JOURNAL OF BACTERIOLOGY, Jan. 1992, p. 254–262
0021-9193/92/010254-09$02.00/0

Vol. 174, No. 1

Drastic Alteration of Cycloheximide Sensitivity by Substitution of One Amino Acid in the L41 Ribosomal Protein of Yeasts

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Received 23 August 1991/Accepted 29 October 1991

Cycloheximide is one of the antibiotics that inhibit protein synthesis in most eukaryotic cells. We have found that a yeast, *Candida maltosa*, is resistant to the drug because it possesses a cycloheximide-resistant ribosome, and we have isolated the gene responsible for this. In this study, we sequenced this gene and found that the gene encodes a protein homologous to the L41 ribosomal protein of *Saccharomyces cerevisiae*, whose amino acid sequence has already been reported. Two genes for L41 protein, named L41a and L41b, independently present in the genome of *S. cerevisiae*, were isolated. L41-related genes were also isolated from a few other yeast species. Each of these genes has an intron at the same site of the open reading frame. Comparison of their deduced amino acid sequences and their ability to confer cycloheximide resistance to *S. cerevisiae*, when introduced in a high-copy-number plasmid, suggested that the 56th amino acid residue of the L41 protein determines the sensitivity of the ribosome to cycloheximide; the amino acid is glutamine in the resistant ribosome, whereas that in the sensitive ribosome is proline. This was confirmed by constructing a cycloheximide-resistant strain of *S. cerevisiae* having a disrupted L41a gene and an L41b gene with a substitution of the glutamine codon for the proline codon.

Cycloheximide (CYH) is an antibiotic that inhibits the peptidyl elongation reaction on the ribosome by binding specifically to the 60S large subunit, and it has been used widely for studies involving inhibition of eukaryotic protein synthesis. For example, wild-type strains of *Saccharomyces cerevisiae* are sensitive to CYH at concentrations lower than 0.5 μg/ml. However, some eukaryotic microorganisms are known to be resistant to this drug at rather high concentrations (100 μg/ml or more), and this property is used as a marker for the classification of some yeasts. Although a mutant strain (*cyh2*) of *S. cerevisiae* which is resistant to this drug at lower concentrations (less than 10 μg/ml) has been isolated and analyzed (2, 13), the molecular mechanism responsible for resistance to CYH at higher concentrations in some eukaryotic microorganisms is still not known. If this could be clarified, it might provide a clue for understanding not only the mechanism of inhibition of protein synthesis by CYH but also the structural and functional differences between CYH-sensitive and CYH-resistant ribosomes in eukaryotes.

Candida maltosa, which we have been studying because of its ability to regulate gene expression in response to a highly hydrophobic carbon source, *n*-alkane, in medium (10), is one of the microorganisms that are resistant to higher concentrations of CYH (16). We previously reported the isolation of a gene (named *RIM-C*) responsible for this resistance by using a CYH-sensitive wild-type strain of *S. cerevisiae* as a host and a YEp-type plasmid as a vector (15). In the present communication, we report that the *RIM-C* gene encodes a ribosomal protein (L41) of the large subunit and suggest that one amino acid residue in this protein determines CYH sensitivity in eukaryotic ribosomes.

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MATERIALS AND METHODS

Strains, media, and plasmids. The yeast strains used were *S. cerevisiae* AH22 (*MATa leu2 his4 can1*), SHY3 (*MATa steVC9 ura3 trp1 leu2 his3 ade1 can1*), and YNN27 (*MATα trp1 ura3 gal2*); *Candida tropicalis* N7Y1; and *Kluyveromyces fragilis* Y610. YPD medium (1% yeast extract, 2% Bacto Peptone, 2% dextrose) and SD medium (0.67% yeast nitrogen base without amino acids, 2% dextrose, and appropriate supplements) were used.

Bacterial strains used were *Escherichia coli* JA221 (*recA1 leuB6 trpE5 hsdR hsdM⁺ lacI thr thi*), MC1061 [*his hsm⁺ araD139* Δ(*ara-leu*)*7697 lacX74 strA galU galK*], MV1190 [Δ(*srl-recA*) *306*::Tn*10* Δ(*lac-pro*) *thi supE*(F′ *proAB lacI^q lacZ*Δ*M15 traD36*)], and CJ236 (*dut-1 ung-1 thi relA1*/ pCJ105), which were grown in Luria-Bertani broth.

The plasmids and helper phage used were pUC19, pUC119, YRp7, YEp13, pAAH5, and M13 KO7. YRpUC 19N, which was used for constructing the genomic library of *K. fragilis* and for subcloning DNA fragments of *C. tropicalis*, was constructed by ligating the mung bean nuclease-treated *Eco*RI fragment of YRp7 containing *ARS1* and *TRP1* with pUC19 previously linearized with *Aat*II and treated with T4 DNA polymerase.

DNA isolation from yeasts and enzymes. Total DNA from yeast cells for construction of genomic libraries was isolated by the method described by Struhl et al. (14). Restriction enzymes, T4 DNA ligase and other enzymes were purchased from Takara Shuzo Co. Ltd., Kyoto, Japan.

Construction of genomic libraries and hybridization conditions. The genomic libraries of *S. cerevisiae* and *C. tropicalis* were constructed in *Bam*HI-digested pUC19 by using about 5- to 10-kb DNA fragments obtained by partial digestion of the total genomic DNA with *Sau*3AI. The genomic library of *K. fragilis* was constructed in the same way, but *Bam*HI-digested YRpUC19N was used as a vector instead of pUC19.

The 1.14-kb DNA fragment containing the *RIM-C* gene (15) labelled with ^{32}P by the random-primer method was used to probe the genomic libraries by colony and Southern

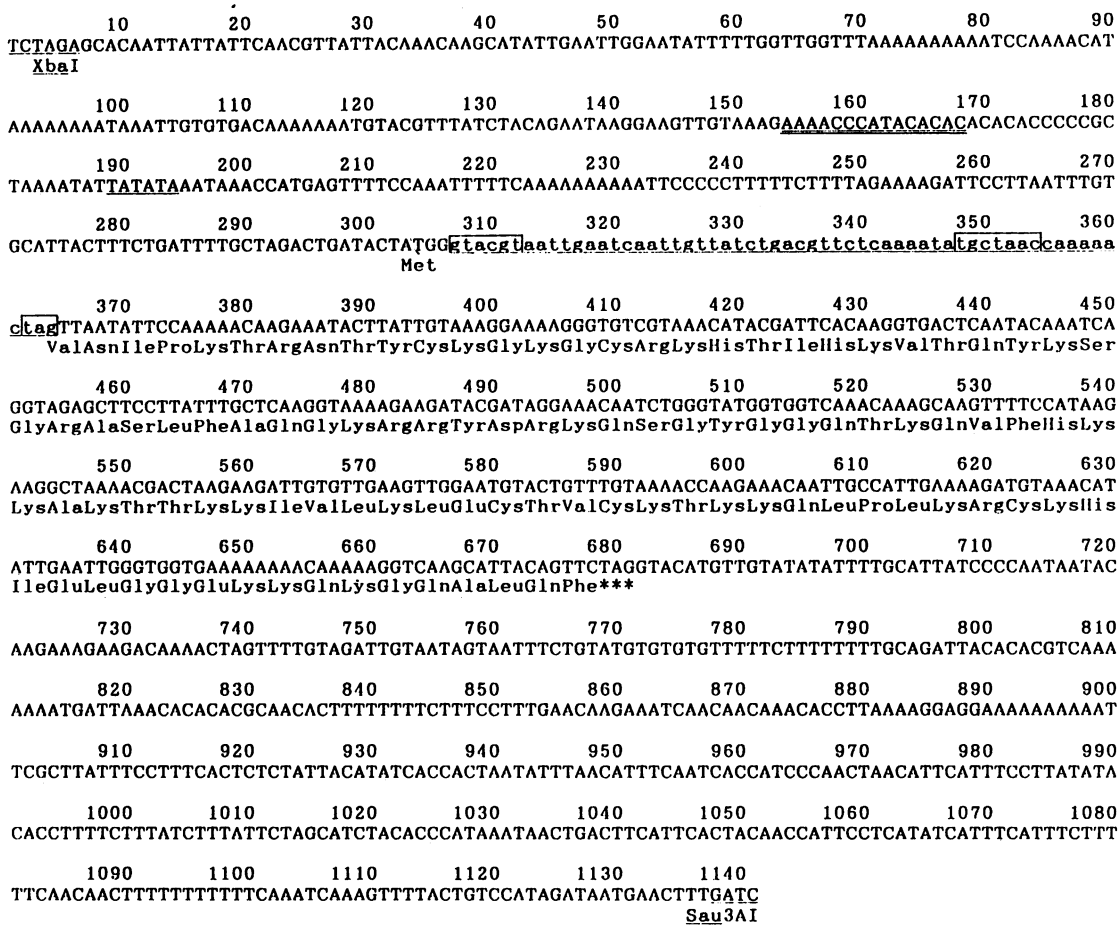

FIG. 1. Nucleotide sequence of the *RIM-C* gene from *C. maltosa* and its deduced amino acid sequence. The genomic nucleotide sequence, beginning at the *Xba*I end of the 1.14-kb *Xba*I-*Sau*3AI fragment (15), is numbered in the 5′ to 3′ direction. The ATG codon, located 303 bp downstream from the *Xba*I end, opens an ORF with an intron (nucleotides 307 to 364). Small underlined letters indicate the sequence of the intron. Open boxes indicate the sequences homologous to the yeast intron consensus sequences. The deduced amino acid sequence is shown below the nucleotide sequence. Double underlining denotes sequence of *S. cerevisiae* similar to an upstream activating sequence of ribosomal protein genes, and single underlining indicates a TATA box.

blot hybridization. For colony hybridization, duplicated filters (Hybond N; Amersham) were hybridized at 56°C in solution containing ^{32}P-labelled *RIM-C* DNA, 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), and 200 μg of denatured and sheared salmon sperm DNA (Sigma) per liter. The membranes were washed at 56°C for 0.5 h with 2× SSC and then at 56°C for 1 h with 2× SSC in the presence of 0.1% SDS. Southern blot hybridization was performed under the same conditions.

Nucleotide sequencing and site-directed mutagenesis. Nucleotide sequences were determined for both DNA strands by the dideoxynucleotide chain termination method (12). Site-directed mutation (6) was introduced into the L41b ribosomal protein gene of *S. cerevisiae* to substitute the glutamine codon for the proline codon at the 56th amino acid residue, by using oligonucleotide GTCAAACCAAGCAA GTTTTCCACAAG (mutation underlined).

Yeast transformation. Yeast transformation was performed by the lithium acetate procedure (4).

Disruption of the L41a gene and replacement of the mutated L41b gene. The *URA3* gene was inserted into the L41a gene in a *Bgl*II-*Sau*3AI fragment in the middle of the open reading frame (ORF). This fragment was used for transformation of *S. cerevisiae* YNN27 (*ura3 trp1*), and a URA$^+$ transformant was selected. Next, the *TRP1* gene was inserted downstream from the L41b gene (either wild-type L41b^P or L41b^Q constructed by site-directed mutagenesis) in an *Xba*I fragment, and the resulting fragment was used for transformation of the URA$^+$ strain described above. Then, a URA$^+$ TRP$^+$ transformant was selected.

Measurement of CYH resistance and growth curve. *S. cerevisiae* was inoculated into SD medium in the presence or absence of 100 μg of CYH per ml at a density of 3×10^7 cells per tube. Growth was measured by automatic recording of the A_{660} of the culture.

Nucleotide sequence accession numbers. The sequences shown in Fig. 1 and 2a to d have been assigned GenBank numbers D90488, D90489, D90490, D90492, and D90491, respectively.

RESULTS

***RIM-C* encodes a yeast ribosomal protein (L41).** The nucleotide sequence of *RIM-C* subcloned in a 1.14-kb DNA fragment (15) was determined (Fig. 1). There was a putative

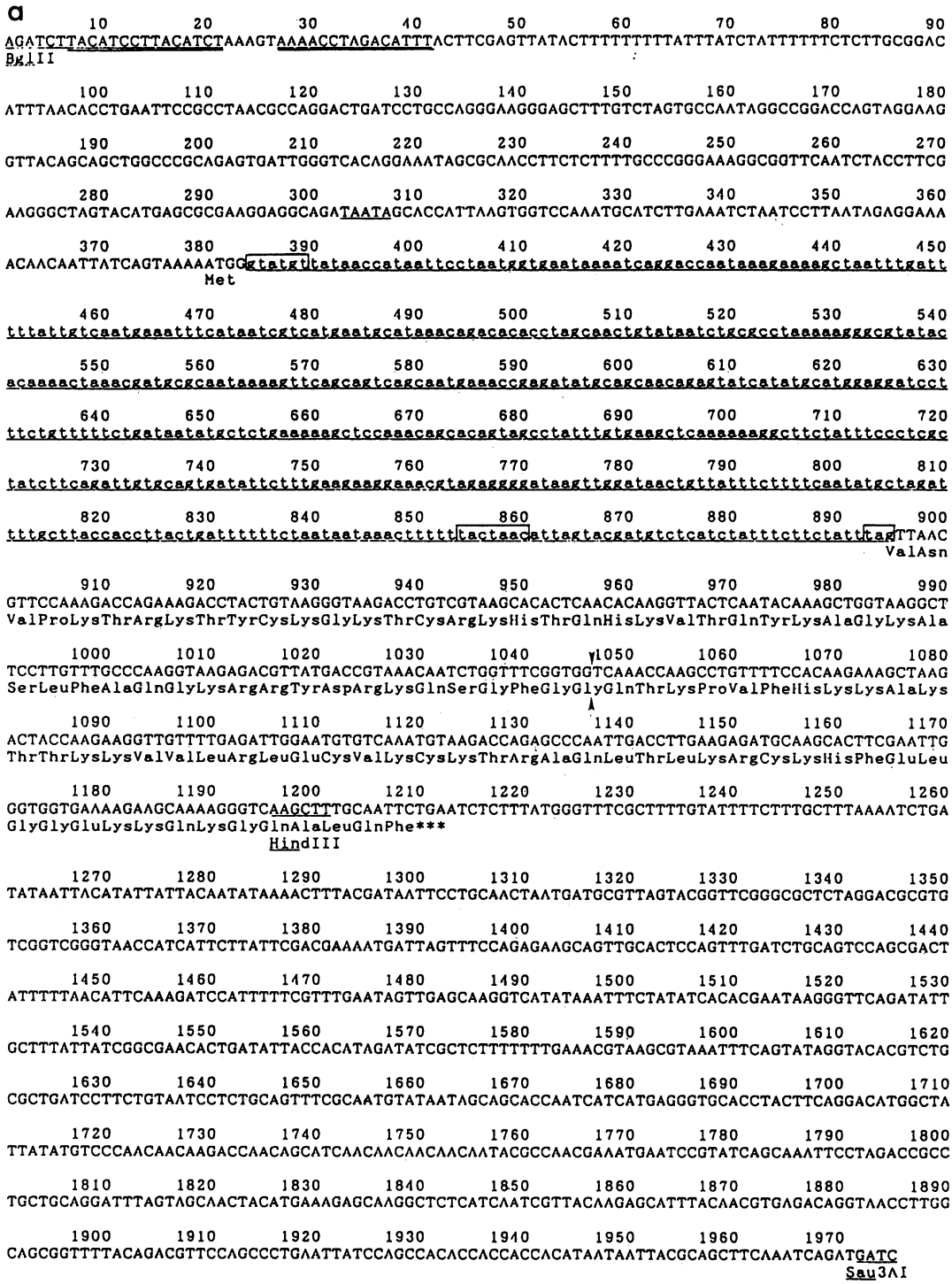

FIG. 2. Nucleotide sequences of four L41-related protein genes from three yeast species and their deduced amino acid sequences. The genomic nucleotides are numbered in the 5′ to 3′ direction. Symbols are same as for Fig. 1. (a) L41a protein gene from *S. cerevisiae*. The genomic sequence begins at the *Bgl*II end of the 2.0-kb *Bgl*II-*Sau*3AI restriction fragment. The ATG codon, located 380 bp downstream from the *Bgl*II end, opens an ORF with an intron (nucleotides 384 to 895). Arrowheads indicate the location of the *URA3* gene shown in Fig. 4. (b) L41b protein gene from *S. cerevisiae*. The genomic sequence begins at the *Xba*I end of the 1.8-kb *Xba*I restriction fragment. The ATG codon, located 229 bp downstream from the 5′ *Xba*I end, opens an ORF with an intron (nucleotides 233 to 673). (c) Gene for the L41-like protein of *C. tropicalis*. The genomic sequence begins at the *Hin*dIII end of the 1.6-kb *Hin*dIII-*Bcl*I fragment. The ATG codon, located 778 bp downstream from the *Hin*dIII end, opens an ORF with an intron (nucleotides 783 to 840). (d) Gene for the L41-like protein of *K. fragilis*. The genomic sequence begins at the 5′ *Eco*RI end of the 1.8-kb *Eco*RI fragment. The ATG codon, located 1,002 bp downstream from the 5′ *Eco*RI end, opens an ORF with an intron (nucleotides 1006 to 1433).

b

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        10         20         30         40         50         60         70         80         90
TCTAGAGAGACGTCGTCGGTGCGGCAACGCTGACGGTTTAGTTGTTCGACGGGATGATGGGTTCCGCCAGGGGGAGGGAAGGCTTTCCAC
 XbaI

       100        110        120        130        140        150        160        170        180
CAAGAGAGGTAAAATTATTCGTCGAAATGAACTCAGAGATGCGTCCATATTGTTGACAATGTATATCTTAATTGATGTGGTATTTTCACT

       190        200        210        220        230        240        250        260        270
GTTTTAACGTAAATTGAAGGAGATTAAGCAAAAAAACAATCAGTAATAATGGgtatgtggacgattaggaatagacaaaccatgttattt
                                                     Met

       280        290        300        310        320        330        340        350        360
atctccattagggcgtgagagtgtaattagtacacaggtactactagaatgctaaagaacttttaaaatatcctgaatcgtagggcaaa

       370        380        390        400        410        420        430        440        450
tccatgtcaagcaagaaactaatagttattaaacttcatttacttttgagctagttaaatattttcatcatttcctaaagtactgaacac

       460        470        480        490        500        510        520        530        540
ctgaatgatacttttattggccctttttaataagaactctggttagaaaaatatattgaggatatcattagtaatactcattagatatttgt

       550        560        570        580        590        600        610        620        630
gaatttagccgtttccccattacagaaaaaagatacaactaattacatgtgcagtcaaattacttttttttaagatcaattactaacaa

       640        650        660        670        680        690        700        710        720
tcaactatcatgctaaatttgctgtgatatcattttgaaccagTTAACGTCCCAAAGACCAGAAAGACCTACTGTAAGGGTAAGACCTGT
                                            ValAsnValProLysThrArgLysThrTyrCysLysGlyLysThrCys

       730        740        750        760        770        780        790        800        810
CGTAAGCACACTCAACACAAGGTTACTCAATACAAAGCTGGTAAGGCTTCCTTGTTCGCTCAAGGTAAGAGACGTTATGACCGTAAACAA
ArgLysHisThrGlnHisLysValThrGlnTyrLysAlaGlyLysAlaSerLeuPheAlaGlnGlyLysArgArgTyrAspArgLysGln

       820        830        840        850        860        870        880        890        900
TCTGGTTTCGGTGGTCAAACCAAGCCTGTTTTCCACAAGAAAGCTAAGACTACCAAGAAGGTTGTTTTGAGATTGGAATGTGTCAAATGT
SerGlyPheGlyGlyGlnThrLysProValPheHisLysLysAlaLysThrThrLysLysValValLeuArgLeuGluCysValLysCys

       910        920        930        940        950        960        970        980        990
AAGACTAGAGCCCAATTAACCTTGAAGAGATGTAAGCACTTCGAATTGGGTGGTGAAAAGAAGCAAAAGGGTCAAGCTTTGCAATTCTGA
LysThrArgAlaGlnLeuThrLeuLysArgCysLysHisPheGluLeuGlyGlyGluLysLysGlnLysGlyGlnAlaLeuGlnPhe***

      1000       1010       1020       1030       1040       1050       1060       1070       1080
GAGTTGTTATTGTTTATTGTTTTATTATATTTTTATAGTTATACTTTGACCATTAACTTTTTGTAAATTTTGCATACTTAACTCTTTAAT

      1090       1100       1110       1120       1130       1140       1150       1160       1170
ATTGAAAACCTGCCTATTTGGCGTAATTTTTCATCCTGCCTTTGAACCTGTTGTAAACGTTCGCGTTGGCAACCATGATAAGTTAACAAG

      1180       1190       1200       1210       1220       1230       1240       1250       1260
ACACGTAGGCGTTCGACTAAAACACAGCATTGCTACTAAAGTACGTTACTAAGAACCAGTAAAAATAAATATGTATAAATAAAATGTAGA

      1270       1280       1290       1300       1310       1320       1330       1340       1350
TACAGCTGATTCCCAATATCTCATTTCAGAAATGATCAACACCAGGCGCCCAGCAACGTTCAATCTATCTACGCTTCAGTACTACTAAAA

      1360       1370       1380       1390       1400       1410       1420       1430       1440
GTGGGAAAAACGTGGGCTTTTAGTCATTCCCTGCAATTTTAAGAAAACTGCATATACTAGTAGTACATTCGCGATATAATTATGATGTTG

      1450       1460       1470       1480       1490       1500       1510       1520       1530
GCCTTTTTAAAACAAAAACCAAAAGGAATTTGATTATAACAAAACTAAAGAGGTGTGGAAAATTAATACAAACCTTCGACTTTTTATAGT

      1540       1550       1560       1570       1580       1590       1600       1610       1620
GCGGTTCCTACACTTTTTTTTTTCCCGCTTACCCTGGTGATTGATTTTTTGGGTTCCGTTGTAGGGCTAGCACGAAAATAAGAATTAAG

      1630       1640       1650       1660       1670       1680       1690       1700       1710
TAGCAAAATTTGAGATTTGCTCACTTTACATACAAAGAGCTTAAATAGTAGTATACTAGTAGTAATGCTAATTCTTATCACCTTTCTTTG

      1720       1730       1740       1750       1760       1770       1780       1790       1800
CCGACTTCTTGATGTATTTTGGCGGAAACTTTTGATAAAATACATAATAAGCCCACGTAATAGTAGCAACAACAACAAATATAATCGACT

      1810       1820
TGTACGGCTCGTTCACGTCTAGA
                 XbaI
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FIG. 2—*Continued.*

ORF interrupted by an intron. The possibility that the ORF encoded a protein homologous to the L41 (5) (also called YP44 or YL27 [11]) protein of the 60S ribosomal subunit of *S. cerevisiae*, suggested from a search of a protein data base (SWISS-PROT), was confirmed by the fact that when the ORF sequence prepared by removing an intron from the *RIM-C* gene was inserted alone into a YEp-type expression vector, pAAH5, the recombinant plasmid conferred resis-

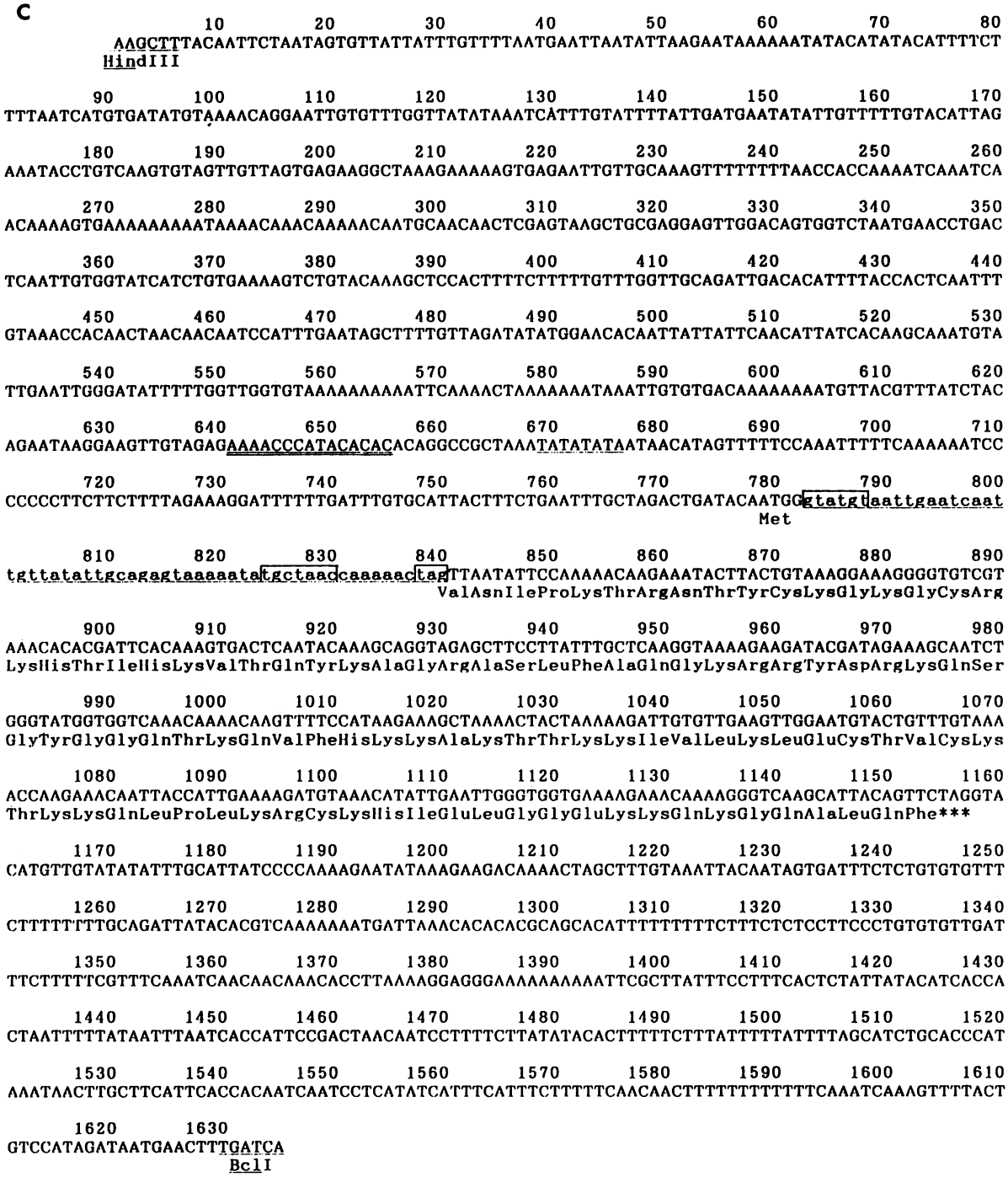

FIG. 2—*Continued.*

tance to 100 μg of CYH per ml on *S. cerevisiae* (data not shown). In the upstream region of *RIM-C*, there are a TATA box and a sequence analogous to the upstream activating sequence of ribosomal protein genes of *S. cerevisiae* (9).

Isolation of L41 protein genes of *S. cerevisiae*. Southern blot analysis with *RIM-C* as a probe indicated that there were at least three copies of *RIM-C*-related sequences in the genome of *C. maltosa*, which is of the diploid type, and two copies of L41-related genes in the haploid genome of *S. cerevisiae*, as in most other basic ribosomal protein genes of this species (8). A genomic library from *S. cerevisiae* AH22 containing about 10,000 independent clones was constructed. By colony hybridization, two different positive clones were obtained, and these were subcloned and sequenced. These two genes, named L41a and L41b (a gene for the L41a protein subcloned as a 2.0-kb *Bgl*II-*Sau*3AI fragment in Fig. 2a, and a gene for the L41b protein subcloned as a 1.8-kb *Xba*I fragment in Fig. 2b), were isolated for the first time from the genome of *S. cerevisiae*, and their deduced amino acid sequences are shown in Fig. 2a and b. These sequences were

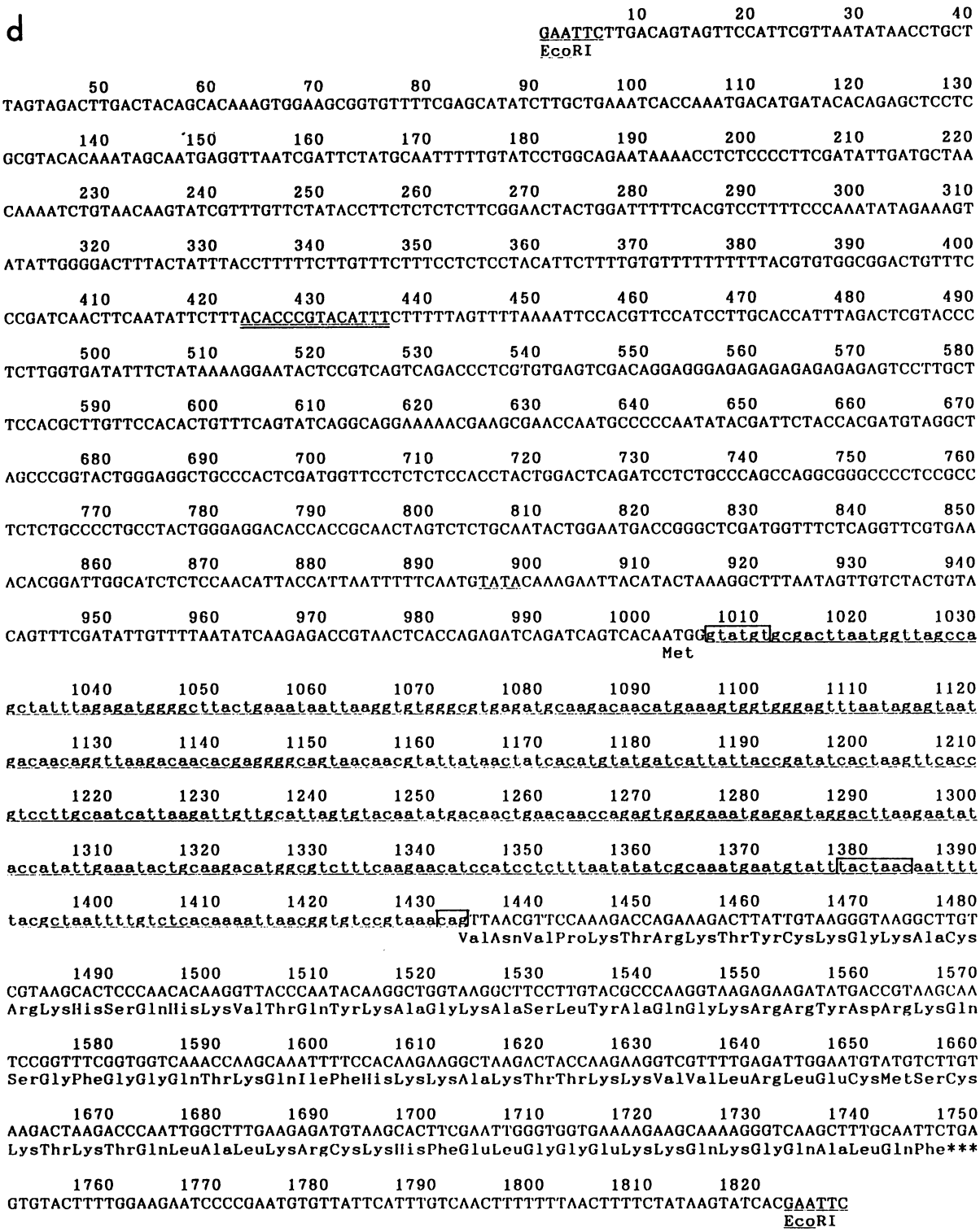

FIG. 2—*Continued.*

identical to each other, but for some unknown reason they showed a few amino acid residues that were different from the published sequence (5). The homology of the deduced amino acid sequences of the CYH-sensitive L41 protein and the CYH-resistant RIM-C protein was about 85% (Fig. 3).

Isolation and phenotype of L41-related protein genes from a few other yeasts. To identify the specific amino acid residue(s) responsible for the difference in CYH sensitivity between these two L41-related ribosomal proteins, a clone with an *RIM-C*-hybridizable sequence was isolated from a

a

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CM   ATG-G 5'/gtacgt---35 bp---tgctaac---7 bp---tag/3' TT
               (intron size 58 bp)
CT   ATG-G 5'/gtatgt---35 bp---tgctaac---7 bp---tag/3' TT
               (intron size 58 bp)
KT   ATG-G 5'/gtatgt---366 bp---tactaac---46 bp---cag/3' TT
               (intron size 428 bp)
SA   ATG-G 5'/gtatgt---464 bp---tactaac---32 bp---tag/3' TT
               (intron size 464 bp)
SB   ATG-G 5'/gtatgt---383 bp---tactaac---42 bp---cag/3' TT
               (intron size 383 bp)
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b

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     1         10        20        30        40        50        60
CM) MVNIPKTRNTYCKGKGCRKHTI-HKVTQYKSGRASLFAQGKRRYDRKQSGYGGQTKQVFHK
CT) ***********************-*******A*************************R****
KT) ***V****K******A****SQ-*******A*K***Y************F*****RI***
SA) ***V****K******T*****Q-*******A*K****************F*****R****
SB) ***V****K******T*****Q-*******A*K****************F*****R****
RT) ***V****R*F*--*K*G**-QP*******K*KD**Y*****************RI*R*
HM) ***V****R*F*--*K*G**-QP*******KEKD**Y***R***************RI*R*
     1         10        20        30        40        50

               70        80        90       100   106
CM) KAKTTKKIVLKLECT--VCKTKKQLPLKRCKHIELGGEKKQKGQALQF    Homology
CT) ***************--*********************************     (99.1%)
KT) *******V**R***M--S****T**A******F***************     (84.0%)
SA) *******V**R***V--K***RA**T******F***************     (84.9%)
SB) *******V**R***V--K***RA**T******F*************-*     (84.9%)
RT) **********R***VEPN*RS*RM*AI*****F****D**R***VI**     (70.4%)
HM) **********R***VEPN*RS*RM*AI*****F****D**R***VI**     (68.5%)
     60        70        80        90       100   106
```

FIG. 3. Structures of introns in five genes for L41-related proteins from four yeast species and the deduced ORF amino acid sequences determined in the present work plus the amino acid sequences of two mammalian L36a proteins described in the literature. Abbreviations: CM, *C. maltosa*; CT, *C. tropicalis*; KT, *K. fragilis*; SA, *S. cerevisiae* (L41a); SB, *S. cerevisiae* (L41b); RT, rat (L36a) (3); HM, human (L36a) (1). (a) Introns in five L41-related protein genes from four yeast species. The 5'- and 3'-junction sequences and branching sequences of introns are shown by underlined letters, except those nucleotides different from the consensus sequences (gtatgt- - -tactaac- - -yag; y means c or t) (7) in *S. cerevisiae* introns. (b) Deduced amino acid sequences of five L41-related protein genes from four yeast species and amino acid sequences of two mammalian L36a proteins. Amino acids are aligned for optimal homology. A dash indicates the absence of an amino acid residue. Amino acid residues identical to those of the *C. maltosa RIM-C* gene product are indicated by asterisks. The boxes indicate the amino acid residues at position 56 (or position 54 in the case of mammalian sequences) which were suggested to be involved in ribosomal CYH resistance (see text). The homology of each amino acid sequence with that of the *RIM-C* gene product was calculated and is shown at the bottom right.

genomic bank of CYH-resistant *C. tropicalis*, and one positive clone obtained by colony hybridization was subcloned as a 1.6-kb *Hin*dIII-*Bcl*I fragment by using YRpUC19N as a vector. Then the ability of the subcloned fragment to confer CYH (100 μg/ml) resistance on *S. cerevisiae* was confirmed. A genomic library of CYH-resistant *K. fragilis* was introduced into *S. cerevisiae* YNN27 and two CYH-resistant clones were isolated from about 7,000 transformants. Both clones had the same plasmid with a 1.8-kb *Eco*RI-inserted fragment. The nucleotide sequences of these 1.6- and 1.8-kb fragments were determined as shown in Fig. 2c and d. Assuming that the intron was at the same position as that in *RIM-C* (Fig. 3a), each sequence had an ORF encoding an L41-related protein. The deduced amino acid sequences of all of these L41-related proteins, together with those of mammalian proteins homologous to the L41 protein which have been sequenced and named L36a (1, 3), are summarized in Fig. 3b.

Determination of the specific amino acid residue responsible for the differences in CYH sensitivity among L41-related ribosomal proteins. When each of these deduced amino acid sequences (Fig. 3b) was classified into either of the two groups according to the ability to confer CYH resistance on *S. cerevisiae* as described above (although neither L41-related genes nor cDNA of mammalian cells have been examined for the ability to confer CYH resistance on *S. cerevisiae*, we considered it reasonable to assume that both would not), the 56th amino acid residue was identical in all sequences of each group but different between the two groups; the amino acid was glutamine in the resistant group and proline in the sensitive one. To confirm that this amino acid residue of the L41 protein determines the sensitivity of the ribosome to CYH, $L41a^P$ was converted to $L41a^Q$ by site-directed mutagenesis, inserted into a YRp-type vector, and used for transformation of *S. cerevisiae*. It was clearly demonstrated that the transformant was resistant to higher

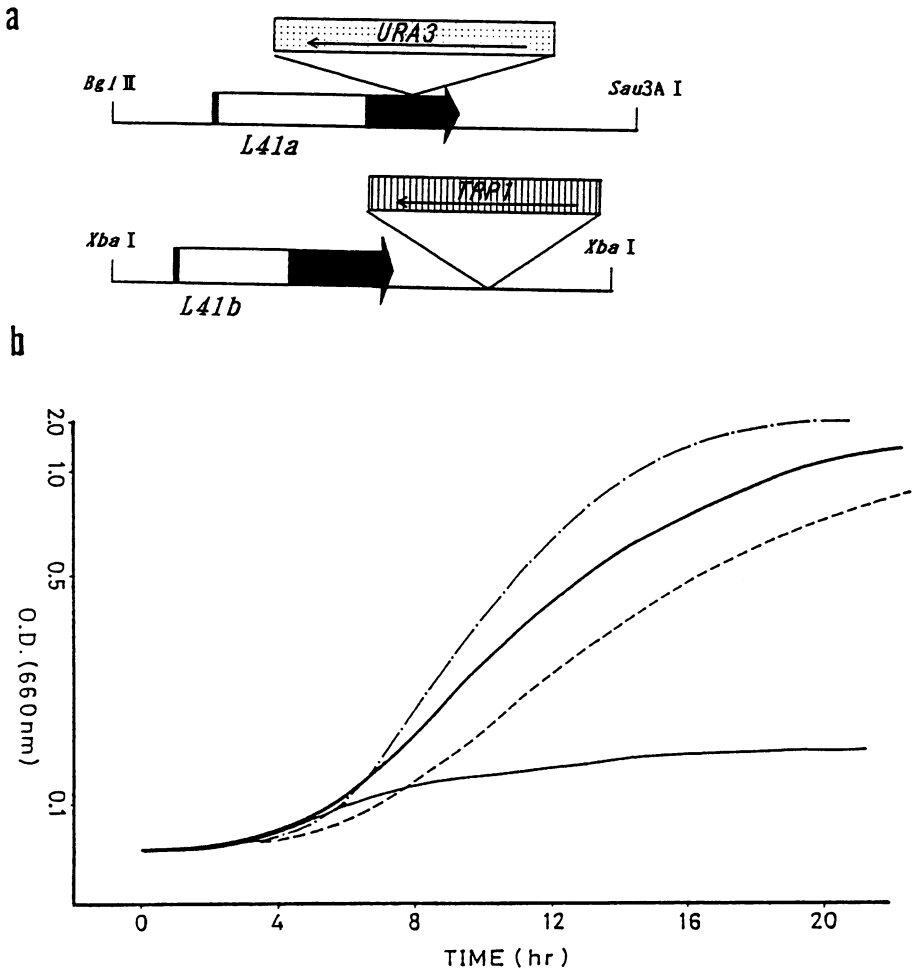

FIG. 4. Structures of DNA fragments used to construct two genetically engineered *S. cerevisiae* strains possessing a disrupted gene for L41a and the wild-type L41b gene (ADBP) or a gene for L41b in which proline is substituted by glutamine at the 56th amino acid residue (ADBQ) (a) and the growth curves of the two strains in the presence or absence of 100 μg of CYH per ml (b). (a) The top diagram shows the structure of the DNA fragment having the L41a protein gene disrupted by the *URA3* gene from *S. cerevisiae*. The bottom diagram shows that of the DNA fragment having two tandem genes, one for L41b (the wild type or a protein with substitution of glutamine for proline at the 56th amino acid residue) and the other the *TRP1* gene from *S. cerevisiae*. The solid box and the clear box in the genes indicate an ORF and an intron, respectively. (b) Growth curves of the two strains in liquid medium in the presence or absence of 100 μg of CYH per ml. Shown are curves for the ADBP strain without (· — · —) or with (——) CYH and the ADBQ strain without (▬) or with (- - - -) CYH.

concentrations of CYH. (We have also examined the effect of replacement of this 56th residue with other amino acids [unpublished data].)

To further confirm the concept described above in a simpler system with only one copy of L41 gene, two strains of *S. cerevisiae* were created by genetic engineering: strain ADBP, with L41a disrupted and with proline in L41b (and with the wild-type $L41a^P$ gene), and strain ADBQ, with L41a disrupted and with glutamine instead of proline L41b at position 56 (Fig. 4a). It was shown that ADBP is sensitive to higher concentrations of CYH, while ADBQ is resistant to higher concentrations of CYH (Fig. 4b).

DISCUSSION

As reported previously, L29 is one of the target proteins of CYH in the ribosome (2, 13), and it is obvious from the present results that L41 is another target protein. In addition to those of L41-related proteins shown in Fig. 3, the amino acid sequence deduced from the nucleotide sequence of cDNA isolated in our laboratory from the cDNA library of the tomato *Lycopersicon escurentum*, which is CYH sensitive, by using *RIM-C* as a probe showed that the 56th amino acid was proline (unpublished data). When the L41-related tomato cDNA sequence in which the proline residue at position 56 had been replaced with glutamine was inserted into a YRp-type expression vector and used for transformation of *S. cerevisiae*, the host became resistant to higher concentrations of CYH (unpublished data). It is suggested that almost all of the higher-order eukaryotes and some of the lower-order eukaryotes that are sensitive to CYH have an $L41^P$-type protein in their ribosomes, whereas the other lower-order eukaryotes which show resistance to CYH at higher concentrations have an $L41^Q$-type protein. It is interesting to speculate how the genes for L41-related ribosomal proteins have evolved in eukaryotes, with one group having the $L41^P$ type and the other having the $L41^Q$ type. We speculate that a ribosome with an $L41^P$-type protein would have appeared first in eukaryotes and would have been

contained in most eukaryotes as observed at present but that some microorganisms would have acquired an L41^{Q}-type protein by a mutation which allowed them to survive in an environment in which CYH was present, as in the case of soil microorganisms such as *C. maltosa*. It might be interesting to screen for useful novel chemical compounds which effectively inhibit translation on ribosomes having an L41^{P}-type, but not an L41^{Q}-type, protein. These could have potential utility as antifungal agents.

Another interesting application would be to utilize the L41^{Q}-type gene as a selection marker for eukaryotic host-vector systems. This possibility is currently being examined with some species of CYH-sensitive yeasts and with cultured tomato cells.

ACKNOWLEDGMENTS

We thank Tadashi Hidaka, Research Institute of Takeda Pharmaceutical Company, for useful discussion.

This work was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. **Davies, M. S., A. Henney, W. H. J. Ward, and R. K. Craig.** 1986. Characterization of an mRNA encoding a human ribosomal protein homologous to the yeast L44 ribosomal protein. Gene **45**:183–191.
2. **Fried, H. M., and J. R. Warner.** 1982. Molecular cloning and analysis of yeast gene for cycloheximide resistance and ribosomal protein L29. Nucleic Acids Res. **10**:3133–3147.
3. **Gallagher, M. J., Y. L. Chan, A. Lin, and I. G. Wool.** 1988. The primary structure of rat ribosomal protein L5. A comparison of the sequence of amino acids in the proteins that interact with 5S rRNA. DNA **7**:269–273.
4. **Itoh, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
5. **Itoh, T., and B. Wittmann-Liebold.** 1978. The primary structure of protein 44 from the large subunit of yeast ribosomes. FEBS Lett. **96**:399–402.
6. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82**:488–492.
7. **Langford, C. J., F. J. Klinz, C. Donath, and D. Gallwitz.** 1984. Point mutations identify the conserved intron-contained TAC TAAC box as an essential splicing signal sequence in yeast. Cell **36**:645–653.
8. **Mager, W. H.** 1988. Control of ribosomal protein gene expression. Biochim. Biophys. Acta **949**:1–15.
9. **Mann, C., J. M. Buhler, I. Treich, and A. Sentenac.** 1987. RPC40, a unique gene for a subunit shared between yeast RNA polymerases A and C. Cell **48**:627–637.
10. **Ohkuma, M., T. Tanimoto, K. Yano, and M. Takagi.** 1991. A cytochrome P450alk [*CYP52*] multigene family in *Candida maltosa*: molecular cloning and nucleotide sequence of the two tandemly arranged genes. DNA Cell Biol. **10**:271–282.
11. **Otaka, E., and S. Osawa.** 1981. Yeast ribosomal proteins. V. Correlation of several nomenclatures and proposal of a standard nomenclature. Mol. Gen. Genet. **181**:176–182.
12. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
13. **Stocklein, W., and W. Peipersberg.** 1980. Altered ribosomal protein L29 in a cycloheximide-resistant strain of *Saccharomyces cerevisiae*. Curr. Genet. **1**:177–183.
14. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High-frequency transformation of yeasts: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76**:1035–1039.
15. **Takagi, M., S. Kawai, I. Shibuya, M. Miyazaki, and K. Yano.** 1986. Cloning in *Saccharomyces cerevisiae* of a cycloheximide resistance gene from the *Candida maltosa* genome which modifies ribosomes. J. Bacteriol. **168**:417–419.
16. **Takagi, M., S. Kawai, Y. Takata, N. Tanaka, M. Sunairi, M. Miyazaki, and K. Yano.** 1985. Induction of cycloheximide resistance in *Candida maltosa* by modifying the ribosomes. J. Gen. Appl. Microbiol. **31**:267–275.