NOTES

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

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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 (leu2-3 leu2-112 his4-519 can1 gal2) (4), *S. cerevisiae* IFO1265 (12), *Escherichia coli* MC1061 [hsdR hsdM+ araD139 Δ(aral-rew)7697 ΔlacX74 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 Sau3A1, and fragments (5 to 6 kilobases [kb]) were isolated in sucrose by density gradient centrifugation and ligated with *BamHI*-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'] clones of *S. cerevisiae* AH22; see Fig. 1) was digested with *HindIII*, and the resulting four fragments containing *C. maltosa* DNA were ligated with *HindIII*-digested plasmid YEp13 and transformed into *S. cerevisiae*. From Cyh' clones, pRIM-C2 was recovered. Then pRIM-C2 was double-digested with XbaI and Sau3A1, and a *BamHI* linker was added to both ends of each fragment, ligated to the *BamHI*-digested YEp13 plasmid, and transformed into *S. cerevisiae*. pRIM-C3 was isolated from Cyh' 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 digested with *BamHI*, and the resulting fragments were ligated with *BamHI*-digested YEp13 (1). Fragments 10 to 20 bp did not hybridize with DNA from *S. cerevisiae* AH22 (leu2) (12). From the DNA remaining after digestion with *BamHI*, fragments of 10 kb were ligated into a plasmid. The plasmid with inserts was designated YEp-C1 (Fig. 1). The plasmids YEp-C1 and pRIM-C3 were transformed into *S. cerevisiae* AH22, and the resulting four fragments containing *C. maltosa* DNA were ligated with *HindIII*-digested plasmid YEp13 and transformed into *S. cerevisiae*. From Cyh' clones, pRIM-C2 was recovered. Then pRIM-C2 was double-digested with XbaI and Sau3A1, and a *BamHI* linker was added to both ends of each fragment, ligated to the *BamHI*-digested YEp13 plasmid, and transformed into *S. cerevisiae*. pRIM-C3 was isolated from Cyh' clones.

![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 Sau3A1 followed by insertion into the *BamHI*-digested plasmid YEp13, a recombinant plasmid was found, which conferred Cyh' 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, *BglII*; H, *HindIII*; S, Sau3A1; X, XbaI.](http://jb.asm.org/Downloaded from October 28, 2017 by guest)
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 Cyh². 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 Cyh² 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

