JOURNAL OF BACTERIOLOGY, Oct. 1986, p. 417–419
0021-9193/86/100417-03$02.00/0

Vol. 168, No. 1

NOTES

Cloning in *Saccharomyces cerevisiae* of a Cycloheximide Resistance Gene from the *Candida maltosa* Genome Which Modifies Ribosomes

MASAMICHI TAKAGI,[1]* SHINYA KAWAI,[1] ICHIRO SHIBUYA,[1]† MASAZUMI MIYAZAKI,[2] AND KEIJI YANO[1]

Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113,[1] and Institute of Molecular Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464,[2] Japan

Received 1 May 1986/Accepted 2 July 1986

We have previously shown that cycloheximide resistance can be induced in a strain of *Candida maltosa* by modifying ribosomes (M. Takagi, S. Kawai, Y. Takata, N. Tanaka, M. Sunairi, M. Miyazaki, and K. Yano, J. Gen. Appl. Microbiol. 31:267–275, 1985). The present paper describes the cloning of the gene involved in this resistance (designated *RIM-C* for ribosome modification by cycloheximide) by using a host-vector system of *Saccharomyces cerevisiae*.

Cycloheximide is an antibiotic which inhibits eucaryotic protein synthesis. Although many yeasts including *Saccharomyces cerevisiae* are sensitive to cycloheximide, some yeast strains are resistant to this drug. Among the resistant strains, *Candida maltosa* IAM12247 has an inducible resistance mechanism, as described in our previous paper (12). Results of in vitro protein synthesis with ribosomal and S-100 fractions prepared from cycloheximide-treated and -nontreated *C. maltosa* cells indicated that ribosomes are modified in the cells during cultivation in the presence of cycloheximide. It was suggested that there is a gene (designated *RIM-C* for ribosome modification by cycloheximide) which functions in the presence of cycloheximide in such a way as to modify ribosomes so that protein synthesis in the cells is no longer inhibited by cycloheximide. In this investigation, we attempted to clone this presumptive gene by using a host-vector system of *S. cerevisiae*. We believe that the cloning of this gene will not only help to analyze the mechanism of cycloheximide resistance related to the *RIM-C* gene in *C. maltosa*, but also will provide a convenient dominant vector marker for recombinant DNA technology with *S. cerevisiae*.

Strains used in this work were *C. maltosa* IAM12247 (12), *S. cerevisiae* AH22 (**a** *leu2-3 leu2-112 his4-519 can1 gal2*) (4), *S. cerevisiae* IFO1265 (12), *Escherichia coli* MC1061 [*hsdR hsdM*$^+$ *araD139* Δ(*ara-leu*)*7697* Δ*lacX74 rpsL* GalK$^-$] (2), and *E. coli* JA221 (*recA1 leuB6 trpE5 hsdR hsdM*$^+$ *lacI thr thi*) (9). The media for cultivation (SD and YPD for yeasts; Luria broth and Davis medium for *E. coli*), transformation, and regeneration of the spheroplasts of yeast cells were as described previously (8). The lithium acetate method was also used for transformation of *S. cerevisiae* (5). For preparation of a gene bank, *C. maltosa* DNA was digested with *Sau*3A1, and fragments (5 to 6 kilobases [kb]) were isolated in sucrose by density gradient centrifugation and ligated with *Bam*HI-digested YEp13 (1) as a vector. *E. coli* MC1061 was used as a recipient of transformation of the ligated DNA. For subcloning of the *RIM-C* gene, pRIM-C1 (15.4 kb; the originally isolated plasmid from cycloheximide-resistant [Cyh^r] clones of *S. cerevisiae* AH22; see Fig. 1) was digested with *Hin*dIII, and the resulting four fragments containing *C. maltosa* DNA were ligated with *Hin*dIII-digested plasmid YEp13 and transformed into *S. cerevisiae*. From Cyh^r clones, pRIM-C2 was recovered. Then pRIM-C2 was double-digested with *Xba*I and *Sau*3A1, and a *Bam*HI linker was added to both ends of each fragment, ligated to the *Bam*HI-digested YEp13 plasmid, and transformed into *S. cerevisiae*. pRIM-C3 was isolated from Cyh^r clones.

In vitro protein synthesis and preparation of salt-washed ribosomes and the S-100 fraction were done as described previously (12). These two fractions were prepared from cells of *S. cerevisiae* AH22(pRIM-C2) grown in the presence of 25 μg of cycloheximide per ml and from cells of *S. cerevisiae* IFO1265 as the control (12).

To clone the presumptive gene of *C. maltosa* responsible for cycloheximide resistance, *S. cerevisiae* AH22 (*leu2*) was

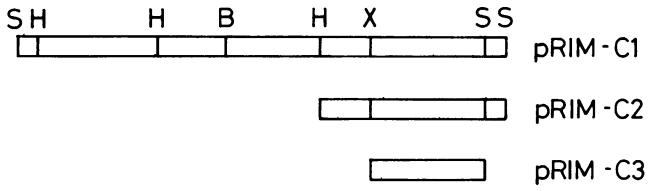

FIG. 1. Subcloning of the *RIM-C* gene. From a gene bank of the *C. maltosa* genome prepared by the partial digestion of the total DNA with *Sau*3A1 followed by insertion into the *Bam*HI-digested plasmid YEp13, a recombinant plasmid was found, which conferred Cyh^r on *S. cerevisiae*. The structure of the plasmid was analyzed by restriction enzyme digestion. The structure of the insert is shown in the top line with the name of the plasmid, pRIM-C1, which consists of the insert (4.7 kb) and YEp13. Subcloning was performed and the plasmids pRIM-C2 and pRIM-C3 were obtained, which have inserts as shown in the middle (1.8 kb) and bottom (1.1 kb) lines, respectively. Abbreviations of the restriction enzymes: B, *Bgl*II; H, *Hin*dIII; S, *Sau*3A1; X, *Xba*I.

* Corresponding author.
† Present address: Institute for Production Research and Development of Nikka Whisky Distilling Co., Ltd., Kashiwa, Chiba 277, Japan.

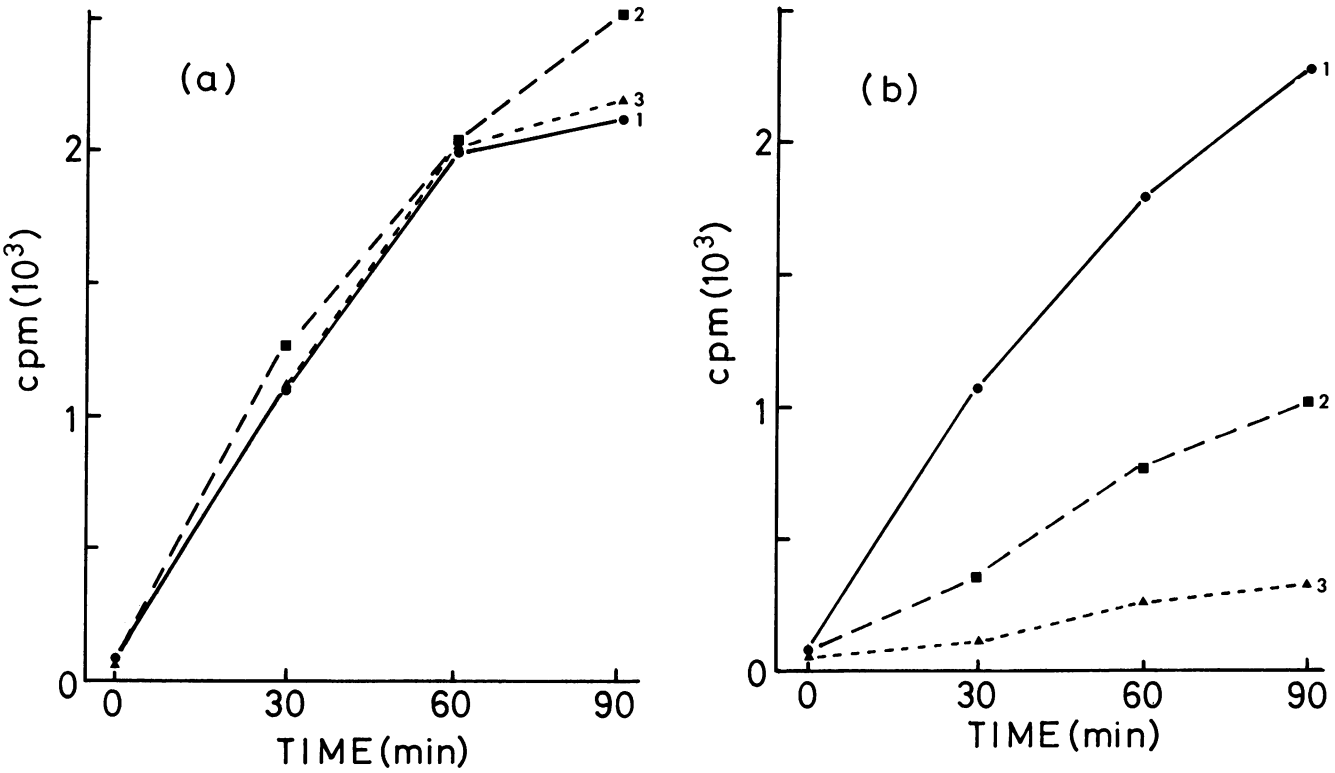

FIG. 2. Effect of cycloheximide on in vitro protein synthesis directed by poly(U). Fraction S-100 was obtained from *S. cerevisiae*(pRIM-C2) cultured in the presence of 25 μg of cycloheximide per ml. Ribosomes were prepared from (a) *S. cerevisiae* AH22(pRIM-C2) cultured as described in the text or (b) nontreated *S. cerevisiae* IFO1256. [^{14}C]phenylalanine incorporation was assayed in the presence of 0 (●), 2.5 (■), or 25 (▲) μg of cycloheximide per ml.

transformed by the lithium acetate method with a gene bank constructed in vector YEp13 (*LEU2*$^+$). *Leu*$^+$ colonies were isolated and confirmed for their *Leu*$^+$ phenotype. They were spread on YPD agar containing 10 μg of cycloheximide per ml. Of 1,050 *Leu*$^+$ clones tested, 2 were Cyh^r. Plasmid DNA was isolated from both of them, transformed, and amplified in *E. coli* JA221, and the structures of both were analyzed by restriction enzymes. It was found that they were identical to each other (Fig. 1). A representative plasmid was designated pRIM-C1 (15.4 kb); it had a 4.7-kb insert of *C. maltosa* genomic DNA in the 10.7-kb YEp13 vector. Subcloning of the fragment was performed as described above (pRIM-C2 was obtained as shown in Fig. 1). *S. cerevisiae* AH22 carrying this plasmid made colonies even in the presence of 1 mg of cycloheximide per ml, but *S. cerevisiae* AH22 itself was sensitive to cycloheximide at 0.2 μg/ml.

To determine whether ribosome modification occurred in *S. cerevisiae*(pRIM-C2), as it did in *C. maltosa*, in vitro protein synthesis was done. When ribosomes from cycloheximide-treated *S. cerevisiae*(pRIM-C2) were used in combination with fraction S-100 from either cycloheximide-treated *S. cerevisiae*(pRIM-C2) (Fig. 2a) or nontreated *S. cerevisiae* IFO1256 (data not shown), the incorporation activity was scarcely influenced by the presence of cycloheximide at 2.5 or 25 μg/ml. In contrast, in the systems containing ribosomes from the control *S. cerevisiae*, the incorporation activity was inhibited by cycloheximide irrespective of the origin of the S-100 [S-100 from cycloheximide-treated *S. cerevisiae*(pRIM-C2), (Fig. 2b) or S-100 from the control *S. cerevisiae* (data not shown)]. From these results, we concluded that the *RIM-C* gene cloned in the pRIM-C2 plasmid modifies ribosomes so that they become resistant to cycloheximide.

Several dominant vector markers have been recognized in *S. cerevisiae* (3, 6, 7, 10, 11). To use the *RIM-C* gene as a selectable marker of plasmids in *S. cerevisiae*, we recommend the following procedure. *S. cerevisiae* AH22 is transformed with pRIM-C2 (or pRIM-C3) by the spheroplast method. The spheroplasts are mixed with about 20 ml of YPD containing 3% agar and 1.2 M sorbitol, poured into a petri dish, and incubated overnight at 30°C. The next morning, about 10 ml of YPD containing 0.6% agar and 5 μg of cycloheximide per ml is poured onto the dish, which is then incubated at 30°C. After 3 to 5 days, only those cells carrying pRIM-C2 (or pRIM-C3) make colonies. By this procedure, it was confirmed that *S. cerevisiae* AH22 carrying either of these plasmids can be isolated without using *LEU2*$^+$ as a selectable marker. The numbers of *LEU2*$^+$ colonies and Cyh^r colonies were almost the same when these colonies were selected and counted separately. The *RIM-C* gene may be useful as a dominant vector marker for recombinant DNA technology of many cycloheximide-sensitive yeasts that have no selectable marker.

LITERATURE CITED

1. **Broach, J. R., J. N. Strathern, and J. B. Hicks.** 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. Gene **8**:121–133.
2. **Casadaban, M., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J. Mol. Biol. **138**:179–207.
3. **Gritz, L., and J. Davis.** 1983. Plasmid-encoded hygromycin B

resistance: the sequence of hygromycin B phosphotransferase gene and its expression in *Escherichia coli* and *Saccharomyces cerevisiae*. Gene **25**:179–188.

4. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. USA **75**:1929–1933.
5. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
6. **Jimenez, A., and J. Davis.** 1980. Expression of a transposable antibiotic resistance element in *Saccharomyces*. Nature (London) **287**:869–871.
7. **Karin, M., R. Najarian, A. Haslinger, P. Valenzuela, J. Welch, and S. Fogel.** 1984. Primary structure and transcription of an amplified genetic locus: the *CUP* locus of yeast. Proc. Natl. Acad. Sci. USA **81**:337–341.
8. **Kawamura, M., M. Takagi, and K. Yano.** 1983. Cloning of a *LEU* gene and an *ARS* site of *Candida maltosa*. Gene **24**:157–162.
9. **Masui, Y., T. Mizuno, and M. Inouye.** 1984. Novel high-level expression cloning vehicles: 10^4-fold amplification of *Escherichia coli* minor protein. Bio/Technology **2**:81–85.
10. **Miyajima, A., I. Miyajima, K.-I. Arai, and N. Arai.** 1984. Expression of plasmid R388-encoded type II dihydrofolate reductase as a dominant selective marker in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:407–414.
11. **Rine, J., W. Hansen, E. Hardeman, and R. W. Davis.** 1983. Targeted selection of recombinant clones through gene dosage effects. Proc. Natl. Acad. Sci. USA **80**:6750–6754.
12. **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.