns) reflects the operation of a specialized splicing factor in the case of *HOP2*. It is also possible that a C at the first position in the intron is tolerated in the *HOP2* gene (though not in other genes) because of the extended base pairing between the 5' splice site and U1 snRNA.

Only 3.5% of all genes in the *S. cerevisiae* genome contain introns (2a, 43, 46, 53). Many of these introns are in genes encoding ribosomal proteins; in this class, about 60% of all genes have introns. Exclusive of the genes encoding ribosomal proteins; only 2.5% of genes contain introns (2a, 43, 46). It is therefore surprising that introns are found in a large fraction of genes expressed specifically in meiotic cells, especially in the early class of meiotic genes preferentially expressed during meiotic prophase (31). Approximately 21 early genes have been identified and examined for expression; of these, six have introns. This frequency (29%) is almost 10 times higher than the frequency of intron-containing genes in the genome as a whole. The meiosis-specific early genes that contain introns include *HOP2*, *MER2*, and *MER3* (discussed above), as well as the *REC114* (29), *ME14* (30), and *DMC1* (4) genes. The *ME14* and *DMC1* genes are similar to the bulk of intron-containing genes with respect to intron position, intron size, and consensus splice signals. However, the intron in *REC114* is unusual in two respects (29): (i) it is located a very long distance (1,242 nt) from the start of the coding region, and (ii) the 3' splice site (AAG) does not conform to the consensus. Splicing of the *REC114* transcript does not require the Mer1 protein, which is not surprising if one assumes (as the data suggest) that Mer1 is involved specifically in promoting the utilization of nonconsensus 5' splice sites.

The reason why so many of the early meiotic genes contain introns remains a mystery. It has been suggested that these introns play an essential role in the proper regulation of meiotic gene expression (43). However, in the five genes for which an intronless version of the gene was constructed and introduced into yeast, the gene lacking the intron was found to complement the corresponding null mutant (16, 25, 29, 30, 33). Thus, the introns in meiotic genes do not appear to have essential functions. Nevertheless, we cannot exclude the possibility that regulation of splicing of the transcripts of the early meiotic genes is required under conditions that induce meiosis and sporulation in nature, even though it is not important under laboratory conditions. A second possible explanation for the high frequency of introns in meiotic genes is based on the assumption that there is selection for a small and compact genome (and therefore for intron loss) in a rapidly dividing organism such as yeast. In this case, meiotic genes would be exposed to less selection pressure than genes expressed in vegetative cells since many cycles of vegetative growth intervene between meioses in nature.

Fink (17) has suggested that introns have been lost from the

yeast genome due to reverse transcription of mRNAs followed by homologous recombination between the resulting intronless cDNAs (17). This model also explains why most introns are located near the 5' ends of genes. Removal of an intron by homologous recombination requires that crossing over take place on both sides of the intron; thus, introns near the ends of pre-mRNAs would be less likely to be removed. Also, introns near the 5' ends of genes would be less likely to be reverse transcribed, since reverse transcription starts at the 3' end of the transcript but does not always proceed all the way to the 5' end. Experimental evidence demonstrates that yeast introns can be removed by reverse transcription followed by homologous recombination. Artificially engineered intron-containing genes undergo intron loss *in vivo*, and this loss depends on an active reverse transcriptase encoded by the transposable element Ty (8, 9). Thus, as suggested previously (39), the high frequency of meiotic genes containing introns might be attributable to the low level of reverse transcriptase present in meiotic cells. Transcription of Ty elements is repressed about 20-fold in *MATa/MAT α* cells grown in a nonfermentable carbon source (one of the conditions required to induce meiosis), and so genes expressed only under these conditions would be exposed to much lower concentrations of reverse transcriptase (56).

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