MOLECULAR AND CELLULAR BIOLOGY, Mar. 1987, p. 1208–1216
0270-7306/87/031208-09$02.00/0

Vol. 7, No. 3

Cloning and Characterization of *LOS1*, a *Saccharomyces cerevisiae* Gene That Affects tRNA Splicing

DEBORAH J. HURT,† SHIRLEY S. WANG, YU-HUEI LIN, AND ANITA K. HOPPER*

Department of Biological Chemistry, The Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, Pennsylvania 17033

Received 23 June 1986/Accepted 11 December 1986

***Saccharomyces cerevisiae* strains carrying *los1-1* mutations are defective in tRNA processing; at 37°C, such strains accumulate tRNA precursors which have mature 5′ and 3′ ends but contain intervening sequences. Strains bearing *los1-1* and an intron-containing ochre-suppressing tRNA gene, *SUP4*^{0}, also fail to suppress the ochre mutations *ade2-1*^{0} and *can1-100*^{0} at 34°C. To understand the role of the *LOS1* product in tRNA splicing, we initiated a molecular study of the *LOS1* gene. Two plasmids, YEpLOS1 and YCpLOS1, that complement the *los1-1* phenotype were isolated from the YEp24 and YCp50 libraries, respectively. YEpLOS1 and YCpLOS1 had overlapping restriction maps, indicating that the DNA in the overlapping segment could complement *los1-1* when present in multiple or single copy. Integration of plasmid DNA at the *LOS1* locus confirmed that these clones contained authentic *LOS1* sequences. Southern analyses showed that *LOS1* is a single copy gene. The locations of the *LOS1* gene within YEpLOS1 and YCpLOS1 were determined by deletion and gamma-delta mapping. Two genomic disruptions of the *LOS1* gene were constructed, i.e., an insertion of a 1.2-kilobase fragment carrying the yeast *URA3* gene, *los1*::*URA3*, and a 2.4-kilobase deletion from the *LOS1* gene, *los1*-Δ*V*. Disruption or deletion of most of the *LOS1* gene was not lethal; cells carrying the disrupted *los1* alleles were viable and had phenotypes similar to those of cells carrying the *los1-1* allele. Thus, it appears that the *los1* gene product expedites tRNA splicing at elevated temperatures but is not essential for this process.**

Primary transcripts of eucaryotic tRNA genes often contain 5′ and 3′ terminal sequences not found in mature tRNAs. Some eucaryotic tRNA genes also contain an internal sequence that is transcribed but not found in mature tRNA molecules. In *Saccharomyces cerevisiae* cells, this intervening sequence varies in length from 14 to 60 nucleotides in different tRNA genes and is located one nucleotide downstream from the 3′ end of the anticodon (5, 19, 24, 29, 30). Many of the details of the removal of intervening sequences from precursor tRNAs of yeast cells have been elucidated (for reviews, see references 8, 10, and 13). tRNA splicing involves at least five enzymatic reactions that are catalyzed by at least two different enzymes, i.e., an endonuclease that excises the intervening sequence and produces a gapped tRNA molecule and a ligase that catalyzes the formation of a phosphodiester bond between the two halves of the tRNA (9, 19, 20, 29, 31, 32). Apparently, all nine different species of intron-containing precursor tRNAs are spliced by the same two enzymes (31). The ligase protein has been purified and characterized and the gene encoding ligase has been cloned (33). It has been shown to be an essential gene (E. Phizicky and J. Abelson, personal communications).

Temperature-sensitive mutations in two yeast genes, i.e., *RNA1* (defined by the lesion *rna1-1*) and *LOS1* (defined by three lesions, *los1-1*, *los1-2*, and *los1-3*), affect the removal of the intervening sequence from precursor tRNAs (14, 15, 16, 19, 29). At the nonpermissive temperatures, both *los1* and *rna1-1* cells accumulate tRNA precursors that have mature 5′ and 3′ termini, including the posttranscriptionally added CCA nucleotides, but contain intervening sequences. Neither gene appears to encode splicing enzymes, since crude extracts from either *rna1-1* or *los1* strains contain significant levels of tRNA-splicing enzyme activities and the activities in these crude extracts are not thermolabile or inactive at elevated temperatures (L. D. Schultz and A. K. Hopper, unpublished results). Although *rna1-1* and *los1* mutants accumulate the same tRNA precursors, they define independent genes. *rna1-1* and *los1-1* complement each other (16), and the two genes map to unlinked genetic loci; *rna1* is on the right arm of chromosome XIII (26), and *los1* is on the left arm of chromosome XI, linked to *ura1* and *trp3* (D. Hurt, C. Peebles, and A. K. Hopper, unpublished results).

los1 cells appear to have a less severe defect than *rna1-1* cells do. The levels of precursor tRNA that accumulate in *los1* cells are much lower than those in *rna1-1* cells, and these precursor tRNAs are transient in pulse-chase experiments, unlike those in *rna1-1* cells (16). *rna1-1* cells do not accumulate mature rRNA and mRNA and do not grow at the nonpermissive temperature (14, 17, 41), whereas the *los1* mutations appear to affect only tRNA processing and *los1* cells are able to grow at temperatures at which tRNA splicing is affected (16). Cells completely defective in tRNA splicing should not be viable, since all eight $tRNA^{Tyr}$ genes contain introns (7, 10). Furthermore, the gene for the tRNA that decodes the rarely used serine codon UCG also contains an intron; loss of this gene function is lethal (3, 5, 6). Thus, *los1* cells appear to be capable of properly splicing precursor tRNAs, but perhaps at a slower rate than wild-type cells.

In addition to the tRNA splicing defect, *los1* cells demonstrate a temperature-sensitive partial loss of suppression. This antisuppressor phenotype was used as a screen to identify the *los1* mutations (16). At nonpermissive temperatures of 34 to 38°C, cells bearing *los1-1* and *SUP4*^{0}, an

* Corresponding author.
† Present address: Uniformed Services University of the Health Sciences, F. Edward Hebert School of Medicine, Bethesda, MD 20814-4709.

ochre-suppressing, intron-containing tRNA gene, fail to suppress the ochre mutations *ade2-1*^{0} and *can1-100*^{0}, which require strong suppression, but are capable of suppressing the mutations *lys1-1*^{0}, *his5-2*^{0}, and *trp5-48*^{0}, which require only weak suppression. At permissive temperatures, *los1-1 SUP4*^{0} cells suppress all the ochre mutations. The same effect is observed with the other seven ochre-suppressing $tRNA^{Tyr}$ genes, which all contain intervening sequences (16). Apparently, *los1-1* cells cannot produce sufficient mature suppressor tRNA at the nonpermissive temperature to overcome ochre mutations in the marker genes.

There are at least four explanations for the phenotype of the *los1* mutations. (i) Perhaps the *los1* mutations result in slightly altered gene products which retain some activity. (ii) The *LOS1* gene may be present in multiple copies in the yeast genome, and the three *los1* mutations isolated thus far may represent lesions in just one copy. (iii) There may be more than one pathway for precursor tRNA splicing, and the *LOS1* gene product may be involved in only one pathway. (iv) The *LOS1* gene product may not be necessary for tRNA splicing but may somehow enhance the process.

To understand the role of the *LOS1* product in tRNA splicing, we initiated a molecular study of the *LOS1* gene. We cloned the *LOS1* gene and used the cloned DNA to determine the *LOS1* gene copy number and to construct genomic disruptions of the *LOS1* gene.

MATERIALS AND METHODS

Strains and DNA libraries. The *S. cerevisiae* strains used in this study are described in the Results section.

The *Escherichia coli* strains used for cloning procedures were RRI (F$^-$ *pro leu thi lacY* Str^r r_k^- m_k^- *hsdR hsdM* endoI) and MC1066 (F$^-$ *leuB trpC pyrF*::Tn*5* r_k^- *mt araT* Δ*lacX74 rpsL*). The *leuB*, *trpC*, and *pyrF* mutations in the strain MC1066 can be complemented by the yeast genes *LEU2*, *TRP1*, and *URA3*, respectively. The *E. coli* strains used for γδ mutagenesis, provided by P. Youderian, were HB101 F′ *lac pro* and HB101 F$^-$ Tn*5*::*pyrA*.

Three libraries of yeast genomic DNA were used to clone the *LOS1* gene. The first library, provided by R. Hamilton (34), is a pool of DNA from yeast strain MP4 1041 in the vector YEp24. The second library, provided by D. Botstein (4), is a pool of DNA from a strain derived from S288C in the vector YEp24. The third library, provided by J. Campbell (21), is a pool of DNA from yeast strain S288C in the vector YCp50.

Genetic methods and media. Yeast cells were grown at 23°C unless otherwise indicated. YEPD contained 1% yeast extract, 2% peptone, 2% glucose, and 40 μg each of adenine and uracil per ml. Complete synthetic medium was as described by Hopper et al. (14), except that (per ml) 100 μg of aspartate, 100 μg of glutamate, 375 μg of serine, and 150 μg of valine were added. Drop-out medium was complete synthetic medium lacking certain bases or amino acids. Can medium was complete synthetic medium lacking arginine but containing 40 μg of canavanine per ml. Solid media also contained 1.5% agar. Selection of diploid yeast strains was accomplished either by prototroph selection or by zygote isolation by micromanipulation. Diploids were sporulated at 23 or 30°C on 1% potassium acetate–0.1% glucose–0.25% yeast extract–0.01% adenine–0.01% uracil–1.5% agar plates. Spore tetrads were dissected by micromanipulation.

E. coli cells were grown at 37°C on YT plates (0.5% yeast extract, 0.8% bactotryptone (Difco Laboratories), 0.5% sodium chloride, and 2% agarose) or in Luria broth (25). Ampicillin-resistant transformants were selected on YT plates supplemented with 30 μg of ampicillin per ml and were grown in Luria broth supplemented with 30 μg of ampicillin per ml. Tetracycline sensitivity of transformants was tested on YT plates supplemented with 20 μg of tetracycline per ml. Uracil-prototrophic transformants of MC1066 were selected on M9 plates (25) supplemented with 0.002% tryptophan and 0.006% leucine. In the γδ mutagenesis procedure, ampicillin-resistant lactose-prototrophic transformants were selected on MacConkey-lactose plates (39) supplemented with 100 μg of ampicillin per ml. Kanamycin- and ampicillin-resistant transconjugates were selected on YT plates supplemented (per ml) with 40 μg of kanamycin and 100 μg of ampicillin.

Transformation of yeast and *E. coli*. Yeast strains were transformed either by the alkali cation method (18) using either CsCl or LiOAc or, if high efficiency was required, by the glusulase method (12). *E. coli* was transformed by the $CaCl_2$ method (25).

Extraction of plasmid DNA from yeast and *E. coli*. Plasmid DNA was extracted from transformants of yeast strain 201-1-5 by the method described by Nasmyth and Reed (28). Plasmid DNA was extracted from *E. coli* RRI transformants by a modification of the alkaline lysis procedure of Birnboim and Doly (2) communicated to us by R. Baker. This modification uses a 15-min incubation with 0.5% diethylpyrocarbonate at 65°C before the ethanol precipitation. Large-scale plasmid preparations were also extracted with phenol and chloroform.

DNA manipulations. Enzymes were purchased from Bethesda Research Laboratories, Inc.; Boehringer Mannheim Biochemicals; New England BioLabs, Inc.; and International Biotechnologies, Inc. Restriction enzyme digests were performed by the instructions of the manufacturers. Linear DNA was dephosphorylated with bacterial alkaline phosphatase (44) or with calf intestinal phosphatase (25). When necessary, overhanging 3′ ends of restriction enzyme-digested DNA were converted to blunt ends with T4 DNA polymerase (25). Ligations with T4 DNA ligase were performed by the optimized conditions described by Lathe et al. (23). Probes were radiolabeled by using a Nick Translation Reagent Kit from Bethesda Research Laboratories with [^{32}P]dCTP or [^{32}P]dTTP. DNA was separated from unincorporated nucleotides by the spun-column procedure (25).

DNA samples were analyzed by electrophoresis through 0.7% agarose in 100 mM Tris (pH 8.6)–99 mM boric acid–2.5 mM EDTA (25). DNA species in the gel were detected with ethidium bromide.

Specific DNA fragments derived from restriction-enzyme-digested plasmids were purified as described by Benson (1). Restriction-enzyme-digested DNA was resolved on agarose gels; the gel fragment was excised and mashed, vortexed with phenol, and frozen on dry ice for 15 min. The aqueous phase was collected and extracted twice with phenol. DNA was precipitated with ethanol and collected by centrifugation.

Plasmid constructions. To construct YIpLOS1, YEpLOS1 was cut with *Bam*HI and *Sma*I. The *Sma*I digestion prevented subsequent recircularization of YEp24 vector DNA. YIp5 was cut with *Bam*HI and dephosphorylated. The digested DNA fragments were ligated. To construct YCpLOS1-ΔV, YCpLOS1 was cut with *Pvu*II and the digested DNA was ligated with T4 DNA ligase under dilute conditions. To construct YEpLOS1-TPL, YEpLOS1 was cut with *Sst*I, the overhanging 3′ ends were trimmed to blunt

ends with T4 DNA polymerase and the DNA was ligated with T4 DNA ligase under dilute conditions. To construct pBRLOS1, YCpLOS1 was cut with *Bgl*II and *Sal*I and the 4.2-kilobase (kb) fragment carrying the *LOS1*-complementing region was isolated. pBR322-HKL (kindly provided by R. Keil), which is a pBR322 derivative in which the *Hin*dIII site has been destroyed by a 4-base-pair (bp) insertion, was cut with *Bam*HI and *Sal*I and dephosphorylated. The digested DNAs were ligated together. To construct pBRLOS1::*URA3*, pBRLOS1 was cut with *Hin*dIII and dephosphorylated. YEp24 was digested with *Hin*dIII, and the 1.2-kb fragment carrying the yeast *URA3* gene was purified. The DNA fragments were ligated together.

For each of these constructions, either *E. coli* RRI or MC1066 was transformed with the ligation mixture and ampicillin-resistant transformants were selected. Transformants were screened for tetracycline sensitivity or uracil prototrophy when applicable. Plasmid DNA was extracted and screened by restriction analysis.

γδ mutagenesis of YEpLOS1 was performed by using a modification of the procedure of Guyer (11) communicated to us by P. Youderian. *E. coli* HB101 F′ *lac pro* was transformed with YEpLOS1, and ampicillin-resistant lactose prototrophs were selected. Cells from a single colony were incubated with *E. coli* HB101 F⁻ Tn*5*::*pyrA*, and kanamycin- and ampicillin-resistant transconjugants were selected. Plasmid DNA was extracted and screened by restriction analysis.

Analysis of precursor tRNAs in yeast cells. Yeast cells were radiolabeled with [5,6-^{3}H]uracil as described by Hopper et al. (16), with the modifications described in the legends to Fig. 2 and 6. RNA was extracted by one of two procedures. Low-molecular-weight RNA species were extracted from the labeled cells by phenol extraction of intact cells as described by Hopper et al. (16). Total cellular RNA was extracted by a modification of the method described by Hopper et al. (14) as described by Najarian et al. (27). RNA was precipitated with ethanol at −20°C and collected by centrifugation. Analytical gel electrophoresis and fluorography were performed essentially as described by Hopper et al. (14), with the modifications described in the legends to Fig. 2 and 6.

Southern analysis. Total cellular yeast DNA for Southern analysis was prepared as described by Struhl et al. (42) except that spheroplasts were prepared by digestion of cells in 1 mg of Zymolyase-5000 per ml (Seikagakau Kogyo Co., Ltd.) in 1 M sorbitol–0.1 M sodium citrate–60 mM EDTA with 0.1% β-mercaptoethanol. The DNA samples were digested with restriction enzymes overnight, a fresh sample of enzyme was then added, and digestion was continued for another 3 h. The DNA samples were electrophoresed through agarose gels as described above. The gels were irradiated with UV light, and DNA was transferred to nitrocellulose (25, 40). Blots were baked for 2 h at 80°C in a vacuum oven and then rinsed in boiling deionized water for 10 min. Prehybridizations and hybridizations were performed as described by Thomas (43). Blots were exposed to XAR-5 X-ray film (Eastman Kodak Co.) with intensifying screens (Du Pont Co.) at −70°C.

Northern analysis. RNA was extracted from yeast cells as described by Najarian et al. (27). Poly(A)$^+$ RNA was prepared by using Hybond-mAP messenger affinity paper (Amersham Corp.) by the procedure described by the manufacturer. Total RNA (10 μg) and poly(A)$^+$ RNA (5 to 10 μg) were resolved on a 1.0 or 1.5% agarose-formaldehyde gel and transferred to Gene Screen Plus membranes (Du Pont) by the procedure described by the manufacturer. Prehybridizations and hybridizations were performed as described by Thomas (43) using nick-translated probes.

RESULTS

Isolation of *LOS1*-containing plasmids. The temperature-sensitive, loss-of-suppression phenotype was used to identify transformants carrying plasmids complementing *los1-1*. Strains bearing *los1-1* and an ochre-suppressing tyrosine-inserting tRNA gene, such as *SUP4*^{0}, fail to suppress the ochre mutation *ade2-1*^{0} at 34°C (16). *ADE2* encodes an enzyme in the adenine biosynthetic pathway; failure to suppress *ade2-1*^{0} renders cells unable to grow on synthetic medium lacking adenine and causes cells to accumulate a red pigment. Thus, *los1-1* cells are white at 23°C on medium lacking adenine, but they are red at 34°C. Although replicas of *los1-1* colonies do not grow significantly at 34°C on medium lacking adenine, the red color can be detected readily (16). *LOS1* cells are white at either temperature on medium lacking adenine.

Plasmids carrying the *LOS1* gene were isolated from yeast DNA libraries in the vectors YEp24 and YCp50. YEp24 is a derivative of pBR322 which contains the yeast *URA3* gene and the yeast 2μm origin of replication and is, therefore, maintained in 5 to 20 copies in yeast cells (46). YCp50 is also a derivative of pBR322 and contains the yeast *URA3* gene but has the yeast ARS1 origin of replication and the yeast centromere CEN4 and is, therefore, maintained in single or low copy numbers in yeast cells (21).

Strain 201-1-5 (relevant genotype, *los1-1 SUP4*^{0} *ade2-1*^{0} *ura3-1*) was transformed with the libraries of yeast genomic DNA described above; uracil-prototrophic transformants were selected, and replicas were screened for adenine prototrophy at 34°C. We identified 20 adenine prototrophs from 3,000 transformants from the Hamilton YEp24 library, 85 adenine prototrophs from 28,000 transformants from the Botstein YEp24 library, and 11 adenine prototrophs from 5,000 transformants from the Campbell YCp50 library. The adenine prototrophs could represent yeast cells transformed with plasmids carrying the *LOS1* gene, the *ADE2* gene, or a gene(s) which could generally enhance suppression. To distinguish among these possibilities, plasmid DNA was extracted from representative isolates of the adenine-prototrophic transformants; the DNA was amplified in *E. coli* and introduced into yeast strains 201-1-5, a$_4$131-20 (relevant genotype, *ade2 ura3*), and H43 (relevant genotype, *mod5-1 SUP7*^{0} *ade2-1*^{0} *ura3*). The *ade2* mutation in a$_4$131-20 is not suppressed by *SUP4*^{0}. *mod5-1* is a tRNA modification mutation which has a partial loss-of-suppression phenotype, i.e., *mod5-1 SUP7*^{0} cells do not suppress *ade2-1*^{0} and are therefore red on medium lacking adenine (22). Our rationale was that we could identify a plasmid carrying *ADE2* by its ability to complement the *ade2* mutation of a$_4$131-20 and that we could identify a plasmid which enhanced suppression by its ability to complement the loss of *ade2-1*^{0} suppression in H43.

The following three classes of plasmids were identified. (i) Several plasmids, which had overlapping restriction maps, conferred adenine prototrophy to *los1-1 SUP4*^{0} *ade2-1* cells, *mod5-1 SUP7*^{0} *ade2-1*^{0} cells, and a$_4$131-20 cells. These plasmids presumably carried the yeast *ADE2* gene. (ii) Two plasmids from the Hamilton YEp24 library, which had overlapping restriction maps, conferred adenine prototrophy to both *los1-1 SUP4*^{0} *ade2-1*^{0} cells and *mod5-1 SUP7*^{0} *ade2-1*^{0} cells but not to a$_4$131-20 cells. However, on integra-

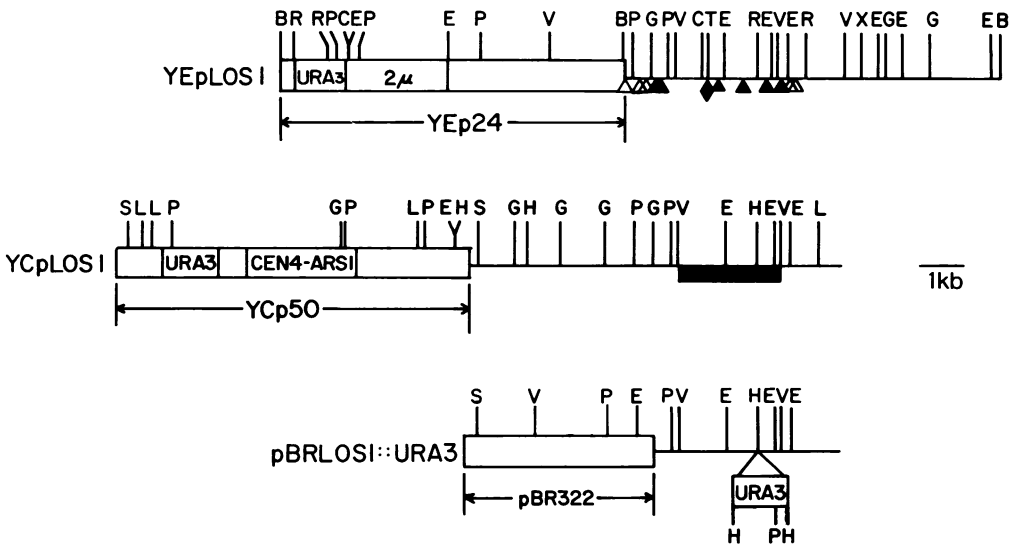

FIG. 1. Restriction maps of plasmids carrying the *LOS1* gene. Symbols: ▭, vector DNA; ——, cloned genomic DNA; ▲ and △, approximate sites of insertions which do and do not prevent *los1-1* complementation, respectively (locations of γδ insertions are approximate in that we know that they lay between the flanking restriction sites, but we do not know the precise locations); ◆, the 4-bp deletion at the *Sst*I site, which prevents *los1-1* complementation; ■, the 2.4-kb deletion of DNA between the *Pvu*II sites, which also prevents *los1-1* complementation. Restriction sites: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; L, *Bgl*I; P, *Pst*I; R, *Eco*RV; S, *Sal*I; T, *Sst*I; V, *Pvu*II; X, *Xho*I.

tion into the yeast genome, the cloned DNA failed to complement the *los1-1* loss-of-suppression phenotype. Further investigations indicated that these plasmids carry a gene that when present in high copy can restore or enhance suppression in a wide variety of strains. (iii) Two plasmids, one from the Botstein YEp24 library and one from the Campbell YCp50 library, restored suppression in *los1-1* cells but not in *mod5-1* cells and did not complement the *ade2* mutation in $a_4$131-20 cells. The genomic DNA inserts cross-hybridized in Southern analysis and colony hybridizations (data not shown) and had overlapping restriction maps (Fig. 1) and thus carried the same DNA sequences. Since the cloned DNA carried on these two plasmids specifically complemented the *los1-1* mutation, in multiple and single copy, it seemed likely that these plasmids carried the *LOS1* gene, and they were designated YEpLOS1 and YCpLOS1. YEpLOS1 had an 8.3-kb insert, and YCpLOS1 had an 8.0-kb insert.

Analysis of precursor tRNA levels in transformants. To determine whether YEpLOS1 and YCpLOS1 complemented the tRNA-splicing defect of *los1-1*, we compared the biosynthesis of tRNAs in *LOS1* cells carrying YEp24 and *los1-1* cells carrying YEp24, YCpLOS1, and YEpLOS1.Cells were labeled with [^{3}H]uracil for 1 h at the nonpermissive temperature (38°C), and low-molecular-weight RNAs were extracted and analyzed on urea-polyacrylamide gels. As can be seen in Fig. 2, YEpLOS1 and YCpLOS1 restored wild-type tRNA splicing in *los1-1* cells. Thus, YEpLOS1 and YCpLOS1 complemented both the loss-of-suppression phenotype and the splicing defect of *los1-1* cells.

Integration of plasmid DNA at the *los1* locus. The identities of the *LOS1* clones were confirmed by integrating plasmid DNA into the yeast genome by homologous recombination and showing that the site of integration was the *los1* locus. To integrate cloned DNA into the yeast genome, we first subcloned sequences from YEpLOS1 into YIp5 (see Materials and Methods). YIp5 is a derivative of pBR322 which contains the yeast *URA3* gene. Since YIp5 lacks a yeast origin of replication, YIp5 and its derivatives are not maintained in yeast cells unless the cloned DNA contains an origin of replication or the plasmid integrates into the yeast genome. Integration occurs via homologous recombination, either at *ura3* or at sites homologous to the cloned DNA insert (42).

Transformants of strain 201-1-5 with YIpLOS1 demon-

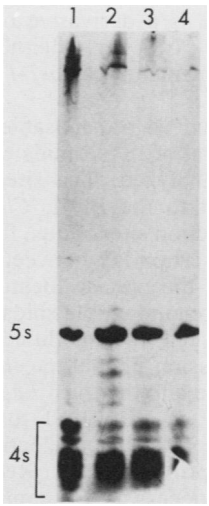

FIG. 2. Analysis of precursor tRNAs in *LOS1* and *los1-1* cells transformed with YEp24, YEpLOS1, or YCpLOS1. Exponentially growing yeast cells were incubated for 30 min at 38°C and then were labeled with [^{3}H]uracil for 60 min at 38°C. Low-molecular-weight RNA species were extracted. Approximately 2×10^5 cpm of each sample was electrophoresed through a 10% polyacrylamide gel containing 7 M urea, 100 mM Tris borate (pH 8.3), and 2.5 mM EDTA. Lanes: 1, *LOS1* cells (strain X2316-3C) transformed with YEp24; 2 to 4, *los1-1* cells (strain 201-1-5) transformed with YEp24 (lane 2), YCpLOS1 (lane 3), or YEpLOS1 (lane 4). Strain 201-1-5 was derived from strain X2316-3C by mutagenesis (16).

strated stable maintenance of uracil prototrophy and restoration of suppression at 34°C. One such integrant, 201-1-5I, was selected for genetic analysis. 201-1-5I was mated to DH15-8d or DH15-9b (relevant genotype, *LOS1 SUP4*^{0} *ade2-1*^{0} *ura3-1*), and the resultant diploids were sporulated and dissected. Of 31 four-spored tetrads, 30 tetrads segregated 4:0 for the ability to suppress *ade2-1* and 2:2 for uracil prototrophy. Southern analysis confirmed that integration had occurred at a site homologous to the cloned DNA and that uracil prototrophy segregated with the altered pattern of restriction fragments in the Southern analysis (not shown). The other tetrad segregated 3:1 for the ability to suppress *ade2-1*^{0} and 1:3 for uracil prototrophy. The spore unable to suppress *ade2-1*^{0} at 34°C was also a uracil auxotroph. The one spore (0.8%) unable to suppress *ade2-1*^{0} at 34°C probably resulted from intrachromosomal recombination between the endogenous *los1-1* and integrated *LOS1* sequence. These data show that YIpLOS1 integrated at, or closely linked to, the *los1* locus. Complementation of both the *los1-1* loss-of-suppression phenotype and tRNA-splicing defect and the genomic integration site indicate that YIpLOS1, and hence YEpLOS1 and YCpLOS1, carried the *LOS1* gene.

Determination of *LOS1* genomic copy number. In the Southern analysis described in the previous section, which used *Pvu*II digests of genomic DNA, the only bands detected were those predicted by the restriction maps of the *LOS1* clones. Similar results were obtained with *Pst*I (see Fig. 4), *Bgl*II (see Fig. 5), *Hin*dIII, and *Eco*RI (data not shown). If multiple copies of *LOS1* existed, the reiterated sequences would have included at least 16 kb of the region surrounding *LOS1*. Therefore, within the limits of this analysis, there was only one copy of *LOS1* in the yeast genome.

Localization of the *los1-1* complementing region. To localize the *los1-1*-complementing region within the cloned sequences, deletions and disruptions of the cloned sequences were constructed. YCpLOS1-ΔV is a derivative of YCpLOS1 deleted for the 2.4-kb region flanked by the *Pvu*II sites. YEpLOS1-TPL is a derivative of YEpLOS1 with a 4-bp deletion at the *Sst*I site. Neither of these deleted plasmids could complement the *los1-1* loss-of-suppression phenotype.

We also constructed γδ transposable element insertions into YEpLOS1. A total of 18 plasmids with insertions in the cloned DNA were analyzed. The sites of insertion were mapped with respect to the *Bgl*II, *Cla*I, *Eco*RI, *Eco*RV, *Pst*I, and *Pvu*II restriction sites. Thus, for any one insertion, we know that the insertion lay between two of these sites, but we do not know the precise location of the insertion. Representative γδ-containing plasmids were transformed into strain 201-1-5 to test their abilities to complement the *los1-1* loss-of-suppression phenotype. Insertions which fell between the *Bgl*II site and the third *Eco*RI site of the insert destroyed the ability of YEpLOS1 to complement *los1-1* (Fig. 1). More precise mapping of selective insertions (the γδ restriction map was provided by R. Reed) showed that the region of YEpLOS1 necessary for complementation was at least 2.8 kb and at maximum 3.4 kb.

Northern analysis of the *LOS1* product. Total and poly(A)$^+$ RNA was extracted from strain 201-1-5 harboring YEpLOS1, a wild-type strain (IIId-2d; see below for decription), and a strain carrying a deletion within *LOS1* (IIId-2c; see below for description). The RNA was resolved on formaldehyde-agarose gels and transferred to a Gene Screen Plus membrane (Du Pont; see Materials and Methods). The RNA on the membranes was hybridized to nick-translated pBRLOS1::*URA3* (Fig. 1). A 2.9- to 3.3-kb low-abundance poly(A)$^+$ RNA was found to be homologous to the probes (Fig. 3). (The estimated size varied in different gel systems.) The size of this species agrees well with the γδ mutagenesis data.

Disruption of the *LOS1* gene at its chromosomal locus by insertion of *URA3*. If the *LOS1* gene is essential for tRNA splicing, it would be expected that disruption or deletions of the *LOS1* gene would be lethal. To test this hypothesis, we constructed two genomic alterations of the *LOS1*-coding sequences, i.e., an insertion of the yeast *URA3* gene into the *LOS1* gene described here and a deletion of the 2.4-kb *Pvu*II fragment containing most of the *LOS1* gene described in the next section.

For the *URA3* insertion, pBRLOS1::*URA3* (Fig. 1), which contained a 1.2-kb *URA3*-bearing *Hin*dIII fragment at the *Hin*dIII site of the *LOS1* gene, was constructed. A gene replacement of chromosomal *LOS1* with the *URA3*-disrupted cloned *LOS1* gene was performed essentially as described by Rothstein (37). A diploid recipient was used in case the disruption was lethal in a haploid. The recipient, 2 × 1d, was constructed by mating 201-1-5 (relevant genotype, *los1-1 SUP4*^{0} *ade2-1*^{0} *can1-100*^{0} *lys1-1*^{0} *trp5-48*^{0} *ura3-1*) with DH15-1d (relevant genotype, *LOS1 SUP4*^{0} *ade2-1*^{0} *can1-100*^{0} *lys1-1*^{0} *trp5-2*^{0} *ura3-1*). The recipient was transformed with a *Pvu*II digest of pBRLOS1::*URA3*. A total of 20 uracil-prototrophic transformants were obtained. Of the diploid transformants, 12 were unable to suppress *ade2-1*^{0} at 34°C, indicating that the *LOS1* gene was converted to a nonfunctional allele, designated *los1*::*URA3*.

To confirm that the transformants carried the disrupted *los1*::*URA3* allele, we performed Southern analyses with *Pst*I-digested genomic DNA from untransformed and transformed cells. DNA from the two parent strains 201-1-5 and DH15-1d and the undisrupted diploid 2 × 1d contained two

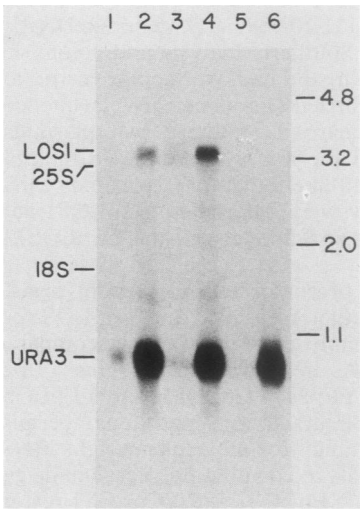

FIG. 3. Northern analysis of RNAs homologous to *LOS1*. Approximately 10 μg of total RNA (lanes 1, 3, and 5) or 5 to 10 μg of poly(A)-enriched RNA (lanes 2, 4, and 6) was loaded onto a 1% agarose-formaldehyde gel. Electrophoresis proceeded for 11 h at 100 V. RNA was transferred to membranes as described in Materials and Methods. After prehybridization, RNA was hybridized overnight at 42°C to the nick-translated (~10^7 cpm/μg) probe pBRLOS1::*URA3* (see Fig. 1). Lanes: 1 and 2, RNA from *los1-1* strain 201-1-5 containing YEpLOS1; 3 and 4, RNA from *LOS1* strain IIId-2d; 5 and 6, RNA from *los1-ΔV* strain IIId-2c. Markers on left are RNAs: 25S (3.3 kb), 18S (1.8 kb), and *URA3* (1.0 kb). Markers on right are DNA standards.

TABLE 1. Meiotic analysis of diploids carrying altered *LOS1* alleles

Diploid	Presumed genotype	No. of tetrads dissected	Four viable spores		Three viable spores			Two viable spores	
			No. of asci	No. segregating 2Ura$^+$:2Ura$^-$	No. of asci	No. segregating 2Ura$^+$:1Ura$^-$	No. segregating 1Ura$^+$:2Ura$^-$	No. of asci	No. segregating 1Ura$^+$:1Ura$^-$
IV2	*los1-1/los1::URA3*	7	6	6	1	1	0	0	0
IV4	*los1-1/los1::URA3*	14	9	9	5	2	3	0	0
22V3	*los1::URA3/LOS1*	6	1	1	4	1	3	1	1
IIId	*los1-ΔV/LOS1*	6	6	NA	0	NA	NA	0	NA

*Pst*I fragments of 8.3 and 0.9 kb which hybridized with the probe pBRLOS1 (Fig. 4). DNA from the uracil-prototrophic transformants 1V2, 1V4, and 22V3 had two new *Pst*I fragments of 6.5 and 3.0 kb which hybridized with pBRLOS1 (Fig. 4) as predicted, since the *URA3*-containing restriction fragment used to construct the disruption was 1.2 kb long and contained a *Pst*I site (Fig. 1).

Two of the three diploids examined, 1V2 and 1V4, were unable to suppress *ade2-1*^{0} at 34°C, indicating that the *LOS1* gene of the diploid had been disrupted, resulting in a diploid with the genotype *los1-1/los1::URA3*. The other diploid examined, 22V3, was able to suppress *ade2-1*^{0} at 34°C, indicating that the *los1-1* gene had been disrupted, resulting in a *los1::URA3/LOS1* genotype. These transformed diploids were sporulated and dissected. The viabilities of the meiotic progeny of these diploids are presented in Table 1. The majority of the progeny were viable, and the viability of uracil-prototrophic progeny was comparable to that of uracil-auxotrophic progeny. If disruption of *LOS1* were lethal, only two spores per tetrad would produce viable progeny and all the progeny would be uracil auxotrophs. The diploids 1V2 and 1V4, which were unable to suppress *ade2-1*^{0} at 34°C, only gave rise to progeny which were unable to suppress *ade2-1*^{0} at 34°C. The diploid 22V3 was able to suppress *ade2-1*^{0} at 34°C. All uracil-prototrophic progeny were incapable of *ade2-1*^{0} suppression at 34°C, and all uracil-auxotrophic progeny were capable of *ade2-1*^{0} suppression. These results indicate that insertion of the 1.2-kb fragment bearing the *URA3* gene into the *Hin*dIII site of the *LOS1* gene inactivated the *LOS1* gene but did not cause lethality.

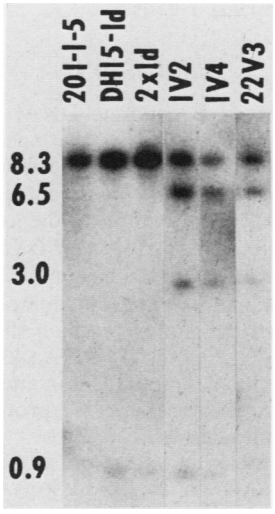

FIG. 4. Disruption of *LOS1* at its chromosomal locus by insertion of *URA3*. In the Southern analysis, *Pst*I-digested genomic DNA was probed with pBRLOS1. Strain 201-1-5 (relevant genotype, *los1-1 ura3-1*) was crossed with strain DH15-1d (relevant genotype, *LOS1 ura3-1*). The resulting diploid, 2 × 1d, was transformed with *Pvu*II-digested pBRLOS1::*URA3* to give rise to 1V2, 1V4, and 22V3.

Deletion of 2.4 kb from the *LOS1* gene at its chromosomal locus. For the deletion of the 2.4-kb *Pvu*II fragment, the 4.1-kb *Hin*dII-*Bgl*II fragment from YCpLOS1-ΔV (Fig. 1), which carried the deleted *LOS1* gene, was used for gene replacement. Gene replacement was accomplished as described by Rothstein (37), with the cotransformation modification described by Rudolph et al. (38). A recipient for the gene replacement, 2 × 8c, was constructed by mating 201-1-5 (*MATα los1-1 SUP4*^{0} *ade2-1*^{0} *can1-100*^{0} *lys1-1*^{0} *trp5-48*^{0} *ura3-1 met*$^-$) with DH15-8c (*MATa LOS1 SUP4*^{0} *ade2-1*^{0} *can1-100*^{0} *lys1-1 trp5-2*^{0} *ura3-1 leu1-12*) and selecting cells that grew on medium lacking methionine and leucine. The recipient was transformed with the fragment and YEp24. Transformation to uracil prototrophy with YEp24 identifies cells that have taken up DNA, including the fragment, during the transformation procedure; these cells are thus more likely to have undergone gene conversion by the deleted fragment (38). Uracil-prototrophic transformants were screened for the loss of *ade2-1*^{0} suppression at 34°C. Southern analysis confirmed that one such transformant had a 2.4-kb deletion at the *los1* locus (Fig. 5). This new allele was designated *los1-ΔV*.

The *los1-ΔV*-bearing transformant 2 × 8c-ΔV was sporulated and dissected. Several lines of data indicate that the transformant was a tetraploid. (i) Leucine and methionine prototrophy and mating type segregated as predicted for ++−−, ++−−, **aa**αα tetraploids (35). (ii) Southern anal-

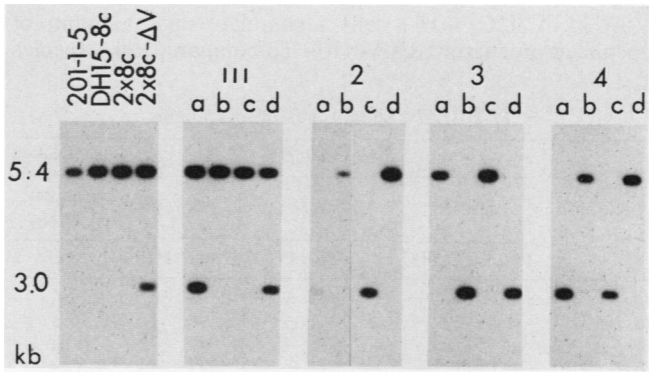

FIG. 5. Disruption of *LOS1* at its chromosomal locus by deletion of 2.4 kb. In the Southern analysis, *Bgl*II-digested genomic DNA was probed with pBRLOS1. Strain 201-1-5 (relevant genotype, *los1-1*) was crossed with strain DH15-8C (relevant genotype, *LOS1*). The cross resulted in tetraploid strain 2 × 8c (see text). 2 × 8c was transformed with the 4.1-kb *Hin*dIII-*Bgl*II fragment from YCpLOS1-ΔV to give rise to 2 × 8c-ΔV. 2 × 8c-ΔV was sporulated, giving rise to the diploid progeny IIIa to d. IIId was sporulated, giving rise to haploid progeny 2a to d, 3a to d, and 4a to d.

ysis indicated that some of the meiotic progeny contained two copies of the *los1* gene, i.e., a deleted and an intact copy (Fig. 5). (iii) Nonmating, presumptive *MAT***a**/*MAT*α progeny were able to sporulate. Genetic analysis of the recipient 2 × 8c indicated that it was also a tetraploid.

One of the progeny of transformed tetraploid IIId was a nonmater, was capable of suppression of *ade2-1*^{0} at 34°C and sporulation, and by Southern analysis had two copies of *LOS1*, i.e., a deleted copy and an intact copy. These data indicate that IIId was a *MAT***a**/*MAT*α *los1*-Δ*V*/*LOS1* diploid. IIId was sporulated, and six tetrads were dissected. All the progeny were viable, and all tetrads segregated 2:2 for adenine prototrophy at 34°C. Southern analysis of three tetrads (Fig. 5) revealed that the deleted *los1*-Δ*V* allele cosegregated with adenine auxotrophy at 34°C, indicating that removal of the 2.4-kb fragment from the *LOS1* gene caused a loss-of-suppression phenotype. These results show that removal of the 2.4-kb *Pvu*II fragment of the 2.8- to 3.4-kb region required for *LOS1* gene function was not lethal.

Northern analysis was performed on two of the haploid segregants of the diploid containing the disruption, i.e., on IIId-2d, containing a wild-type *LOS1* allele, and on IIId-2c, containing the *los1*-Δ*V* allele. RNA from the strain carrying the deletion allele did not show any species homologous to the probe (Fig. 3). The results confirm that the 2.9- to 3.3-kb species was the *LOS1*-encoded RNA.

Phenotypes of cells with disrupted *los1* alleles. Since the disruptions of the *los1* gene were not lethal, phenotypes of cells bearing the two new *los1* alleles, i.e., *los1*::*URA3* and *los1*-Δ*V*, could be compared with the cells bearing the previously identified alleles, i.e., *los1-1*, *los1-2*, and *los1-3*. At 34°C, *SUP4*^{0} *los1-1*, *los1-2*, and *los1-3* cells were unable to suppress *ade2-1*^{0} and *can1-100*^{0} but were able to suppress *lys1-1*^{0} *and trp5-48*^{0}. At 23°C, *los1-1 SUP4*^{0} cells were able to suppress all the ochre alleles (16), but *los1-2* and *los1-3* were unable to suppress *can1-100*. In both disruption experiments, the parent strains carried the ochre-suppressible markers *ade2-1*^{0}, *can1-100*^{0}, *lys1-1*^{0}, and either *trp5-48*^{0} or *trp5-2*^{0}. We tested the abilities of the strains bearing the two new *los1* alleles to suppress these ochre suppressible markers at 23, 30, and 34°C (Table 2). *los1*::*URA3* and *los1*-Δ*V* strains had phenotypes very similar to those of *los1-2* and *los1-3*.

At 34 to 38°C, *los1-1* cells accumulate small amounts of unspliced precursor tRNAs (16). To compare tRNA species synthesized in *LOS1*, *los1-1*, *los1*::*URA3*, *los*-Δ*V*, and the *rna1-1* strain, cells were labeled with [^{3}H]uracil for 1 h at 38°C. Total cellular RNA was extracted and analyzed on urea-polyacrylamide gels. As can be seen in Fig. 6, *los1*::*URA3* and *los1*-Δ*V* cells accumulated small RNA species which comigrated with the precursor tRNA species found in *los1-1* and *rna1-1* cells.

TABLE 2. Phenotypes of *los1* alleles

Strain	Suppression[a]									Accumulation of intron-containing pre-tRNA
	ade2-1^0			*can1-100*^{0}			*lys1-1*^{0} *trp5*^{0}			
	23°C	30°C	34°C	23°C	30°C	34°C	23°C	30°C	34°C	38°C
LOS1	+	+	+	+	+	+	+	+	+	No
los1-1	+	+/−	−	+	+	−	+	+	+	Yes
los1-2	+	NT	−	−	NT	−	+	NT	+	Yes
los1-3	+	NT	−	−	NT	−	+	NT	+	Yes
los1::*URA3*	+	+/−	−	−	−	−	+	+	+	Yes
los1-Δ*V*	+	+/−	−	−	−	−	+	+	+	Yes

[a] *ade2-1*^{0}, *lys1-1*^{0}, and *trp5*^{0} are ochre mutations in three biosynthetic pathways for adenine, lysine, and tryptophan, respectively. Failure to suppress these mutations renders cells incapable of growth on medium lacking those nutrients. Additionally, *ade2* cells accumulate a red pigment. *CAN1* encodes arginine permease; failure to suppress *can1-100* confers resistance to the toxic arginine analog canavanine. NT, Not tested; +, suppression of mutation; +/−, weak suppression; −, no suppression.

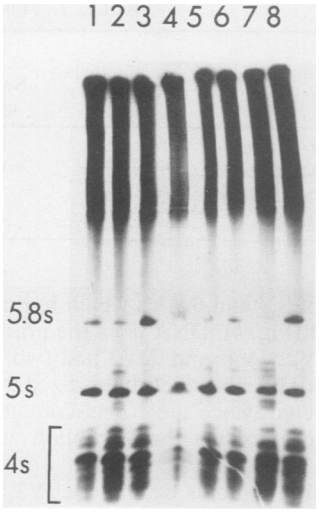

FIG. 6. Analysis of precursor tRNAs in *LOS1*, *los1-1*, *los1*::*URA3*, *los1*-Δ*V*, and *rna1-1* cells. Exponentially growing yeast cells were incubated for 90 min at 38°C and then radiolabeled with [^{3}H]uracil for 60 min at 38°C. Total cellular RNA was extracted. Approximately 10^5 cpm of each sample was electrophoresed through a 10% polyacrylamide gel with a 5% polyacrylamide stacking gel; both gels contained 7 M urea, 100 mM Tris borate (pH 8.3), and 2.5 mM EDTA. Unspliced precursor tRNAs migrated above 5S rRNA and between 5S rRNA and mature 4S tRNAs. Lanes: 1, *LOS1* cells (strain DH15-1d); *los1-1* cells (strain 201-1-5); 3 and 4, *los1*::*URA3* cells (strains 1V4-3a and 1V4-3c, respectively); 5 and 6, *los1*-Δ*V* cells (strains IIId-2a and IIId-3d, respectively); 7, *rna1-1* cells (strain 2bgp); 8, *los1-1* cells (strain 201-1-5) transformed with YEpLOS1.

DISCUSSION

From libraries of yeast genomic DNA, we isolated two clones, YEpLOS1 and YCpLOS1, that specifically complemented the *los1-1* partial loss-of-suppression phenotype and the *los1-1* precursor tRNA-splicing defect. Integration of the cloned DNA into the yeast genome at the *los1* locus showed that the cloned sequences carried the *LOS1* gene. Mutagenesis of YEpLOS1 showed that a region of 2.8 to 3.4 kb was necessary to complement *los1-1*. The complementation data agree with the Northern analysis, which showed that *LOS1* encoded a 2.9- to 3.3-kb poly(A)$^+$ RNA. Assuming that this RNA was translated in a simple fashion and that the protein product was not processed, the *LOS1* protein was approximately 116 to 130 kilodaltons.

A major reason for undertaking the cloning of *LOS1* was to explain the phenotype of *los1* mutations. The explanation that the three *los1* alleles previously identified (15, 16) only partially inactivated the *LOS1* product now seems unlikely. If the *LOS1* product were essential for tRNA splicing, then disruptions or deletions of the gene should have resulted in lethality. Yet neither an insertion of sequences into a region necessary for complementation nor a deletion of a large part of *LOS1* resulted in lethality. In fact, both of these alter-

ations resulted in a phenotype which is very similar or identical to the three ethyl methanesulfonate-induced presumptive point mutations (15, 16). It is possible that the insertion may not have totally inactivated the *LOS1* product. However, judging from the γδ mutagenesis and Northern analysis, the 2.4-kb deletion left at maximum ~750 bp to the left and ~250 bp to the right of the *LOS1* functional region; it seems unlikely that this deletion would not have inactivated the *LOS1* product.

A second explanation, that the *LOS1* gene is present in multiple copies in the genome, is also unlikely, since Southern analysis of genomic yeast DNA detected only fragments predicted by the restriction maps of the *LOS1* clones consistent with there being only one copy of the *LOS1* gene in the yeast genome. Thus, the role of *LOS1* in tRNA splicing cannot be explained by leaky mutations or duplicate genes.

Temperature-sensitive mutations are usually explained as mutations which produce temperature-sensitive gene products. However, if we assume that *los1::URA3* and *los1-ΔV* produce inactive or no gene products, then the temperature-sensitive phenotypes must be explained differently. Perhaps there are two or more pathways for tRNA splicing, i.e., one pathway, involving *LOS1*, which is normally not affected by high temperatures and another pathway(s) which does not involve *LOS1* and which is less active at high temperatures. Another possibility is that the *LOS1* gene product is not required for tRNA splicing but facilitates the process and this effect is more pronounced at higher temperatures. In the latter case, *LOS1* may be either directly or indirectly involved in precursor tRNA splicing. For instance, the *LOS1* gene product may participate in the tRNA-splicing reactions as a noncatalytic component, perhaps as a scaffolding protein that holds the precursor tRNAs in the correct conformation for splicing. Or the *LOS1* gene may encode a regulatory element. It is also conceivable that *los1* mutations affect some step in cell metabolism unrelated to RNA processing and that this secondarily results in slowed precursor tRNA splicing. For example, it has recently been suggested that a function of the heat shock proteins of *Drosophila melanogaster* is to protect mRNA splicing at elevated temperatures (45). Further elucidation of the role of *LOS1* in precursor tRNA splicing will require isolation and analysis of the *LOS1* gene product and addition of this product to in vitro splicing systems as well as the identification and characterization of other genes that affect precursor tRNA splicing.

ACKNOWLEDGMENTS

We thank N. Atkinson, W. Bajwah, C. Hill, J. Hopper, R. Keil, and T. Torchia for their critical reading of the manuscript. We are also grateful to G. Fabian and R. Dunst for helpful discussions and to R. Reed for supplying the restriction map and sequence of the γδ insertion element.

This research was supported by a Public Health Service grant to A.K.H. from the National Institutes of Health and a National Institutes of Health Postdoctoral Fellowship to D.J.H.

LITERATURE CITED

1. **Benson, S. A.** 1984. A rapid procedure for isolation of DNA fragments from agarose gels. Biotechniques **2**:66–68.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
3. **Brandriss, M. C., L. Soll, and D. Botstein.** 1975. Recessive lethal amber suppressors in yeast. Genetics **79**:551–560.
4. **Carlson, M., and D. Botstein.** 1982. Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell **28**:145–154.
5. **Etcheverry, T., D. Colby, and C. Guthrie.** 1979. A precursor to a minor species of $tRNA^{Ser}$ contains an intervening sequence. Cell **18**:11–26.
6. **Etcheverry, T., M. Salvatto, and C. Guthrie.** 1982. Recessive lethality of yeast strains carrying the *SUP61* suppressor results from loss of a tRNA with a unique decoding function. J. Mol. Biol. **158**:599–618.
7. **Goodman, H. M., M. V. Olson, and B. D. Hall.** 1977. Nucleotide sequences of a mutant eukaryotic gene; the yeast tyrosine inserting suppressor, *SUP4-0*. Proc. Natl. Acad. Sci. USA **74**:5453–5457.
8. **Greer, C. L., and J. Abelson.** 1984. RNA splicing: rearrangement of RNA sequences in the expression of split genes. Trends Biochem. Sci. **9**:139–141.
9. **Greer, C. L., C. L. Peebles, P. Gegenheimer, and J. Abelson.** 1983. Mechanism of action of a yeast RNA ligase in tRNA splicing. Cell **32**:537–546.
10. **Guthrie, C., and J. Abelson.** 1982. Organization and expression of tRNA genes in *Saccharomyces cerevisiae*, p. 487–528. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Guyer, M.** 1983. Uses of the transposon γδ in the analysis of cloned genes. Methods Enzymol. **101**:362–369.
12. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. USA **75**:1929–1933.
13. **Hopper, A. K.** 1984. Genetic and biochemical studies of RNA processing in yeast, p. 91–118. *In* D. Apirion (ed.), Processing of RNA. CRC Press, Inc., Boca Raton, Fla.
14. **Hopper, A. K., F. Banks, and V. Evangelidis.** 1978. A yeast mutant which accumulates precursor tRNAs. Cell **14**:211–219.
15. **Hopper, A. K., S. L. Nolan, J. Kurjan, and A. Hama-Furukawa.** 1981. Genetic and biochemical approaches to studying *in vivo* intermediates in tRNA biosynthesis, p. 302–319. *In* D. von Wettstein, J. Friis, M. Kielland-Brandt, and A. Stenderup (ed.), Molecular genetics in yeast. Alfred Benzon Symposium 16. Munksgaard International Publishers, Ltd., Copenhagen.
16. **Hopper, A. K., L. D. Schultz, and R. A. Shapiro.** 1980. Processing of intervening sequences: a new yeast mutant which fails to excise intervening sequences from precursor tRNAs. Cell **19**:741–751.
17. **Hutchison, H. T., L. H. Hartwell, and C. S. McLaughlin.** 1969. Temperature-sensitive yeast mutant defective in ribonucleic acid production. J. Bacteriol. **99**:807–814.
18. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
19. **Knapp, G., J. S. Beckmann, P. F. Johnson, S. A. Fuhrman, and J. Abelson.** 1978. Transcription and processing of intervening sequences in yeast tRNA genes. Cell **14**:221–236.
20. **Knapp, G., R. C. Ogden, C. L. Peebles, and J. Abelson.** 1979. Splicing of yeast tRNA precursors: structure of the reaction intermediates. Cell **18**:37–46.
21. **Kuo, C.-L., and J. L. Campbell.** 1983. Cloning of *Saccharomyces cerevisiae* DNA replication genes: isolation of the *CDC8* gene and two genes that compensate for the *cdc8-1* mutation. Mol. Cell. Biol. **3**:1730–1737.
22. **Laten, H., J. Gorman, and R. M. Bock.** 1978. Isopentenyladenosine deficient tRNA from an antisuppressor mutant of *Saccharomyces cerevisiae*. Nucleic Acids Res. **5**:4329–4342.
23. **Lathe, R., M. P. Kieny, S. Skory, and J. P. Lecocq.** 1984. Linker tailing: unphosphorylated linker oligonucleotides for joining DNA termini. DNA **3**:173–182.
24. **Lee, M.-C., and G. Knapp.** 1985. Transfer RNA splicing in *Saccharomyces cerevisiae*: secondary and tertiary structures of the substrates. J. Biol. Chem. **260**:3108–3115.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Mortimer, R. K., and D. Schild.** 1982. Genetic map of *Saccha-*

romyces cerevisiae, p. 639–650. *In* J. N. Strathern, E. W. Jones, and J. B. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

27. **Najarian, D., M. E. Dihanich, N. C. Martin, and A. K. Hopper.** 1987. DNA sequence and transcript mapping of *MOD5*: features of the 5′ region which suggest two translational starts. Mol. Cell. Biol. **7**:185–191.
28. **Nasmyth, K. A., and S. I. Reed.** 1979. Isolation of genes by complementation in yeast: molecular cloning of a cell-cycle gene. Proc. Natl. Acad. Sci. USA **77**:2119–2123.
29. **O'Farrell, P. Z., B. Cordell, P. Valenzuela, W. J. Rutter, and H. M. Goodman.** 1978. Structure and processing of yeast precursor tRNAs containing intervening sequences. Nature (London) **274**:438–445.
30. **Ogden, R. C., M.-C. Lee, and G. Knapp.** 1984. Transfer RNA splicing in *Saccharomyces cerevisiae*: defining the substrates. Nucleic Acids Res. **12**:9367–9382.
31. **Peebles, C. L., P. Gegenheimer, and J. Abelson.** 1983. Precise excision of intervening sequences from precursor tRNAs by a membrane associated yeast endonuclease. Cell **32**:525–536.
32. **Peebles, C. L., R. C. Ogden, G. Knapp, and J. Abelson.** 1979. Splicing of yeast tRNA precursors: a two-stage reaction. Cell **18**:27–35.
33. **Phizicky, E. M., R. C. Schwartz, and J. Abelson.** 1986. *Saccharomyces cerevisiae* tRNA ligase: purification of the protein and isolation of the structural gene. J. Biol. Chem. **261**:2978–2986.
34. **Post-Beittenmiller, M. A., R. W. Hamilton, and J. E. Hopper.** 1984. Regulation of basal and induced levels of the *MEL1* transcript in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:1238–1245.
35. **Roman, H., M. M. Phillips, and S. M. Sands.** 1955. Studies of polyploid *Saccharomyces*. I. Tetraploid segregation. Genetics **40**:546–551.
36. **Rose, M., and D. Botstein.** 1983. Structure and function of the yeast *URA3* gene. J. Mol. Biol. **170**:883–904.
37. **Rothstein, R.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
38. **Rudolph, H., I. Koenig-Rauseo, and A. Hinnen.** 1985. One-step gene replacement in yeast by transformation. Gene **36**:87–95.
39. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experimentation with gene fusions, p. 218. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
40. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
41. **St. John, T., and R. Davis.** 1981. The organization and transcription of the galactose cluster of *Saccharomyces*. J. Mol. Biol. **152**:285–315.
42. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76**:1035–1039.
43. **Thomas, P.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
44. **Ullrich, A., J. Shine, J. Chirgwin, R. Pictet, E. Tischer, W. Rutter, and H. Goodman.** 1977. Rat insulin genes: construction of plasmids containing the coding sequences. Science **196**:1313–1319.
45. **Yost, H. J., and S. Lindquist.** 1986. RNA splicing is interrupted by heat shock and is rescued by heat shock protein synthesis. Cell **45**:185–193.
46. **Zakian, V. A., and J. F. Scott.** 1982. Construction, replication, and chromatin structure of TRP1 RI circle, a multiple-copy synthetic plasmid derived from *Saccharomyces cerevisiae* chromosomal DNA. Mol. Cell. Biol. **2**:221–232.