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

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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 $0.5 \,\mu$ g/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 CYHsensitive 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.

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 adel can1), and YNN27 (MATa 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 (recAl leuB6 trpE5 hsdR hsdM⁺ lacI thr thi), MC1061 [his hsm⁺ araD139 Δ (ara-leu)7697 lacX74 strA galU galK], MV1190 [Δ (srl-recA) 306::Tn10 Δ (lac-pro) thi supE(F' proAB lacI^q lacZ Δ M15 traD36)], and CJ236 (dut-1 ung-1 thi relAl/ 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 BamHI-digested pUC19 by using about 5- to 10-kb DNA fragments obtained by partial digestion of the total genomic DNA with Sau3AI. The genomic library of K. fragilis was constructed in the same way, but BamHIdigested 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

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TCTAGA Xbal	10 Agcacaattat Î	20 TATTCAACGT	30 Гаттаслаас	40 AAGCATATTGA	50 AATTGGAATAT	60 FTTTTGGTTG	70 GTTTAAAAAA	80 AAAATCCAAAA	90 CAT
****	100 \ataataattgt	110 GTGACAAAAA	120 ANTGTACGTT	130 TATCTACAGA	140 \taggaagt	150 Igtaaag <u>aaa</u>	160 ACCCATACAC	170 <u>AC</u> ACACACCCC	180 CGC
TAAAA	190 Гат <u>татата</u> аа	200 TAAACCATGA	210 GTTTTCCAAA	220 TTTTTCAAAA	230 Ааааааттсс(240 CCCTTTTTCT	250 TTTAGAAAAG	260 Аттссттаатт	270 TGT
GCATT	280 ACTTTCTGATT	290 TTGCTAGACT	300 GATACTAŢGG Met	310 gtacgtaatt	320 gaatcaattg	330 ttatctgacg	340 <u>ttctcaaaat</u>	350 Algctaaccaa	360 1888
ctagT Va	370 FAATATTCCAA lAsnileProl	380 AAACAAGAAA ysThrArgAs	390 TACTTATTGT nThrTyrCys	400 AAAGGAAAAG LysglyLysg	410 GGTGTCGTAA LyCysArgLy:	420 ACATACGATT sHisThrIle	430 Cacaaggtga HislysValt	440 CTCAATACAAA hrGlnTyrLys	450 ATCA sSer
GGTAG GlyAr	460 AGCTTCCTTAT gAlaSerLeuP	470 TTGCTCAAGG heAlaGlnGl	480 TAAAAGAAGA yLysArgArg	490 Tacgatagga Tyraspargl	500 AACAATCTGG ysGlnSerGl	510 GTATGGTGGT yTyrGlyGly	520 CAAACAAAGC GlnThrLysG	530 AAGTTTTCCAT InValPhellis	540 [AAG slys
AAGGC LysAla	550 FAAAACGACTA aLysThrThrL	560 AGAAGATTGT ysLysIleVa	570 GTTGAAGTTG lleuLysLeu	580 GAATGTACTG GluCysThrV	590 TTTGTAAAAC alCysLysTh	600 CAAGAAACAA rLysLysGln	610 TTGCCATTGA LeuProLeuL	620 AAAGATGTAAA ysArgCysLys	630 ACAT silis
ATTGA Ilegi	640 ATTGGGTGGTG uLeuGlyGlyG	650 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	660 AAAAGGTCAA nLýsGlyGln	670 GCATTACAGT AlaLeuGlnP	680 TCTAGGTACA he***	690 TGTTGTATAT	700 ATTTTGCATT	710 ATCCCCAATAA	720 Atac
AAGAA	730 Agaagacaaaa	740 .CTAGTTTTGT	750 Agattgtaat	760 Agtaatttct	770 GTATGTGTGT	780 GTTTTTCTTT	790 TTTTTGCAGA	800 TTACACACGTC	810 CAAA
АЛЛАТ	820 Gattaacaca	830 Cacgcaacac	840 TTTTTTTTCT	850 TTCCTTTGAA	860 Сладалатса	870 ACAACAAACA	880 CCTTAAAAGG	890 Aggaaaaaaa	900 1887
TCGCT	910 TATTTCCTTTC	920 ACTCTCTATT	930 Acatatcaco	940 Асталтаттт	950 AACATTTCAA	960 TCACCATCCC	970 AACTAACATT	980 CATTTCCTTAI	099 0777
CACCT	1000 ГТТСТТТАТСТ	1010 ТТАТТСТАСС	1020 ATCTACACCC	1030 Сатаалтааст	1040 Gacttcattc	1050 ACTACAACCA	1060 TTCCTCATAT	1070 1 CATTTCATTTC	1080 CTTT
ттсаа	1090 Саастттттт	1100 TTTTCAAATC	1110 AAAGTTTTAC	1120 TGTCCATAGA	1130 Taatgaactt <u>S</u>	1140 T <u>GATC</u> au3AI			
Muslaa	tida agavamaa	of the DIM (anna fram (~ maltasa an	hasubab stil	amino acid se	equence The	genomic nucl	entid

ic nucleotide sequence. 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 *XbaI* end of the 1.14-kb *XbaI-Sau3AI* fragment (15), is numbered in the 5' to 3' direction. The ATG codon, located 303 bp downstream from the XbaI 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 ³²P-labelled *RIM-C* DNA, $6 \times$ SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), $5 \times$ 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 $2 \times$ SSC and then at 56°C for 1 h with $2 \times$ 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 BglII-Sau3AI 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 XbaI 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 μ g 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

a 10	20	30	40	50	60	70	80	90
AGATCTTACATC BglII	CTTACATCTAAA	GTAAAACCT/	<u>GACATTT</u> ACT	TCGAGTTATA	CTTTTTTTT	TATTATCTAT	TTTTTTCTCTT	GCGGAC
100	110	120	130	140	150	160	170	180
ATTTAACACCTG	AATTCCGCCTAA	CGCCAGGAC	FGATCCTGCCA	.GGGAAGGGAC	SCTTTGTCTAC	Stgccaatago	GCCGGACCAGT	AGGAAG
190	200	210	220	230	240	250	260	270
GTTACAGCAGCT	GGCCCGCAGAGI	GATTGGGTC	Acaggaaatag	CGCAACCTTC	CTCTTTTGCCC	CGGGAAAGGCC	Gottcaatcta	CCTTCG
280	290	300	310	320	330	340	350	360
Angggctagtac	Atgagcgcgaag	GAGGCAGA <u>T</u>	AATAGCACCAT	TAAGTGGTCC	CANATGCATCI	Гтдлаатстая	Атсстталтас	Aggaaa
370	380	390	400	410	420	430	440	450
Асалсааттатс	Agtaaaatgg	tatgutata	accataattco	taatggtgag	Ataaatcag	Caccaataaas	zaaaagctaat	ttgatt
460	Met 470	480	490	500	510	520	530	540
tttattgtcaat	gaaatttcataa	tcgtcatga	atgcataaaca	gacacacct	agcaactgta	taatctgcgco	ctaaaaagggc	<u>gtatac</u>
550	560	570	580	590	600	610	620	630
<u>acaaaactaaac</u>	gatgcgcaatae	aagttcagc	agtcagcaate	asaccgaga	tatgcagcaa	cagagtatcat	<u>tatgcatggag</u>	<u>gatcct</u>
640 <u>ttctgtttttct</u>	650 gataatatgete	660	670 tccaaacagca	680 LCagtagccta	690 Atttgtgaag	700 ctcaaaaagg	710 <u>scttctatttc</u>	720
730	740	750	760	770	780	790	800	810
<u>tatcttcagatt</u>	gtgcagtgatat	tctttgaag	aaggaaacgta	gaggggata	agttggataa	ctgttatttci	ttttcaatatg	<u>ctagat</u>
820	830	840	850	860	870	880	890	900
<u>tttgcttaccac</u>	cttactgattti	ttctaataa	taaacttttt	actaacatta	Agtacgatgt	ctcatctatt	tcttctattta	TTAAC
								ValAsn
910	920	930	940	950	960	970	980	990
GTTCCAAAGACC	AGAAAGACCTAC	TGTAAGGGT	AAGACCTGTCC	STAAGCACAC	FCAACACAAG	GTTACTCAAT	ACAAAGCTGGT	AAGGCT
ValProLysThr	ArgLysThrTyi	CyslysGly	LysThrCysAi	gLysHisTh	rGlnHisLys	ValThrGlnT	yrLysAlaGly	LysAla
1000	1010	1020	1030	1040	¥1050	1060	1070	1080
TCCTTGTTTGCC	CAAGGTAAGAG/	CGTTATGAC	CGTAAACAATC	TGGŤTTCGG	FGGTCAAACC	AAGCCTGTTT	TCCACAAGAAA	GCTAAG
SerLeuPheAla	GlnGlyLysArg	ArgTyrAsp	ArgLysGlnSe	TGÍyPheGl	yGlyGlnThr	LysProValP	hellisLysLys	AlaLys
1090	1100	1110	1120	1130	1140	1150	1160	1170
ACTACCAAGAAG	GTTGTTTTGAGA	TTGGAATGT	GTCAAATGTAA	GACCAGAGCO	CCAATTGACC	TTGAAGAGAT(GCAAGCACTTC	GAATTG
ThrThrLysLys	ValValLeuArg	LeuGluCys	VallysCysly	SThrArgAlo	aGlnLeuThri	LeuLysArgC;	ysLysHisPhe	GluLeu
1180 GGTGGTGAAAAG GlyGlyGluLys	1190 AAGCAAAAGGG7 LysGlnLysG13	1200 TC <u>AAGCTT</u> TG GlnAlaLeu <u>Hin</u> dIII	1210 CAATTCTGAAT GlnPhe***	1220 TCTCTTTATG	1230 GGTTTCGCTT	1240 TTGTATTTTC'	1250 TTTGCTTTAAA	1260 ATCTGA
1270	1280	1290	1300	1310	1320	1330	1340	1350
Татааттлсата	TTATTACAATAT	TAAAACTTTA	CGATAATTCC1	Igcaactaat	BATGCGTTAG	TACGGTTCGG	GCGCTCTAGGA	CGCGTG
1360	1370	1380	1390	1400	1410	1420	1430	1440
TCGGTCGGGTAA	CCATCATTCTT	ATTCGACGAA	AATGATTAGTT	TTCCAGAGAA	GCAGTTGCAC	ICCAGTTTGA	TCTGCAGTCCA	GCGACT
1450	1460	1470	1480	1490	1500	1510	1520	1530
ATTTTTAACATT	CAAAGATCCATT	TTTTCGTTTG	AATAGTTGAGG	Caaggtcata	TAAATTTCTA	Fatcacacga	Ataaggttca	GATATT
1540	1550	1560	1570	1580	1590	1600	1610	1620
GCTTTATTATCG	GCGAACACTGA	TATTACCACA	TAGATATCGC1	CTTTTTTTG	AAACGTAAGC	GTAAATTTCA	Gtataggtaca	CGTCTG
1630	1640	1650	1660	1670	1680	1690	1700	1710
CGCTGATCCTTC	TGTAATCCTCTC	Scagtttcgc.	AATGTATAAT/	AGCAGCACCA	ATCATCATGA	GGGTGCACCT	ACTTCAGGACA	ATGGCTA
1720	1730	1740	1750	1760	1770	1780	1790	1800
TTATATGTCCCA	ACAACAAGACC/	Acagcatca	ACAACAACAAC	Cantacgcca	Acganatgaa	TCCGTATCAG	Caaattcctag	ACCGCC
1810	1820	1830	1840	1850	1860	1870	1880	1890
TGCTGCAGGATT	TAGTAGCAACTA	Catgaaaga	GCAAGGCTCTC	CATCAATCGT	Tacaagca	TTTACAACGT	GAGACAGGTAA	CCTTGG
1900 CAGCGGTTTTAC	1910 Agacgttccago	1920 CCTGAATTA	1930 TCCAGCCACAC	1940 Сслосассло.	1950 Аталталтта	1960 CGCAGCTTCA	1970 AATCAGAT <u>GAT</u> Sau3	1 <u>C</u> 3A I

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 Bg/II end of the 2.0-kb Bg/II-Sau3AI restriction fragment. The ATG codon, located 380 bp downstream from the Bg/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 Solution of the 5' XbaI 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 XbaI end of the 1.8-kb XbaI restriction fragment. The ATG codon, located 229 bp downstream from the 5' XbaI 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 HindIII end of the 1.6-kb HindIII-Bc/I fragment. The ATG codon, located 778 bp downstream from the 5' EcoRI end of the 1.8-kb EcoRI fragment. The ATG codon, located 1,002 bp downstream from the 5' EcoRI end, opens an ORF with an intron (nucleotides 783 to 840). (d) Gene for the L41-like protein of K. fragilis.

b	10	20	30	40	50	60	70	80	90
<u>TCTAGA</u> XbaI	JAGACGTCG'	rcggtgcggc/	ACGCTGACG	GTTTAGTTGTT	CGACGGGATG	ATGGGTTCCG	CCAGGGGGGAG	GGAAGGCTTT	CCAC
CAAGAG	100	110	120	130	140	150	160	170	180
	Aggtaaaat'	FATTCGTCGA	Атдластса	GAGATGCGTCC	CATATTGTTGA	Caatg <u>tata</u> t	CTTAATTGAT	GTGGTATTTT	CACT
GTTTTA	190 Acgtaaatt	200 Gaaggagatt/	210 AAGCAAAAAA	220 Acaatcagta/	230 ATAATGG <mark>gtat</mark> Met	_240 gtggacgatt	250 <u>aggaatagac</u>	260 <u>aaaccatgtt</u>	270 <u>attt</u>
atctcc	280	290	300	310	320	330	340	350	360
	<u>attagggcg</u>	tgagagtgta	attagtacac	aggtactacte	agaatgctaaa	gaacttttta	aaatatcctg	aatcgtaggg	caaa
tccatg	370	380	390	400	410	420	430	440	450
	tcaagcaag	aaactaatag	ttattaaact	tcatttactt	ttgagctagtt	aaatattttc	atcatttcct	aaagtactga	lacac
ctgaat	460	470	480	490	500	510	520	530	540
	gatactttt	attggccctt	ttaataagaa	ctctggttag	maaatatattg	aggatatcat	tagtaatact	cattagatat	ttgt
gaattt	550	560	570	580	590	600	610	620	630
	agccgtttc	cccattacag	aaaaagata	caactaatta	catgtgcagtc	aaattacttt	tttttaaga	tcaattacte	acaa
tcaact	640 atcatgcta	650 aatttgctgt	660 gatatcattt	670 tgaaccagTT Val	680 AACGTCCCAAA AsnValProLy	690 GACCAGAAAG sThrArgLys	700 ACCTACTGTA ThrTyrCysL	710 AGGGTAAGAC ysGlyLysTh	720 CTGT IrCys
CGTAAG ArgLys	730 CACACTCAA HisThrGln	740 CACAAGGTTA HislysValT	750 CTCAATACAA hrGlnTyrLy	760 AGCTGGTAAG sAlaGlyLys	770 GCTTCCTTGTT AlaSerLeuPh	780 CGCTCAAGGI eAlaGlnGly	790 AAGAGACGTT LysArgArgT	800 ATGACCGTAA YrAspArgLy	810 ACAA sGln
TCTGGT SerGly	820 TTCGGTGGT PheGlyGly	830 CAAACCAAGC GlnThrLysP	840 CTGTTTTCCA roValPheHi	850 CAAGAAAGCTA sLysLysAla	860 AAGACTACCAA LysThrThrLy	870 GAAGGTTGT1 sLysValVal	880 TTGAGATTGG LeuArgLeuG	890 AATĞTGTCAA luCysValLy	900 ATGT sCys
AAGACT. LysThr	910 AGAGCCCAA ArgAlaGln	920 TTAACCTTGA LeuThrLeuL	930 Agagatgtaa ysargCysLy	940 GCACTTCGAA sHisPheGlu	950 FTGGGTGGTGA LeuGlyGlyGl	960 AAAGAAGCAA ulyslysGlr	970 AAGGGTCAAG LysGlyGlnA	980 CTTTGCAATT laLeuGlnPh	990 CTGA 1e***
GAGTTG	1000	1010	1020	1030	1040	1050	1060	1070	1080
	TTATTGTTT	ATTGTTTTAT	Гататттта	TAGTTATACT	Гтдассаттаа	CTTTTTGTAA	Attttgcata	CTTAACTCTT	TAAT
ATTGAA	1090	1100	1110	1120	1130	1140	1150	1160	1170
	AACCTGCCT	Atttggcgta	ATTTTTTCATC	CTGCCTTTGA	ACCTGTTGTAA	ACGTTCGCG1	TIGGCAACCAT	Gataagttaa	ACAAG
ACACGT	1180	1190	1200	1210	1220	1230	1240	1250	1260
	AGGCGTTCG	Actaaacac	Agcattgcta	Сталадтасс ⁷	FTACTAAGAAC	Састалала	YAAATATGTAT	Халатаааатс	Jtaga
TACAGC	1270	1280	1290	1300	1310	1320	1330	1340	1350
	Tgattccca	ATATCTCATT	FCAGAAATGA	TCAACACCAG	GCGCCCAGCAA	CGTTCAATCI	ATCTACGCTI	CAGTACTACT	Maaa
GTGGGA	1 36 0	1370	1380	1390	1400	1410	1420	1430	1440
	AAAACGTGG	GCTTTTAGTC	Attccctgca	Attttaagaa	Nactgcatata	Ctagtagtac	Cattcgcgata	\TAATTATGAT	Igttg
GCCTTT	1450	1460	1470	1480	1490	1500	1510	1520	1530
	Ттлаласлл	AAACCAAAAG	Gaatttgatt	Аталсаалас'	FAAAGAGGTGT	Ggaaaattaa	MTACAAACCT1	CGACTTTTTA	\tagt
GCGGTT	1540	1550	1560	1570	1580	1590	1600	1610	1620
	CCTACACTT	TTTTTTTTTC	CCGCTTACCC	TGGTGATTĠA	ITTTTTGGGTT	CCGTTGTAGO	GCTAGCACGA	\AAATAAGAAT	Tag
TAGCAA	1630	1640	1650	1660	1670	1680	1690	1700	1710
	AATTTGAGA	TTTGCTCACT	Гтасатасаа	Agagettaaa	Гастастатас	TAGTAGTAAT	Igctaattct1	CATCACCTTTC	CTTTG
CCGACT	1720	1730	1740	1750	1760	1770	1780	1790	1800
	TCTTGATGT	Attttggcgg	AAACTTTTGA	Таллатасат.	AATAAGCCCAC	GTAATAGTAC	GCAACAACAAC	Хааататлатс	CGACT
TGTACG	1810 GCTCGTTCA	1820 CG <u>TCTAGA</u> <u>Xba</u> I							

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-

С	10	20	30	40	50	60	70	80
AA(Hing	<u>CTT</u> TACAATTO	CTAATAGTGT	FATTATTTGT	ΓΤΤΛΛΤΘΑΛΤΊ	TAATATTAAGA	АТАААААТ	АТАСАТАТАС/	ATTTTCT
90	100	110	120	130	140	150	160	170
TTTAATCATGTG/	Atatgtļaaac	Aggaattgtg:	FTTGGTTATA'	Гаалтсаттт(Этатттаттс	Atgaatata	FTGTTTTTGT/	Acattag
180	190	200	210	220	230	240	250	260
AAATACCTGTCAA	\gtgtagttgt	TAGTGAGAAG	GCTAAAGAAA	AAGTGAGAAT	Igttgcaaagt	TTTTTTTTAA(CCACCAAAAT	Caaatca
270	280	290	300	310	320	330	340	350
Acaaaagtgaaa	Алалааталаа	Саласалала	Caatgcaaca	ACTCGAGTAA	GCTGCGAGGAG	TTGGACAGTO	GGTCTAATGA/	ACCTGAC
360	370	380	390	400	410	420	430	440
TCAATTGTGGTA	Icatctgtgaa	AAGTCTGTAC	AAAGCTCCAC	TTTTCTTTTT(Stttggttgca	Agattgacaca	ATTTTACCAC	TCAATTT
450	460	470	480	490	500	510	520	530
GTAAACCACAAC	Faacaacaatc	Catttgaata	GCTTTTGTTA	GATATATGGA/	\cacaattatt	TATTCAACAT	Fatcacaagc/	4447674
540	550	560	570	580	590	600	610	620
TTGAATTGGGAT	ATTTTTGGTTG	Gtgtaaaaa	Аалаттсала	ACTAAAAAA	FAAATTGTGTG	Bacaaaaaa	Atgttacgtt	Гатстас
630	640	650	660	670	680	690	700	710
Agaataaggaag'	FTGTAGAG <u>aaa</u>	ACCCATACAC	<u>AC</u> ACAGGCCG	Стала <u>татат</u> /	<u>Ata</u> ataacata	AGTTTTTCCA	AATTTTTCAA	AAAATCC
720	730	740	750	760	770	780	790	800
CCCCCTTCTTCT	FTTAGAAAGGA	TTTTTTGATT	Tgtgcattac	TTTCTGAATT	Fgctagactga	ATACAATGGE	tatgtaattgi	aatcaat
810 tgttatattgca	820 gagtaaaaata	830 tgctaadcaa	840 <u>aaactag</u> TTA ValA	850 ATATTCCAAA snlleProLys	860 AACAAGAAATA sThrArgAsn7	870 ACTTACTGTA ThrTyrCysL;	880 AAGGAAAGGG ysGlyLysGl;	890 GTGTCGT yCysArg
900	910	920	930	940	950	960	970	980
AÁACACACGATT	CACAAAGTGAC	TCAATACAAA	GCAGGTAGAG	CTTCCTTATT	FGCTCAAGGTA	AAAAGAAGAT	ACGATAGAAA	GCAATCT
LysHisThrlle	HislysValTh	rGlnTyrLys	AlaGlyArgA	laSerLeuPho	eAlaGlnGlyI	JysArgArgT	yrAspArgLy:	sGlnSer
990	1000	1010	1020	1030	1040	1050	1060	1070
GGGTATGGTGGT	CAAACAAAACA	AGTTTTCCAT	AAGAAAGCTA	AAACTACTAA	AAAGATTGTGT	ITGAAGTTGG	AATGTACTGT	TTGTAAA
GlyTyrGlyGly	GlnThrLysGl	nValPheHis	LysLysAlaL	ysThrThrLy:	sLysIleVall	LeuLysLeuG	luCysThrVa	lCysLys
1080	1090	1100	1110	1120	1130	1140	1150	1160
ACCAAGAAACAA	TTACCATTGAA	AAGATGTAAA	CATATTGAAT	TGGGTGGTGAA	AAAGAAACAA/	AAGGGTCAAG	CATTACAGTT(CTAGGTA
ThrLysLysGln	LeuProLeuLy	sArgCysLys	HislleGluL	euGlyGlyGl	uLysLysGlnI	LysGlyGlnA	laLeuGlnPho	e***
1170	1180	1190	1200	1210	1220	1230	1240	1250
CATGTTGTATAT	ATTTGCATTAT	CCCCAAAAGA	Ататаладаа	Gacaaacta	GCTTTGTAAA	FTACAATAGT	GATTTCTCTG	TGTGTTT
1260	1270	1280	1290	1300	1310	1320	1330	1340
CTTTTTTTTGCA	Gattatacacg	Тсаалааат	Gattaaacac	Acacgcagca	CATTTTTTTT	ГСТТТСТСТС	CTTCCCTGTG	TGTTGAT
1350	1360	1370	1380	1390	1400	1410	1420	1430
ТТСТТТТТССТТ	Тсалатсааса	ACAAACACCT	TAAAAGGAGG	GAAAAAAAA	ATTCGCTTAT	FTCCTTTCAC	TCTATTATAC	ATCACCA
1440	1450	1460	1470	1480	1490	1500	1510	1520
Сталтттттата	ATTTAATCACC	Attccgacta	ACAATCCTTT	Тсттататас	ACTTTTTCTT	Гаттттатт	TTAGCATCTG	Cacccat
1530	1540	1550	1560	1570	1580	1590	1600	1610
Алатласттест	TCATTCACCAC	AATCAATCCT	Сататсаттт	CATTTCTTTT	TCAACAACTT	FTTTTTTTTT	Caaatcaaag	TTTTACT
1620 Gtccatagataa	1630 TGAACTT <u>TGAT</u> <u>Bcl</u>	CA I						

FIG. 2-Continued.

tance to 100 μ g 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 *Bg/II-Sau3AI* fragment in Fig. 2a, and a gene for the L41b protein subcloned as a 1.8-kb *XbaI* 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

EcoRI 50 70 80 90 100 110 120 130 TAGTAGACTTGACAGAGAGGGGAAGTGGAAGCGGTGTTTTCGAGGCATATCTTGCGGAAAATAGACTGACGACAGAGGGCTCCTC 140 150 160 170 180 190 200 210 220 SGGTACACAAATAGGGTTATCGATTCTATGCAATTTTTTTT	d				GAATTC	10 TTGACAGTAG	20 TTCCATTCGT	30 Taatataacc	40 TGCT
50 60 70<					EcoRI				
140 150 160 170 180 200 210 220 220 CACAAATAGAGGTTAATGGATTETATACGATTETTTTTTTTTT	50 TAGTAGACTTGACTAC	60 Agcacaaagt	70 GGAAGCGGTG	80 TTTTCGAGCA	90 FATCTTGCTG	100 AAATCACCAA	110 ATGACATGAT	ACACAGAGCT	CCTC
2007ACACAAATAGCAAATGCAGGTTAATCGATTCTATCCAATTTTTUTATCUTUGUAQAAATAAAAACUTUCUUGUAQAATAAAAACUTUCUUGUAQAATAAAAACUTUCUUGUAQAATAAAAACUTUCUUGUAQAATAAAAACUTUCUUGUAQAATAAAAACUTUCUUGUAQAAATAAAAACUTUCUUGUAQAAATAAAAACUTUCUUGUAQAAATAAAAACUTUCUUGUAQAAATAAAAACUTUCUUGUAQAAATAAAAACUTUCUUGUAQAAATATGGGGAACTTTTCCTTTCTTTTTTTTTTTTTTT	140	150	160	170	180	190	200	210	220
230 240 250 240 250 250 240 250 250 250 250 250 250 250 250 250 25	GCGTACACAAATAGCAA	ATGAGGTTAA	TCGATTCTAT	GCAATTTTTG	FATCCTGGCA	GAATAAAACC		GATATTGATG	
320 330 340 350 360 370 380 390 400 ATATTGGGGGACTTATTACCTTTTTGTGTTTTTTGTTTTTGTTTTTGTTTTTTGTTTTTT	230 CAAAATCTGTAACAAG	240 FATCGTTTGI	250 TCTATACCTT	260 CTCTCTCTTC	GGAACTACTG	GATTTTTCAC	GTCCTTTTCC	CANATATAGA	AAGT
410 420 430 440 450 460 470 480 490 CCGATCAACTTCAATTTCTTTACCCGTACATTTCTTTTTAAAATTCCACGGTTCCACGGTTCCACGTTCCAGCCGTACGTCAGCGGTTCCACGGTTCCAGCCCGTGGTGGTCGACGGAGGGGGGGG	320	330	340	350	360	370	380	390	400
	Atattggggactttac	Гатттасст1	TTTTCTTGTTI	CTTTCCTCTC	Стасаттстт	TTGTGTTTTT	TTTTTACGTG	TGGCGGACTO	3TTTC
500 510 520 530 540 550 560 570 580 TCTTGGTGATATTTCATATAAAGGATACTCCGTCAGTCAG	410	420	430	440	450	460	470	480	490
	CCGATCAACTTCAATA	TTCTTT <u>ACAC</u>	CCGTACATTI	CTTTTTAGTT	Гталааттсс	ACGTTCCATC	CTTGCACCAT	TTAGACTCG1	TACCC
590 600 610 620 630 640 650 660 670 TCCACGCTTGTTCCACACTGTTTCAGTATCAGGCAGGAAAAACGAAGCAAACCAATGCCCACTAGTACGCATTACACACAC	500	510	520	530	540	550	560	570	580
	TCTTGGTGATATTTCT	Ataaaggaa	Atactccgtca	GTCAGACCCT	CGTGTGAGTC	Gacaggaggg	Agagagagag	Agagagtcc'i	TTGCT
680 690 700 710 720 730 740 750 760 AGCCCGGTACTGGGAGGGCTGGCCACTCGATGGTTCCTCTCCACCTACTGGATGCTCAGATCCTCTGCCAGCCA	590	600	610	620	630	640	650	660	670
	TCCACGCTTGTTCCAC	Actgtttcag	Tatcaggcag	Igaaaaacgaa	GCGAACCAAT	GCCCCCAATA	Tacgattcta	CCACGATGT	AGGCT
770 780 790 800 810 820 830 840 850 TCTCTGCCCTGCCTACTGGGAGGACACCACCGCGAACTAGTCTCTGCAATACTGGAATGACCGGGCTCGAATGGTTTCTCAGGTTCCTAGGTCACCACGCGCCGCAACCACGCGCACCACGCACCACGCACCAC	680	690	700	710	720	730	740	750	760
	Agcccggtactgggag	GCTGCCCAC1	CCGATGGTTCC	CTCTCTCCACC	Tactggactc	CAGATCCTCTG	ICCCAGCCAGG	ICGUGCCCCT	CCGCC
860 870 880 890 900 910 920 930 940 ACACGGATTGGCATCTCTCCAACATTACCATTACCATATTTTTCAATGTATACAAAGAATTACATACTAACGACATCAGGCTTTAATAGTTGTCTACTGTA 950 960 970 980 990 1000 1010 1020 1030 CAGTTTCGATATTGTTTTAATATCAAGAGACCGTAACTCACACGAGGACAGTCAGT	770	780	790	800	810	820	830	840	850
	TCTCTGCCCCTGCCTA	Ctgggaggag	Caccaccgcaa	CTAGTCTCTG	Caatactgga	ATGACCGGGC	TCGATGGTT1	CTCAGGTTC	GTGAA
950 960 970 980 990 1000 1010 1020 1030 CAGTTTCGATATTGTTTAATATCAAGAGACCGTAACTCACCAGAGATCAGATCAGTCAG	860	870	880	890	900	910	920	930	940
	Acacggattggcatct	CTCCAACATT	Гассаттаат	TTTTCAATG <u>TA</u>	<u>Ta</u> caaagaat	TACATACTAA	AggCtttant	Agttgtcta	Ctgta
Interview 1040 1050 1060 1070 1080 1090 1100 1110 1120 gctatttagagatggggcttactgaaataattaaggtgtgggcgtgagatgcaagaacaacatgaaagtggtgggggttaaatgagtagggggggg	950	960	970	980	990	1000	1010	1020	1030
	CAGTTTCGATATTGTT	TTAATATCA/	Ngagaccgta/	Actcaccagag	Atcagatcag	TCACAATGG	tatgtgcgad	ttaatggtte	agcca
1130 1140 1150 1160 1170 1180 1190 1200 1210 gacaacaggttaagaacaacggaggcagtaacaacgtattataactataacatgtatgatcattattaagcaacaggaggagaatgaggaaatgaggataggagaatgaggaatgaggaatgaggaatgaggaatgaggaatgaggaatgaggaatgaggaatgaggaatgaggaatgaggaatgaggag	1040	1050	1060	1070	1080	1090	1100	1110	1120
	gctatttagagatggg	gcttactgas	aataattaagg	stgtgggcgtg	agatgcaaga	Icaacatgaaa	gtggtgggag	tttaatagag	gtaat
1220 1230 1240 1250 1260 1270 1280 1290 1300 gtccttgcaatcattaagattgttgcattagtgtacaatatgacaactgaacaaccagagtgaggaaatgaggtaggaatggagtaggaatat 1310 1320 1330 1340 1350 1360 1370 1380 1390 accatattgaaatactgcaagacatggcgtctttcaagaacatccatc	1130	1140	1150	1160	1170	1180	1190	1200	1210
	gacaacaggttaagac	aacacgaggg	gcagtaacaa	Acgtattataa	ctatcacate	tatgatcatt	attaccgata	Atcactaagt	t <u>cacc</u>
131013201330134013501360137013801390accatattgaaatactgcaagacatggcgtctttcaagaacatccatc	1220	1230	1240	1250	1260	1270	1280	1290	1300
	gtccttgcaatcatta	agattgttgd	cattagtgtad	saatatgacaa	ctgaacaaco	agagtgagga	Maatgagagta	aggacttaag	aatat
140014101420143014401450146014701480tacgctaatttgtctcacaaaattaacggtgtccgtaaacagTTAACGTTCCAAAGACCAGAAAGACTTATTGTAAGGGTAAGGCTTGT ValAsnValProLysThrArgLysThrTyrCysLysGlyLysAlaCys149015001510152015301540155015601570CGTAAGCACTCCCAACACAAGGTTACCCAATACAAGGCTGGTAAGGCTTCCTTGTACGCCCAAGGTAAGAGAAGATATGACCGTAAGCAA ArgLysHisSerGlnHisLysValThrGlnTyrLysAlaGlyLysAlaSerLeuTyrAlaGlnGlyLysArgArgTyrAspArgLysGln158015901600161016201630164016501660TCCGGTTTCGGTGGTCAAACCAAGCAAAGTTTTCCACAAGAAGGCTAAGACTACCAAGAAGATGGAATGTATGT	1310	1320	1330	1340	1350	1360	1370	1380	1390
	accatattgaaatact	gcaagacat	gcgtctttca	Magaacatcca	tcctctttaa	itatatcgcas	atgaatgtat	ttactaada	<u>atttt</u>
ValAsnValProLysThrArgLysThrTyrCysLysGlyLysAlaCys 1490 1500 1510 1520 1530 1540 1550 1560 1570 CGTAAGCACTCCCAACAAGGTTACCCAATACAAGGCTGGTAAGGCTTGCTT	1400 tac <u>gctaattttgtct</u>	1410 cacaaaatta	1420 aacggtgtccg	1430 taaacagTTA	1440 ACGTTCCAAA	1450 Ngaccagaaaa	1460 ACTTATTGT/	1470 AAGGGTAAGG	1 480 CTTGT
149015001510152015301540155015601570CGTAAGCACCACACACACAGGTTACCCAATACAAGGCTGGTAAGGCTACCTTGTACGCCCAAGGTAAGAGAAGATATGACCGTAAGCAA ArgLysHisSerG1nHisLysValThrG1nTyrLysAlaGlyLysAlaSerLeuTyrAlaGlnGlyLysArgArgTyrAspArgLysG1n158015901600161016201630164016501660TCCGGTTTCGGTGGTCAAACCAAGCAAAGTTTTCCACAAGAAGGCTAAGACTACCAAGAAGGTCGTTTTGAGATTGGAATGTATGT				ValA	snValProLy	sThrArgLys	ThrTyrCysI	JysGlyLysA	laCys
158015901600161016201630164016501660TCCGGTTTCGGTGGTCAAACCAAGCAAGCAAATTTTCCACAAGAAGGCTAACCAAGAAGGTCGTTTTGAGATTGGAATGTATGT	1490	1500	1510	1520	1530	1540	1550	1560	1570
	CGTAAGCACTCCCAAC	ACAAGGTTAG	CCCAATACAAG	GCTGGTAAGG	CTTCCTTGTA	ACGCCCAAGG1	MAGAGAAGA	MATGACCGTAA	AGCAA
	ArgLysHisSerGlnH	isLysValTI	hrGlnTyrLys	sAlaGlyLysA	laSerLeuTy	/rAlaGlnGl3	LysArgArg7	MyrAspArgL	ysGln
1670 1680 1690 1700 1710 1720 1730 1740 1750 AAGACTAAGACCCAATTGGCTTTGAAGAGATGTAAGCACTTCGAATTGGGTGGTGAAAAGAAGCAAAAGGGTCAAGCTTTGCAATTCTGA LysThrLysThrGlnLeuAlaLeuLysArgCysLysHisPheGluLeuGlyGlyGluLysLysGlnLysGlyGlnAlaLeuGlnPhe*** 1760 1770 1780 1790 1800 1810 1820 GTGTACTTTTGGAAGAATCCCCGAATGTGTTATTCATTTGTCAACTTTTTTAACTTTTCTATAAGTATCAC <u>GAATTC</u>	1580 TCCGGTTTCGGTGGTC SerGlyPheGlyGlyG	1590 AAACCAAGCA lnThrLysGI	1600 AAATTTTCCAC LnIlePhellis	1610 CAAGAAGGCTA sLysLysAlaL	1620 AGACTACCAA ysThrThrLy	1630 AGAAGGTCGT1 /sLysValVal	1640 TTTGAGATTGO LeuArgLeu(1650 BAATGTATGT(BluCysMetS)	1660 CTTGT erCys
1760 1770 1780 1790 1800 1810 1820 GTGTACTTTTGGAAGAATCCCCCGAATGTGTTATTCATTTGTCAACTTTTTTAACTTTTCTATAAGTATCAC <u>GAATTC</u>	1670 AAGACTAAGACCCAAT LysThrLysThrGlnL	1680 TGGCTTTGA euAlaLeuL	1690 Agagatgtaac ysargCysLy:	1700 GCACTTCGAAT sHisPheGluL	1710 TGGGTGGTGA euGlyGlyGl	1720 AAAAGAAGCAA LuLysLysGlr	1730 AAGGGTCAAG LysGlyGln/	1740 GCTTTGCAAT AlaLeuGlnP	1750 TCTGA he***
<u>Eco</u> RI	1760 GTGTACTTTTGGAAGA	1770 Atccccgaa'	1780 Igtgttattc/	1790 Атттстасласт	1800 TTTTTTTAAC1	1810 ГТТТСТАТААС	1820 Statcac <u>gaa</u> <u>Eco</u> i	<u>rtc</u> 31	

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

ATG-G 5'/gtacgt---35 bp---tgctaac---7 bp---tag/3' TT CM (intron size 58 bp) СТ 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) ATG-G 5'/gtatgt---464 bp---tactaac---32 bp---tag/3' TT SA (intron size 464 bp) SB ATG-G 5'/gtatgt---383 bp---tactaac---42 bp---cag/3' TT (intron size 383 bp) ъ 10 20 30 40 50 60 CM) MVNIPKTRNTYCKGKGCRKHTI-HKVTQYKSGRASLFAQGKRRYDRKQSGYGGQTKØVFHK SA) ***V****K*****T****Q-*****A*K****************F****F****F RT) ***V****R*F*--*K*G**-QP******K*KD**Y************FII*R* HM) ***V***R*F*--*K*G**-QP******KEKD**Y***R************ 40 10 20 30 50 70 80 90 100 106 CM) KAKTTKKIVLKLECT--VCKTKKQLPLKRCKHIELGGEKKQKGQALQF Homology (99.1%) (84.0%)(84.9%)(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 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 HindIII-BclI 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 EcoRI-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 L41related 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 μ g 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 μ g 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.

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