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Molecular cloning and analysis of yeast gene for cycloheximide resistance and ribosomal protein L29

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ABSTRACT

A cosmid clone bank of yeast DNA has been used to isolate the cycloheximide resistance gene cyh2 of Saccharomyces cerevisiae. A cosmid carrying this gene was identified by cross hybridization to another cloned gene, tsm437. The two genes, which are tightly linked genetically are both present on a 31 kb segment of cloned DNA. The cyh2 gene encodes ribosomal protein L29, a component of the large subunit. Blot hybridization analysis reveals that this gene is present as a single copy in the yeast genome, unlike many other yeast ribosomal protein genes which appear to be duplicated. The cyh2 gene also appears to contain an intervening sequence, a characteristic common to most yeast ribosomal protein genes that have been cloned.

INTRODUCTION

Ribosome biosynthesis in both prokaryotes and eukaryotes is a highly regulated process in which each of 50-70 ribosomal proteins are synthesized at the same rate in growing cells. Furthermore, the rate of synthesis for each protein is adjusted coordinately in response to changes in growth conditions (reviewed in 1,2). This regulation ensures the correct molar synthesis of each protein in the absence of any significant pool of free ribosomal components. In Escherichia coli, a form of autogenous regulation of large operons appears to be primarily responsible for this regulation (3,4,5,6, see 7 for review). In eukaryotes the molecular mechanisms regulating ribosomal protein synthesis are unknown.

To investigate the regulation of ribosomal protein synthesis in yeast, a number of investigators have isolated genes for yeast ribosomal proteins (8,9,10). Analysis of these genes reveals that they are not clustered, many may be present in more than one copy per genome and most appear to contain an intervening sequence. For physiological studies of ribosomal protein biosynthesis it is useful to use a cloned gene which expresses an easily distinguishable phenotype and has a known genetic locus. One such gene we have used

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is tcm1, which encodes a mutant allele of ribosomal protein L3 and which confers resistance to the protein synthesis inhibitor trichodermin (10).

Another example of a ribosomal protein gene which expresses a distinct phenotype may be cyh2, in which mutations confer resistance to the inhibitor of protein synthesis, cycloheximide (11). A particular allele of cyh2, cyhx32, has been shown to produce an electrophoretically altered form of ribosomal protein L29 (12). Genetic analysis showed that this cycloheximide resistance mutation and the electrophoretic alteration were linked. Furthermore, diploid cells which produce both the wild type and the altered L29 protein showed an intermediate level of drug resistance, suggesting that such cells contained a mixture of wild type and resistant ribosomes. Stocklein and Piepersberg concluded that the cyhx32 mutation is located within the structural gene for ribosomal protein L29 (12).

In this report we describe the molecular cloning of the CYH2 locus of Saccharomyces cerevisiae. This gene was obtained by first isolating a nearby gene, TSM437. The cloned DNA spans 31 kilobases, yet encodes only one ribosomal protein, i.e., L29. A cloned cyh2 cycloheximide resistance gene produces the same semidominant phenotype as a chromosomal cyh2 gene, e.g., wild type cells carrying a plasmid with a resistant allele of cyh2 show an intermediate level of resistance to cycloheximide, suggesting that they contain a mixture of sensitive and resistant ribosomes. Since the cyhx32 mutation was found to be allelic to cyh2, our results confirm independently those of Stocklein and Piepersberg and we conclude that one type of resistance to cycloheximide in S. cerevisiae is due to mutations in the structural gene for ribosomal protein L29.

MATERIALS AND METHODS

Strains and Plasmids: S. cerevisiae strains tsm437 (α tsm437 ade1 ura1 his7 lys2 tyr1 gal1) and XS144-S22 (α met13 leu1 trp5 aro2 lys5 ade5 cyh2) were obtained from the Yeast Genetic Stock Center, Berkeley, California and strain DB703 (α ura3-52 his3-Δ1 trp1-289) was obtained from D. Botstein. These strains served as the source for the tsm437 temperature sensitive mutation, the cyh2 cycloheximide resistance mutation and the non-reverting his3 and ura3 mutations used in construction of strains for this study. Strain A10700B (α thr4) was from G. Fink and strain NP6B (α ura3-52 his3-Δ1 trp1-289 lys2 ade2) was from N. Pearson. Plasmids YEp6 and YIp5 were from D. Botstein. A clone bank of wild type yeast DNA in a pBR325-2μ shuttle vector was used to isolate the tsm437 gene. A clone bank in phage λ was prepared as described (9). A cosmid clone bank (13) consisting of partial EcoR1* restriction enzyme fragments was generously supplied by Drs. A. Hinnen and G. Fink.

Yeast Transformation: Yeast cells were transformed essentially as described by Hinnen et al. (14). Since the clone bank of DNA used to isolate the wild type allele of tsm437 was constructed in a plasmid lacking a selectable marker, temperature sensitive cells transformed with this DNA were placed immediately at 37° to select transformants able to grow at the non-permissive temperature. Plasmid pTSM437, which allowed this growth, was maintained in yeast cells by growth at 37°. Plasmids carrying either the URA3 or HIS3 genes were maintained in Ura$^-$ or His$^-$ hosts by growth in media lacking these nutritional requirements. To clone the cyh2 cycloheximide resistance allele of CYH2, the "integration-excision" method of Roeder and Fink was used (15). Strain HF168x7 (a ura3-52 his3-Δ1 tsm437 cyh2) was transformed with 50 μg of plasmid YIp5-S2, which carries a 12 kb SalI fragment containing CYH2 (see Results). The plasmid was linearized by digestion with XbaI prior to transformation (see Fig. 6). Approximately 7.5 μg of DNA from HF168x7 transformed with YIp5-S2 was digested with KpnI to completion. The digested DNA was diluted and ligated at a final concentration of 25 μg/ml in 70 mM Tris-HCl pH 7.5-7 mM $MgCl_2$-10 mM DTT and 1 mM ATP with 7.5 units/ml T4 ligase at 16° for 18 hours. This DNA was used to transform 4 mls of $CaCl_2$ treated E. coli C600 to ampicillin resistance. Nineteen E. coli transformants were obtained. Plasmid DNA prepared from several of these transformants was used to transform yeast strain NP6B to cycloheximide resistance to demonstrate recovery of cyh2.

Hybridizations: Cloned DNA, labeled with ^{32}P by nick translation, was hybridized to λ plaques by the procedure of Benton and Davis (16) and to bacterial colonies essentially as described by Grunstein and Hogness (17). Restriction enzyme digested DNA was blotted to nitrocellulose and hybridized according to Southern (9). To prevent hybridization of the endogenous, multi-copy 2 μ yeast plasmid to plasmid probes carrying 2 μ segments, genomic DNA was prepared from strain DCO4 (from J. Broach) which lacks 2 μ DNA (18).

RNA Blot Analysis: Yeast RNA was prepared form either wild type cells or from strain J23 as described (19). The latter is a strain carrying a temperature sensitive allele of rna2, a gene which is required for processing of ribosomal protein gene transcripts (28). Two to four micrograms of total RNA in 10 mM $NaPO_4$ (pH 7), 6% formaldehyde, 50% formamide were heated at 50° C for 10 minutes and electrophoresed in 1.5% agarose gels in 10 mM $NaPO_4$, 6% formaldehyde. The RNA was transferred to nitrocellulose and hybridized to nick translated probes as described by Thomas (20). The sizes of RNA molecules which hybridized to the probes were estimated by their mobility

relative to that of the fragments of ØX174RF DNA produced by cleavage with Hae III restriction enzyme and run in parallel with the RNA samples.

Hybridization-Translation Assays: Poly A^+ RNA was purified as described (19) and hybridized to cloned DNA in solution under R-loop conditions (8). Unhybridized RNA was separated from the DNA on a column of Bio Rad A150m. The hybridized RNA was recovered from the DNA by heating and was subsequently translated in vitro in a wheat germ extract. The products of translation were analyzed on two dimensional gels with carrier yeast ribosomal protein as described (21,22). RNA was also hybridized to purified restriction enzyme fragments immobilized on DBM paper discs exactly as described by Fried et al. and analyzed by in vitro translation (9).

Determination of Growth of Strains as a Function of Cycloheximide Concentration

Yeast cells were grown to about 2×10^7/ml in synthetic media lacking histidine. Approximately 200 cells were spread on plates lacking histidine and containing from 0 to 50 µg/ml cycloheximide. All plates were incubated for 4 days after which colonies were counted (regardless of size). As a control for viable cell number, an equivalent aliquot of cells was spread on rich media (without cycloheximide); the number of colonies which appeared on these non-selective plates was found to equal the number appearing on media lacking histidine (without cycloheximide).

RESULTS

Isolation of CYH2

The isolation of the yeast gene tcm1, which encodes ribosomal protein L3, was accomplished by transforming a trichodermin sensitive yeast strain with cloned DNA segments derived from a trichodermin resistant strain; subsequent selection for drug resistant transformants yielded the cloned tcm1 gene (10). Since the yeast strain from which our clone bank was derived also carried the cycloheximide resistance gene cyh2, we attempted a similar selection to isolate this gene. This selection however, failed to produce any cycloheximide resistant transformants.

The cyh2 mutation has been mapped very close to a temperature sensitive mutation, tsm437 (23). Thus, we decided to isolate the tsm437 locus in the hope that the cloned DNA which complemented tsm437 might also contain cyh2. Initially we used plasmids carrying wild type yeast DNA to transform a strain containing tsm437, and isolated several plasmids which permitted growth at the restrictive temperature. One such plasmid, pTSM437, carrying a 5 kb fragment of yeast DNA (Fig. 1), was hybridized to poly A^+

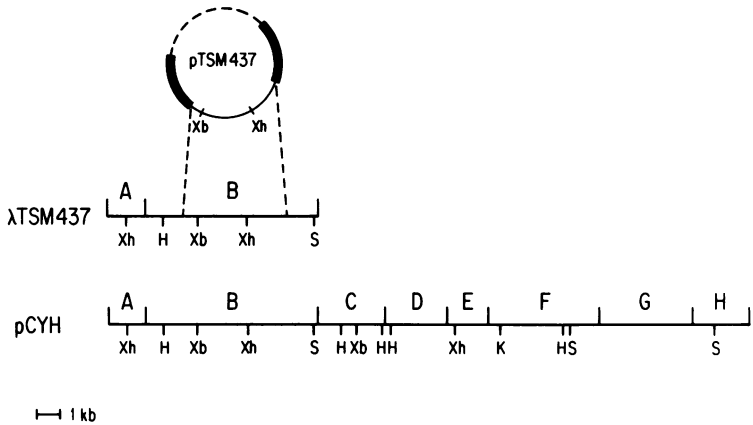

Figure 1. Summary of cloned DNA used in this study. pTSM437 carries the TSM437 gene in a pBR325 (solid boxes) -2μ DNA (dashed line) plasmid vector. λTSM437 carries two EcoR1 fragments of yeast DNA in a λgt vector (16). The vertical dashed lines show the region common to pTSM437. pCYH is a cosmid (13) obtained also by cross hybridization to pTSM437. Only the insert is shown which consists of EcoR1 fragments A through H. Restriction enzyme sites: S, Sall; X, Xhol; Xb, Xbal; K, Kpnl; H, Hind III. Except for Sall, not all sites are shown for the enzymes listed here.

RNA. The complementary RNA was translated in vitro but analysis of the products showed that this fragment did not contain a ribosomal protein gene. Hybridization of pTSM437 to a clone bank in phage λ produced a phage carrying a 10 kb yeast insert homologous to the 5 kb segment which, when analyzed by the same translation assay, still did not contain a ribosomal protein gene. Finally the 5 kb fragment was used to detect by hybridization a cosmid, pCYH (Fig. 1), from a bank provided by A. Hinnen (submitted). As described below pCYH contained a 31 kb insert containing the wild type alleles of both tsm437 and cyh2.

Analysis of pCYH

When introduced into HF168x7, a tsm437 cyh2 strain of yeast, the cosmid pCYH not only complemented the temperature sensitive mutation but also rendered cells less resistant to cycloheximide (Table 1).

The cosmid vector alone, pYC1, did neither. This reduced level of drug resistance is the same as that observed for heterozygous diploid cells which contain both a wild type and resistance allele of CYH2 (11). Such semidominance of CYH2 alleles results from cells containing a mixture of sensitive and resistant ribosomes. Figure 2 shows that when plasmid YEpCYH (12), a subclone of pCYH (see below), is introduced into HF168x7, the

Table 1. Phenotype of HF168x7 Transformed with Various Plasmids

Plasmid	23°	36°	1 μg/ml Cyclohex. 23°	10 μg/ml Cyclohex. 23°	1 μg/ml Cyclohex. 36°	10 μg/ml Cyclohex. 36°
None *	+	-	+	+	-	-
pYCI	+	-	+	+	-	-
pTSM437	+	+	+	+	+	+
pCYH	+	+	+	-	+	-
YEpCYH (12)	+	-	+	-	-	-

* Strain HF168x7 was tested on synthetic media containing all required nutrients. Strain HF168x7 transformed with the plasmids listed above was tested on the same media but lacking histidine.

transformants are inhibited by 50% at 3 μg/ml cycloheximide, a dose similar to the 3 μg/ml required to inhibit a heterozygous diploid to the same extent. This concentration is intermediate between the mean inhibitory concentration of the sensitive and resistant haploid strains (0.1 and 20 μg/ml, respectively). These results show that pCYH includes both the CYH2 locus as well as the TSM437 locus and that the cloned gene produces cycloheximide sensitive ribosomes when introduced into a resistant host.

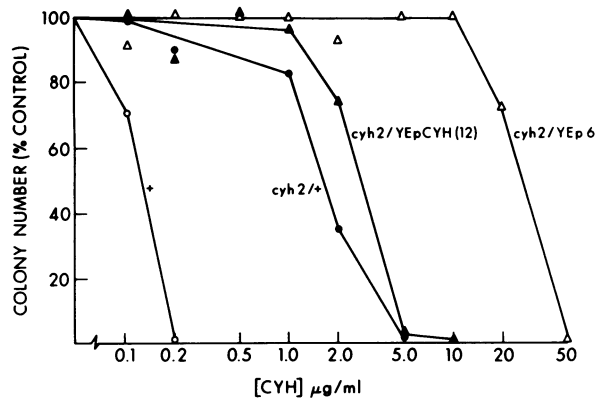

Figure 2. Effect of cycloheximide concentration on growth of various strains. + Refers to haploid strain A10700B, wild type at CYH2 locus. cyh2/YEp6 and cyh2/YepCYH (12) refers to haploid strain HF168x7, which carries the cyh2 chromosomal resistance mutation and either the vector YEp6 or YEp6 carrying the subcloned CYH2 gene. cyh2/+ is a diploid formed by mating of HF168x7 with A10700B. Equal aliquots of cells were plated on media lacking histidine and containing various amounts of cycloheximide. The control is the number of colonies which grew on rich media (lacking cycloheximide).

To determine the physical relationship between the two genes detected in pCYH, we constructed a partial restriction map of the yeast insert (Fig. 1). The insert consists of eight EcoR1 fragments totaling 31 kb. The two EcoR1 fragments obtained from our λ bank by cross hybridization to pTSM437 (A and B) are located at one end of the insert in pCYH. Fragment B carries the TSM437 gene. Since these two fragments do not contain CYH2, we subcloned into the vector YEp6 a 12 kb Sal1 fragment, one end of which begins 0.5 kb from the end of EcoR1 fragment B. Table 1 shows that when introduced into HF168x7, this cloned fragment, in plasmid YEpCYH (12), does not yield temperature resistant transformants but does cause reduced resistance to cycloheximide. Thus, the 12 kb Sal1 fragment contains the CYH2 gene but not the TSM437 gene.

pCYH2 Encodes Ribosomal Protein L29

Having identified a cosmid containing CYH2, we wished to determine if it indeed encodes a ribosomal protein. Yeast poly A^+ RNA was hybridized to pCYH (see Methods), eluted from the DNA and translated in vitro (8). The products of the translation were analyzed by two dimensional acrylamide gel electrophoresis in two solvent systems (2). Figures 3A and 3B show that pCYH carries a sequence which hybridizes to mRNA for a ribosomal protein identified as [44] in this gel system (21). In this hybridization, the DNA from pTCM, which encodes protein [1] (10) was added as a positive control. In this 2D gel system there were no detectable translation products from hybridization to the cosmid vector pYCI. In a second gel system (22), Figures 3C and 3D show that the protein encoded by pCYH comigrates with protein L29 (24). L29 and [44] are the same protein (2). Thus, the genomic DNA in pCYH encodes the gene for ribosomal protein L29, a component of the 60S subunit. Note that L29 is the only ribosomal protein encoded within the 31 kb of cloned DNA.

To locate the coding sequence for protein L29, we purified EcoR1 fragments C, D, E, and F, which are either partly or completely contained within the subcloned Sal1 fragment carrying CYH2. The fragments were immobilized on DBM paper discs and each was used to select by hybridization mRNA for subsequent in vitro translation. Only EcoR1 fragment E was capable of hybridizing to RNA encoding protein L29 (data not shown). This fragment alone, however, when subcloned and reintroduced into yeast, failed to provide a functional CYH2 gene. Apparently a small portion of the gene must extend into an adjacent restriction fragment (see below).

Genome Hybridization Analysis with pCYH

We have shown previously that many cloned DNA segments carrying yeast ribosomal

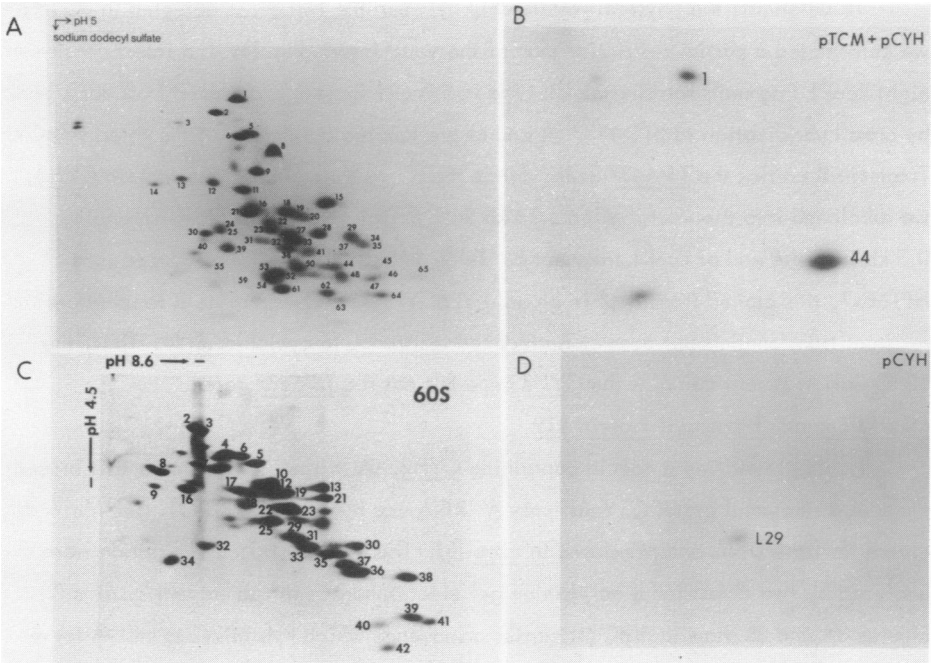

Figure 3. Two dimensional gel electrophoresis of yeast ribosomal proteins. (A) Coommassie Blue stained gel of total ribosomal protein separated as described (2). (B) Autoradiogram of in vitro translation products of RNA hybridized to pCYH and pTCM, separated as in (A). The other spots seen in the autoradiogram are products of endogenous wheat germ RNA as they are seen in controls without added RNA. (C) Coommassie Blue stained gel of 60S ribosomal proteins separated as described (24). (D) Autoradiogram of in vitro translation product of RNA hybridized to pCYH, separated as in (C).

protein genes are homologous to other DNA segments in the yeast genome (9), suggesting that many of the genes are duplicated in either a functional or nonfunctional form. To determine if the DNA sequence corresponding to the gene for L29 is present more than once in the genome, an electrophoretically fractionated EcoR1 digest of yeast DNA was probed with nick translated pCYH (Fig. 4).

Plasmid pCYH revealed eight complementary EcoR1 fragments in the yeast genome. The uppermost 10 kb fragment contains HIS3 (25) (identified by hybridization of the same DNA to pYCI), which comigrates fortuitously with the cosmid vector. The remaining seven fragments are identical in size to those contained in the 31 kb insert of pCYH (fragments A and E comigrate in these gels). No additional complementary segments are found. Since the 2 kb EcoR1 fragment E comigrates with another EcoR1 fragment in these

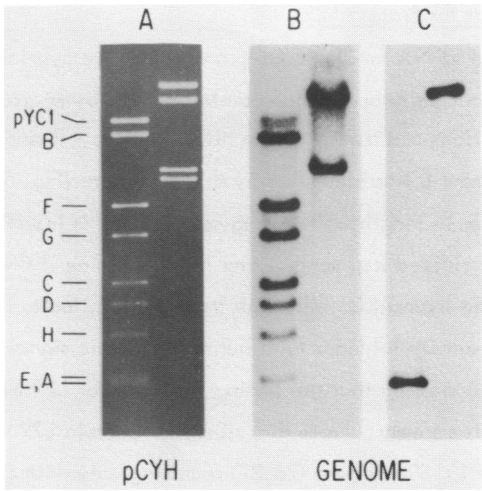

Figure 4. Genome hybridization analyses with pCYH. (A) Ethidium bromide stained gel of plasmid pCYH digested with restriction enzyme EcoR1 (left) and Sal1 (right). The eight EcoR1 fragments which constitute the 31 kb of cloned DNA are indicated (refer to Fig. 1). (B) Autoradiogram of yeast DNA, digested with either EcoR1 or Sal1 and hybridized to ^{32}P labeled pCYH. (C) Same as (B) but hybridized to ^{32}P labeled EcoR1 fragment E.

gels, we hybridized the same restriction digest of yeast DNA to the purified fragment E. Again, only one fragment of equivalent size was observed complementary to the EcoR1 fragment carrying the gene for protein L29. Hybridization of pCYH or fragment E to yeast DNA digested with other restriction enzymes also fails to reveal additional complementary segments (see Fig. 4). Thus, not only does the gene for protein L29 appear to be unique in the yeast genome, but a total of about 30 kb of DNA surrounding this gene is also unique.

Since the construction of the cosmid clone bank could have joined normally non-adjacent EcoR1 segments, we checked to see that the arrangement of fragments in pCYH is the same as that in the genome. Yeast DNA was digested with restriction enzymes whose sites in the cloned DNA span several EcoR1 fragments and the DNA was hybridized to pCYH. Figure 4 shows that when the enzyme Sal1 is used, two genomic fragments are found, identical in size to the two internal Sal1 fragments of the insert in pCYH. [In other blots it was sometimes possible to see two additional very large Sal1 fragments, which represent the segments originating from within EcoR1 fragments B and H and ending in the genome beyond that cloned in pCYH.] From such analyses, we conclude that the linkage of EcoR1 fragments in pCYH is the same as that in the genome.

Characteristics of mRNA for Protein L29

To characterize the mRNA for ribosomal protein L29, we hybridized EcoR1 fragment E, labeled with ^{32}P, to total cellular RNA, separated by size by agarose gel electrophoresis under denaturing conditions and transferred to nitrocellulose. A single RNA species complementary to fragment E was identified by this procedure (Fig. 5A) and estimated to be about 550 nucleotides in length. Since fragment E alone is insufficient to express the CYH2 gene, we also hybridized fragments D and F to determine if the coding sequence for L29 might extend beyond segment E. Although fragment F failed to hybridize to the 550 nucleotide RNA, fragment D yielded a faint autoradiographic signal coincident with this RNA (data not shown) suggesting that the coding sequence for L29 might indeed extend a short distance into this segment. Note that ribosomal protein L29 has been estimated to be between 17,500 and 19,500 daltons (26,27) requiring only 440-490 nucleotides of coding sequence; thus, our estimate of 550 nucleotides for the L29 mRNA is consistent with the size of this protein.

Previous investigations in this laboratory have described a set of temperature sensitive

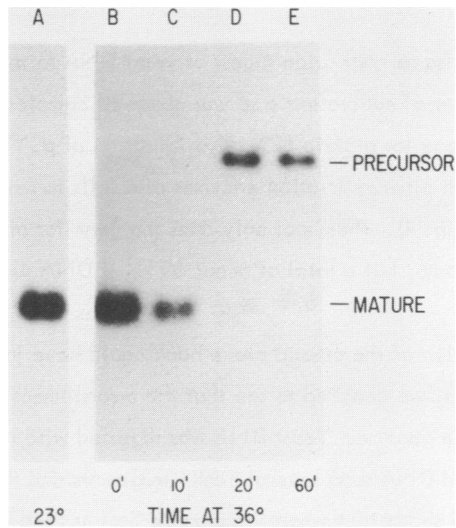

Figure 5. Blot hybridization analysis of ribosomal protein L29 mRNA. (A) Hybridization of electrophoretically separated RNA from wild type yeast to ^{32}P labeled EcoR1 fragment E. (B) through (E) the same probe hybridized to RNA obtained from strain J23, which carries the rna2 temperature sensitive mutation (see results). The RNA was obtained from cells at the permissive temperature (0') and after 10, 20 and 60 minutes following a shift to the non-permissive temperature of 36°.

mutations, known as rna mutations, which cause cells maintained at the restrictive temperature to become deficient in the mRNA for most ribosomal proteins (19). This defect is due apparently to the inability of the cells to remove an intervening sequence in the nuclear transcript of ribosomal protein genes (28). To determine if the gene for ribosomal protein L29 might contain an intron, we hybridized the 2 kb EcoR1 fragment E to RNA obtained from one of these ts strains (J23) which carries the rna2 mutation. Within 10 min after temperature shift there is a sharp reduction in the mature mRNA for L29 (Fig. 5). By 20 min virtually no mature mRNA is observed but a larger RNA of about 1050 nucleotides is seen, which persists at 60 min. This pattern is the same as that observed for other ribosomal protein mRNAs (28,9, Kim and Warner, submitted) and thus the gene for protein L29 probably contains an intervening sequence.

Isolation of Resistance Allele of CYH2

Since it is more convenient to use the resistance phenotype of CYH2 instead of its wild type counterpart to demonstrate expression of the gene, we cloned the resistance allele of CYH2 by the "integration-excision" method of Roeder and Fink (15). This method allows one to isolate alleles of cloned genes without the necessity of preparing new clone banks.

The 12 kb Sal1 fragment containing CYH2 was subcloned into plasmid YIp5 which carries the yeast URA3 gene. The resulting plasmid, YIp5-S2, was cut at a unique site with Xba1 and used to transform a ura3-52 cyh2 strain by homologous recombination (Fig. 6). Orr-Weaver et al. have shown that the ends of such linear molecules enhance recombination (29); integration of a plasmid into the genome in yeast almost always occurs at the site of the cut. As expected Ura^+ transformants had an intermediate level of cycloheximide resistance similar to a heterozygous diploid (Fig. 2). That the plasmid had integrated at the cyh2 locus was verified by mating the transformed strain to a strain carrying the ura3-52 mutation and the tsm437 mutation. Meiotic progeny from the resulting diploid showed co-segregation of the Ura^+ and intermediate resistance phenotypes; furthermore neither marker recombined with tsm437, demonstrating that the transforming plasmid was linked to this locus and that the cloned DNA is indeed derived from this region. Finally, the arrangement of DNA in the transformant (Fig. 6) was confirmed by Southern blot analysis (data not shown).

The resistance allele of CYH2 was recovered by digesting the chromosomal DNA of the transformed strain with Kpn1, religating the DNA at a dilute concentration and trans-

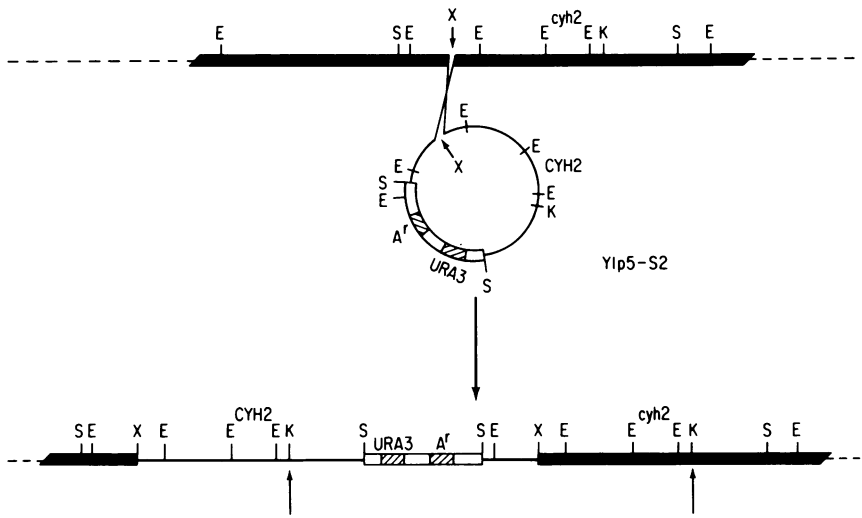

Figure 6. Integration-excision isolation of cyh2 resistance allele. A Ura$^-$ yeast strain carrying a chromosomal cyh2 gene (top of figure) was transformed with plasmid YIp5-S2, which contains the yeast URA3 gene, the bacterial β-lactamase gene (A^r) and a 12 kb segment containing CYH2. The plasmid was linearized with the restriction enzyme XbaI prior to transformation to stimulate its recombination with the genome (29). The bottom of the figure shows the resulting arrangement of chromosomal DNA in one Ura$^+$ transformant. This DNA was digested with KpnI and the fragments were joined by intramolecular ligation. The joining of one such fragment (see arrows at bottom) produces a plasmid which is recovered by transforming E. coli to ampicillin resistance and which now carries the cyh2 cycloheximide resistance gene.

forming E. coli to ampicillin resistance with the ligated DNA. When the plasmid was recovered from E. coli and used to transform a ura3-52 CYH2 strain of yeast, the Ura$^+$ transformants were found to have become partially resistant to cycloheximide, demonstrating successful recovery of the resistance allele of CYH2.

DISCUSSION

This report details the molecular cloning of the region including TSM437 and CYH2 from S. cerevisiae. These two genes, which map to chromosome VII, were found by genetic techniques to be very closely linked (23), and we have demonstrated their linkage physically by isolation of 31 kb DNA segment containing both genes. Restriction enzyme analysis of the yeast genome demonstrated that the 31 kb segment constitutes a unique sequence and that the arrangement of segments in the cloned DNA is the same as that in the genome. The presence of the two genes in this cloned DNA was shown by its ability to complement

the tsm437 temperature sensitive defect and simultaneously to reduce the level of resistance of cells carrying a chromosomal cyh2 mutation to the level characteristic of bonafide diploids heterozygous for cyh2. Genetic mapping of a transformant in which the cloned DNA was integrated into the genome by homologous recombination confirmed that the 31 kb segment is derived from this locus.

The physical distance separating tsm437 and cyh2 was found to be somewhat greater than expected. Mortimer and Hawthorne found no recombination between the two genes in 25 meiotic asci (23). Using different strains carrying the same two mutations, we found 2/67 tetratype asci (data not shown), giving about 1.5 cM separating the genes. The minimum length of the yeast genetic map has been placed at 4600 cM (30) and its physical size at 1.4×10^4 kb (31), yielding a ratio of 3 kb/cM, a value close to the 2.7 kb/cM determined by Strathern et al. from analysis of chromosome III (32). Thus, one would expect tsm437 and cyh2 to be about 4.5 kb apart. However, the minimum distance we find between the two genes is 8 kb, giving a minimum ratio of 5.3 kb/cM for this region. A rigorous physical and genetic analysis of the CYC1-sup4 interval of chromosome X by Shalit et al. yielded 5.6 kb/cM (33); so our observation is not unique. It remains to be determined whether the reduced recombination frequency of the TSM437-CYH2 region has some physiological significance. Some preliminary data (not shown) suggests that at least two additional genes lie between TSM437 and CYH2 since RNA blot hybridizations using EcoR1 fragment D reveal two other transcripts.

We wished originally to isolate cyh2 in order to obtain a gene which was likely to encode a ribosomal protein which expresses a unique phenotype. Mutations at many loci in S. cerevisiae lead to cycloheximide resistance (30). In vitro assays using purified ribosomal components have shown that many resistance mutations, in S. cerevisiae as well as in other fungi, are associated with the 60S ribosomal subunit (34). In addition, the 60S ribosomal subunit of S. fragilis is responsible for the naturally occurring cycloheximide resistance of this organism (35). Nevertheless, the inability to detect mutationally altered ribosomal components has hampered identification of the sites of such mutations. Since the cyh2 gene was the only genetically mapped cycloheximide resistance mutation exhibiting semidominance in vivo and in vitro (C. McLaughlin, personal communication), we selected this gene as the object of our cloning effort. During this work, Stocklein and Piepersberg demonstrated convincingly that a particular cyh2 resistance allele co-segregated with an alteration in electrophoretic mobility of protein L29 (12), an alteration

more recently defined as an amino acid replacement in this protein (36). Our results showing that the cloned DNA encodes CYH2 and protein L29 corroborate the conclusion that CYH2 is the structural gene for ribosomal protein L29. These data, as well as our previous identification of ribosomal protein L3 as the product of the TCM1 gene (10), make it clear that resistance to protein synthesis inhibitors in yeast can be due to mutations in specific ribosomal proteins. In light of the finding that the yeast genome may contain multiple copies of many yeast ribosomal protein genes (8,9), it is reassuring that, of the three genetically mapped antibiotic resistance loci expected to encode ribosomal proteins (11), we find that TCM1 and CYH2 are present in only a single copy.

The mechanisms which coordinate the synthesis of the many ribosomal proteins are unknown for eukaryotes. The coordinate expression of over 50 E. coli ribosomal proteins is due in part to their organization into operons (1). In yeast, coordinate expression of ribosomal protein genes cannot be due to their organization into operons. We have described previously 15 cloned DNA segments totalling 120 kb and containing 15 different yeast ribosomal protein genes (9). All but two were found not closely linked to any other ribosomal protein gene. Woolford and Rosbash used clones for 4 ribosomal proteins to analyze an additional 71 kb of DNA adjacent to the 4 genes: no additional ribosomal protein genes were found in the additional DNA (37). The identification of CYH2 in the center of 31 kb of DNA, in which it is the only ribosomal protein gene, adds to the demonstration that yeast ribosomal protein genes are not tightly clustered as they are in bacteria.

In a series of temperature sensitive (rna) mutants defective in the synthesis of mature ribosomal protein mRNAs at the restrictive temperature, a larger transcript from many ribosomal protein genes accumulates (28,9); one of these larger species contains an intervening sequence whose removal is blocked at the restrictive temperature (28). As shown in Figure 5, a large transcript of the CYH2 gene accumulates in one such rna mutant, suggesting that it also contains an intron. The fact that this gene is expressed when carried on a plasmid in wild type cells implies that its transcript is processed properly. Because of its easily identifiable phenotype, this gene may prove useful in studying mRNA processing in yeast.

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