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Inducible Expression of a Gene Encoding an L41 Ribosomal Protein Responsible for the Cycloheximide-Resistant Phenotype in the Yeast *Candida maltosa*

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In a previous paper (S. Kawai, S. Murao, M. Mochizuki, I. Shibuya, K. Yano, and M. Takagi, J. Bacteriol. 174:254–262, 1992), we showed that in each genome of several yeast species, there is one of two types of L41 gene, one for an L41 (Q-type) protein which confers cycloheximide (CYH) resistance or one for an L41 (P-type) protein which does not. These genes have been suggested to be responsible for the CYH response used in taxonomy. For example, *Saccharomyces cerevisiae*, which is CYH sensitive, has a P-type L41 gene, while *Kluyveromyces fragilis* and *Candida maltosa*, which are CYH resistant, have Q-type L41 genes. However, in contrast to *K. fragilis*, which is constitutively resistant to CYH, *C. maltosa* is inducibly resistant to CYH. Here, we show that *C. maltosa* has both types of the L41 gene in its genome and that expression of the Q-type L41 gene is induced by CYH while the P-type L41 gene is constitutively expressed.

High-level cycloheximide (CYH) resistance is a taxonomic marker in yeasts. In *Candida maltosa*, as we have previously reported (20), it is associated with an allele of the gene for ribosomal protein L41 that specifies a glutamine at residue 56 (Q-type L41). Another mechanism of resistance (17) may affect CYH transport. CYH-sensitive yeasts, such as *Saccharomyces cerevisiae* (20), contain P-type L41 (proline instead of glutamine). *S. cerevisiae* has two alleles, both for the P-type protein, and a mutation to relatively low-level CYH resistance in *S. cerevisiae* is associated with altered ribosomal protein L29 (4, 19). We have also shown that the resistant yeast *C. maltosa* is phenotypically CYH sensitive but acquires resistance after exposure to CYH (6, 21). Now we show that *C. maltosa* contains both a Q-type L41 and a P-type L41. The former is inducible, and the latter is constitutive.

A gene encoding Q-type L41 is induced by CYH. The simplest explanation for why *C. maltosa* shows CYH-inducible resistance to CYH is that a gene encoding a Q-type L41 is induced by CYH, leading to the synthesis of CYH-resistant ribosomes. To test this possibility, Northern (RNA) hybridizations were done (15), with the L41-Q1b gene (previously named *RIM-C* [20]) (the *Xba*I-*Sau*3AI fragment) as a probe. The results shown in Fig. 1A clearly indicate that a Q-type L41 gene either is not expressed or is weakly expressed in the absence of CYH but is induced by the addition of CYH to the medium. In contrast, the L41 gene of the constitutively CYH-resistant yeast *Kluyveromyces fragilis* (1) was expressed at similar levels regardless of the presence or absence of CYH (data not shown). To examine whether expression of the Q-type L41 gene is directly related to the CYH-resistant phenotype, a plasmid, pMEA1-Q, in which the L41-Q1b encoding region was inserted at the *Eco*RV site between the promoter and terminator of the phosphoglycerate kinase gene from *C. maltosa*, which we had previously proved to be constitutively expressed (11), was constructed and introduced into *C. maltosa* CHA1. In Fig. 2, the growth curves of CHA1 carrying either the vector pMEA1 or pMEA1-Q in the presence and absence of CYH are shown. CHA1 became constitutively resistant to CYH by replacing the native promoter of the L41-Q1b gene with the constitutive phosphoglycerate kinase gene.

It is known that the rates of ribosome synthesis in yeasts are positively related to cell growth rates (22). The syntheses of some ribosomal components have been shown to have positive correlations with cell growth (8). Therefore, the fact that the Q-type L41 gene encoding a ribosomal component is induced by the treatment of cells with an inhibitor of protein synthesis (CYH) seems to be unusual. Some mammalian genes, such as c-*fos* and c-*jun* (3, 9), have been known to be superinduced by CYH, and some yeast genes, such as *YPT1* (10, 13) and *PDR5* (*YDR1*) (2, 5, 12), are induced by CYH.

There is a gene encoding a P-type L41 in the genome. The next question to be answered was what gene is responsible for the synthesis of L41 in the absence of CYH if a Q-type L41 gene is CYH inducible. We hypothesized the presence of a second L41 gene, presumably a P-type L41 gene, detectable by

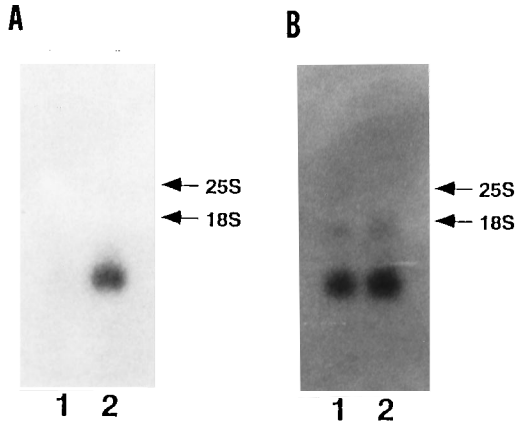

FIG. 1. Northern blot analyses of total RNAs (18) from *C. maltosa* IAM12247 cells grown in the absence (lanes 1) or presence (lanes 2) of 25 μg of CYH per ml for 2 h. Hybridizations were performed at 42°C (15). The *Xba*I-*Sau*3AI restriction enzyme fragment of the L41-Q1b gene (20) (A) and the *Hin*dIII restriction enzyme fragment of clone A (Fig. 4B) containing L41-P1a (B) were used as probes. The positions of 18S and 25S rRNAs are indicated by arrows.

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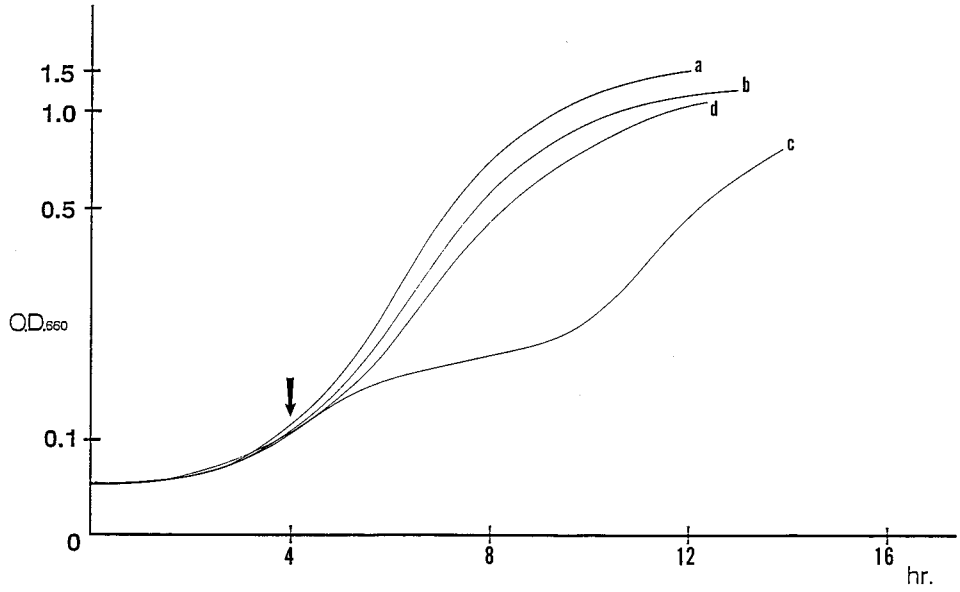


FIG. 2. Growth curves of *C. maltosa* CHA1 (*his5 ade1*) (6) carrying pMEA1 (11) (a and c) or pMEA1-Q (b and d) in the presence (c and d) or absence (a and b) of 25 μg of CYH per ml. The growth of *C. maltosa* cells was automatically recorded by a biophotorecorder (model TN-112D; Toyo Co.). An arrow indicates the time of CYH addition to the medium. $O.D._{660}$, optical density at 660 nm.

genomic Southern blot hybridization under low-stringency hybridization conditions (15), with the L41-Q1b gene as a probe. The results of this experiment (Fig. 3) supported our hypothesis by indicating that multiple bands in addition to those derived from the L41-Q1b gene itself hybridized with the probe. To analyze the primary structure of an L41 protein other than the L41-Q1b protein, the hybridizable DNA fragments were cloned and sequenced (16). About 10,000 plaques of the gene library derived from *C. maltosa* genome fragments cloned into vector EMBL3 were screened (14), with the L41-Q1b gene as a probe, and six hybridizable clones were isolated. After partial sequencing of some of them, we focused on two clones as candidates for containing a P-type L41 gene. Figure 4A and B (clones A and B, respectively) show restriction maps of the inserts in these clones. The 2.0-kb *Hpa*I fragment and the 1.1-kb *Hin*dIII fragment of clone A were separately cloned into pUC119, and the regions containing open reading frames (ORFs) were sequenced completely for both strands. As shown in Fig. 4C, there are two ORFs. The sequence of the upstream ORF, with one predicted intron, has proline at the deduced 56th amino acid residue, suggesting that this ORF encodes a P-type L41. This gene was designated the L41-P1a gene. As described previously (20), when the L41-Q1b gene was introduced into *S. cerevisiae* in a multicopy vector, the recipient strain became resistant to CYH at 10 μg/ml. In contrast, when the L41-P1a gene isolated here was introduced into *S. cerevisiae* in the same way, the recipient strain remained sensitive to CYH (less than 1 μg/ml). In this experiment, we demonstrated the presence of an mRNA of reasonable size that is hybridizable to the L41-P1a gene in recipient cells (data not shown). These results indicate that in the genome of *C. maltosa*, there is at least one gene encoding a P-type L41 which cannot confer CYH resistance on *S. cerevisiae* cells.

The sequence of the downstream ORF (Fig. 4C) also has one predicted intron and is 96% identical to the L41-Q1b gene that we previously isolated and sequenced (20). Its 56th amino acid residue is glutamine, indicating that it is a Q-type L41 gene. Therefore, we conclude that this gene is allelic to the L41-Q1b gene in the diploid genome of *C. maltosa*. This gene was designated L41-Q1a.

We also sequenced a part of the insert in clone B. There are two ORFs (Fig. 4D). One is identical to the ORF of L41-Q1b, and the other is highly homologous to L41-P1a. The gene encoding the latter ORF was designated L41-P1b. We conclude that the inserts in clones A and B are allelic to each other.

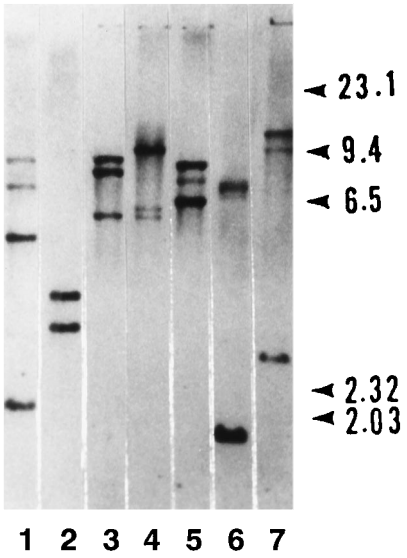
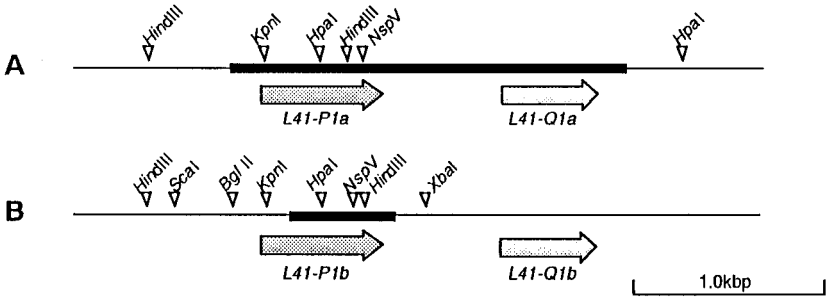


FIG. 3. Southern blot analyses of total DNAs from *C. maltosa* cells after digestion with the following restriction enzymes: *Eco*RV (lane 1), *Dra*I (lane 2), *Bam*HI (lane 3), *Kpn*I (lane 4), *Bgl*II (lane 5), *Nsp*V (lane 6), and *Xba*I (lane 7). The probe was the same as that used in Fig. 1A. Arrowheads and numbers indicate the positions and sizes (in kilobases), respectively, of molecular size markers.



C

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CCCCACAGGA AAAACAGTTA CGGGATTGAG AAAAGAGAAT TTGTTGNAAA ATCCTCCGCA  120
CTACATTTTT AAGTTAATAG TAACCTTTGA AAGTATAGAC AAGATGGGTA TGTTCAATGT  180
GGTACCATAG TGTTTTATGA ATATGGATTA AGTCCCTGAT ACGTTTGGAT ATATGAATAT  240
GAGAGAAACG AATGGATATT ATTACCGTAT TGTTGTTGTT TTGAAGGAAT AAGNAAAATT  300
CAAGATTTGA TGAAAAAGTT TATGTAAAGT CATGTCAGGT CATCTCGAGT GGAATAAAGA  360
AAGAAAATGA CGTTGAATAG GGAGAATACG TTGCAGTGGA TAAGACCATA ATCATAAAAG  420
ACTACTTTAA CATTATCAAT AATTCAAATA TACTAACAAT CATTTTTTCA TTATAGTTAA  480
CGTTCCAAAA ACTAGAAAGA CCTACTGTAA AGGTAGAGAA TGTCGTAAAC ACTCACAACA  540
CAAAGTTACC CAATACAAAG CTGGTAAATC ATCTTTGTTC GCTCAAGGTA AGAGAAGATA  600
TGACAGAAAA CAATCCGGTT TCGGTGGTCA AACCAAACCA GTTTTCCACA AAAAAGCTAA  660
AACTACCAAA AAAGTTGTGT TAAGATTGGA ATGTGTTGTC TGTAAAACCA AAGCTCAATT  720
AGCTTTAAAG AGATGTAAAC ATTTCGAATT GGGTGGTGAC AAAAAACAAA AAGGTCAAGC  780
TTTACAATTC TAATAGTGTT ATTATTTGTT TTAATGAATT AATATTAAGA ATAAAAAATA  840
TACATATACA TTTCCTTTTA ATCATGTGAC ATGTAAAACA GGAATTGTGT TTGGTTATAT  900
AAATCATTTG TATTTAATTA ATGAATATAT TGTTTTNGTA CATTAGAAAT ACCTGTCAAG  960
CGTAGTTGTT AGTGAGAAGG CTAAAGAAAA AATGAGAATT GTTGCAAAGT TTTTTTTTCT 1020
TTTTNACCAC CAAAATCAAA TCAACAAAAG TGAAAATATA ATAAAACAAA CATAAACAAT 1080
GCAACAACTC ATGTAACCTG TAAGTGTTTG GTCATTGGTA CAATGAACCT GACTCAATTG 1140
TGGTATCATC TGTTAATTGT CGGTGTACAT AATCACTTTT CTTTTTGTTT GGTTGCAGAT 1200
TGATACATTT TACCACTCAA TTTGTAATAA CTTTCGTTAG GTTTCTGGAA CACAATTATT 1260
ATTCAACATT ATTACATTCA AATGTATTGA ATTGGAATAT TTTTGGTTGG TGTAAAAAAA 1320
AAAAATCCAA ACATAAAAAA AATAAATTGT GTGACAAAAA AATGTACGTT TATCTACAGA 1380
ATAAGGAAGT TGTAAAGAAA ACCCATACAC ACACACCCCC GCTAAATTAT TATATAAATA 1440
AACCATAAGT TTTCCAAATT TTTCAAAAAA AAAATCCCCC TTTTTCTTTT AGAAAGGTTG 1500
CTAGACTGAT ACAATGGGTA TGTAATTGAA TCAATTGCTA TCTGACATTG TCAAAATATG 1560
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GTCGTAAACA TACCATTCAT AAGGTGACTC AATACAAAGC AGGTAGAGCT TCCTTATTTG 1680
CTCAAGGTAN AAGAAGATAC GATAGAAAAC AATCTGGTTA TGGTGGTCAA ACAAAACAAG 1740
TTTTCCATAA GANGGCTAAG ACTACTAAAA AGATTGTGTT GAAATTGGAA TGTACTGTTT 1800
GTAAAACCAA GAAACAATTG CCATTGAAAA GATGTAAGCA TATTGAATTG GGTGGTGAAA 1860
GAAAACAAAA GGGTCAAGCA TTACAGTTCT AAGTGCATGT ATATATTTGC ATTATCTCCA 1920
ATAATACAAA AAAGAAGACA AAACTAGTTT TGTAAATTAC AATAGTGATT TCTCTATGTG 1980
TTTCTTTTTT TTTGCAGATT ATACACGTCA AAAAAAATGA TTAAACACAC ACGCAGCACT 2040
TTTTTTTTCT TTCTCTCTCA ACAAACACCT TAAAAGGAGG GAAAAAAAAT TCGC
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D

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GGGTCATCTC AACTAGAATG AACCAACGAA ATGATGTTGA ATAGGGAGNA GACATTGATA   60
CGTTGCAGTG GATAAGACCA TAATCATAAA AAACTACTTT AACATTATCA ATAATTCAAA  120
TATACTAACA ACCATTTTTT TCATTATAGT TAACGTTCCA AAAACTAGAA AGACCTACTG  180
TAAAGGTAGA GAATGTCGTA AACACACTCA ACACAAAGTT ACCCAATACA AAGCTGGTAA  240
ATCATCTTTA TTCGCTCAAG GTAAGAGAAG ATATGACAGA AAACAATCCG GTTTCGGTGG  300
TCAAACCAAA CCAGTTTTCC ACAAAAAAGC TAAAACTACC AAAAAAGTTG TTTTAAGATT  360
GGAATGTGTT GTCTGTAAAA CCAAGGCTCA ATTAGCTTTA AAGAGATGTA AACATTTCGA  420
ATTGGGTGGT GACAAAAAAC AAAAAGGTCA AGCTTTACAA TTCTAATAGT GTTATTATTT  480
GTTTTAATGA ATTAATATTA AGAATAAAAA ATATACATAT ACATTTTCTT TTAATCATGT  540
GATATGTAAA
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FIG. 4. Restriction maps of two DNA clones isolated by using the L41-Q1b gene as a probe, clone A containing the L41-P1a and L41-Q1a genes in a head-to-tail manner (A) and its allelic clone, clone B, containing the L41-P1b and L41-Q1b genes in a head-to-tail manner (B). Arrows indicate the positions of these genes. Thick lines show the regions whose nucleotide sequences are shown in panels C and D. Some restriction sites are indicated by open arrowheads. (C and D) Nucleotide sequences of the inserts in clones A and B, respectively. The initiation and termination codons are indicated by black boxes with white letters. The sequences of predicted introns are underlined, and boxes in these introns show the consensus sequences for yeast introns. The codons for the 56th amino acid residues are overlined.

The P-type L41 gene is constitutively expressed in *C. maltosa*. We examined whether the L41-P1a gene is constitutively expressed in *C. maltosa* in the absence of CYH. Figure 1B shows the results of Northern blot hybridizations of total RNAs isolated from *C. maltosa* cells grown in the presence and absence of CYH, with the L41-P1a gene as a probe. It was found that the gene is constitutively expressed. Further experiments should clarify the mechanism that makes cells CYH resistant when both the Q-type L41 gene and the P-type L41 gene operate in the presence of CYH (when CYH-resistant and CYH-sensitive ribosomes coexist in polysomes, those polysomes are expected to be sensitive to CYH).

Nucleotide sequence accession numbers. The nucleotide sequences reported in this paper have been submitted to the DDBJ, EMBL, and GenBank databases under accession numbers D43686 and D43687.

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