Proc. Natl. Acad. Sci. USA
Vol. 78, No. 1, pp. 238–242, January 1981
Biochemistry

Cloning of yeast gene for trichodermin resistance and ribosomal protein L3

(*Saccharomyces cerevisiae*/eukaryotic antibiotic resistance/yeast transformation)

HOWARD M. FRIED* AND JONATHAN R. WARNER*†

Departments of *Biochemistry and †Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461

Communicated by Harry Eagle, October 13, 1980

ABSTRACT **Yeast cells sensitive to the eukaryotic protein synthesis inhibitor trichodermin have been transformed with autonomously replicating recombinant plasmids carrying DNA fragments of the genome of a trichodermin-resistant yeast strain. After selection for trichodermin-resistant cells, several transformants yielded a plasmid containing a 13.5-kilobase (kb) DNA fragment that encodes the trichodermin resistance gene, *tcm1*, and the gene for ribosomal protein L3, the largest of the yeast ribosomal proteins. Cells carrying this plasmid are resistant to trichodermin and to the related drug verrucarin A as well as to the unrelated drug anisomycin. This pattern of resistance is similar to that exhibited by strains carrying a chromosomal copy of *tcm1*. Moreover, polyribosomes prepared from transformed cells are resistant to trichodermin when tested in an *in vitro* protein synthesis assay. Subcloning of the 13.5-kb DNA fragment revealed that the gene for *tcm1* and the gene for protein L3 are contained within a 3.2-kb segment. These results suggest that the gene for trichodermin resistance in yeast specifies ribosomal protein L3.**

Studies of *Escherichia coli* have yielded a wealth of information on cell strategies for efficient modulation of production of ribosomes (1). Similar work with *Saccharomyces cerevisiae* has been hampered by lack of information on the genetic arrangement of ribosomal components. Recently, however, Petes (2) has shown that all 100 copies of the rRNA genes are present in tandem array on chromosome XII. In contrast, Woolford *et al.* (3) isolated the genes for four ribosomal proteins and found them all to be unlinked.

For our studies on the regulation of ribosomal protein biosynthesis, it was necessary to isolate a ribosomal protein gene that could express a phenotype at the level of the intact cell. A likely candidate was the gene specifying resistance to trichodermin. Trichodermin, a substituted 12,13-epoxytrichothecene, has been shown to inhibit eukaryotic protein synthesis by blocking the peptidyl transferase (elongation) step (4). In *S. cerevisiae*, resistance to trichodermin is conferred by a single gene, *tcm1*, located on chromosome XV (5). The 60S ribosomal subunits of strains carrying *tcm1* exhibit altered binding of trichodermin (6), and thus *tcm1* appears to be a genetic marker for a component of the 60S ribosomal subunit.

This report describes the isolation of a plasmid which carries a fragment of yeast DNA specifying resistance to trichodermin (Tcm^R). The plasmid, pTCM, was obtained by the transformation of intact yeast cells (7) with a clone bank of DNA from a strain carrying *tcm1*. When introduced into yeast cells, pTCM renders sensitive cells partially resistant to trichodermin. Furthermore, polyribosomes from such transformed cells are also partially resistant to trichodermin. The plasmid carries the gene for the 60S ribosomal protein L3. Both the resistance gene and ribosomal protein gene are contained within the same small segment of DNA. Therefore, we suggest that L3 is the protein responsible for Tcm^R in yeast.

METHODS

Strains, Media, and Chemicals. The following strains were used: *E. coli*, C600 (F^- *leuB pro*$^-$ *thi*$^-$ *lacY rK*$^-$ *mK*$^-$); *S. cerevisiae*, HF-T1 (*α his4-864 tcm1*), a derivative of a spontaneous Tcm^R strain provided by C. McLaughlin [the Tcm^R trait of HF-T1 was found to be tightly linked to *pet17* (data not shown) and it is therefore allelic to the *tcm1* locus described previously (5)]; HF100-8A (*α his4-846 lys5 aro2 trp5 cyh2 tcm1*); JC 27 (*α leu2-3 leu2-112 his4*) obtained from J. Cohen; HF-D100, a diploid obtained by mating JC27 with HF100-8A; and DBY703 (*α his3-Δ1 trp1-289 ura3-52*) obtained from David Botstein. LB (rich medium) for *E. coli* and YPD (rich medium) and SD (minimal medium) for yeast were used throughout. Trichodermin was the generous gift of W. O. Godtfredsen.

Construction of Clone Bank and DNA Preparation and Isolation. The yeast transformation vector YEp13, obtained from J. Broach, consists of the bacterial plasmid pBR322 (carrying the genes for resistance of *E. coli* to ampicillin and tetracycline) joined to a portion of the yeast 2-μm plasmid (allowing autonomous replication in yeast) and also carrying the yeast *LEU2* gene (allowing selection in *leu2* strains) (8). DNA from the Tcm^R strain HF100-8A was prepared by the method of Cryer *et al.* (9), purified further by buoyant density centrifugation in KI (10), digested with *Bam*HI, ligated to YEp13 at the *Bam*HI site within the tetracycline gene, and used to transform *E. coli* C600. This process yielded 4200 ampicillin-resistant, tetracycline-sensitive transformants which were divided into eight different pools of about 500 clones. Each pool of clones was grown on sterile nitrocellulose filters and the filters were incubated in liquid LB (containing ampicillin at 20 μg/ml), providing precultures for preparation of plasmid DNA by standard procedures. Plasmid DNA from yeast transformants was prepared according to a procedure developed by J. Cohen (personal communication). Plasmid YEp6 (obtained from David Botstein) carries the yeast HIS3 gene. The gene was purified by digesting YEp6 with *Bam*HI and recovering the 1.7-kilobase (kb) fragment (containing HIS3) from an agarose gel.

Yeast Transformation. Approximately 10^9 spheroplasts of the strain JC27 were transformed with 25 μg of each pool of plasmid DNA by the procedure of Hinnen *et al.* (7) to yield about 10^4 Leu^+ transformants per pool. Tcm^R cells were selected from among these transformants by homogenizing the agar medium, allowing the cells to grow out for several generations in liquid SD, and then plating about 10^6 cells per pool on SD plates containing histidine and trichodermin at 2 μg/ml.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: Tcm^R and Tcm^S, trichodermin resistance (resistant) and sensitivity (sensitive); kb, kilobase pairs.

Table 1. Maintenance of plasmids pTCM and YEp13 in yeast transformants

Cloning vector	Growth conditions		Viable colonies, no.	Phenotype, %		
	Cells grown in	Cells plated on		Leu$^+$,Tcm^R	Leu$^+$,Tcm^S	Leu$^-$,Tcm^R
pTCM	YPD	YPD	400	5	0	0
	YPD	YPD + Tcm	20	100	0	0
	YPD + Tcm	YPD	400	50	0	0
	YPD + Tcm	YPD + Tcm	200	100	0	0
YEp13	YPD	YPD	400	0	50	0
	YPD	YPD + Tcm	0	—	—	—

Strain JC27, harboring cloning vector YEp13 or pTCM, was grown in liquid SD medium containing histidine. The cultures were then diluted into nonselective (YPD) or selective [YPD + trichodermin (Tcm)] medium for growth for 10–12 doublings (to $\approx 10^8$/ml). Then they were diluted and approximately 500 cells were plated on the solid medium. "Viable colonies" shows the numbers of colonies that grew on the solid media. These colonies were replica plated to SD + His (to determine Leu phenotype) and to YPD + trichodermin (to determine Tcm phenotype). The percentage of the replica-plated colonies that grew is shown under "Phenotype." The trichodermin concentration was 10 μg/ml throughout.

R Loop Positive Translation. Supercoiled plasmid DNA was relaxed by heating to 70°C, hybridized under R-loop conditions with yeast poly(A)$^+$ RNA, and separated from unhybridized RNA as described by Woolford *et al.* (3). RNA was released from the DNA by incubating at 100°C for 1 min and was translated in a wheat germ extract. The translation products were analyzed on one- and two-dimensional polyacrylamide gels as described (11).

RESULTS

Strategy for Transformation of Yeast to Tcm^R. Antibiotic-resistance mutations which lead to alterations in the primary structure of ribosomal proteins are generally recessive because ribosomes do not function alone but as polyribosomes. Nevertheless, using diploids heterozygous for *tcm1*, we found that Tcm^R can be codominant at low drug concentrations. This observation suggested that it might be possible to select resistant transformants from a sensitive recipient, provided that the preexisting (sensitive) ribosomes are first diluted by several generations of growth after transformation. Therefore, we constructed a clone bank of DNA from a Tcm^R strain, using as a vector a plasmid carrying the yeast *LEU2* gene. A leu2, Tcm^S strain was transformed with this plasmid DNA and transformants were obtained by selecting first for Leu$^+$ colonies in a medium lacking leucine. These Leu$^+$ transformants were then screened for Leu$^+$, Tcm^R cells on medium lacking leucine and containing 2 μg of trichodermin per ml, the lowest concentration that prevented growth of the recipient strain while permitting growth of a diploid heterozygous for *tcm1*.

Analysis of Tcm^R Transformants. When colonies of Leu$^+$ transformants were recovered and plated on trichodermin-containing medium, several colonies of resistant transformants were found. To determine if the resistance of these transformants resulted from a plasmid-associated gene or a spontaneous chromosomal mutation, we examined the segregation of the Leu$^+$ and Tcm^R phenotypes.

A useful characteristic of yeast cells transformed with 2-μm DNA-containing plasmids is the loss of these plasmids during mitotic growth under nonselective conditions (12–14). If Tcm^R is due to the presence of a YEp13 plasmid carrying the resistance gene, this trait should be mitotically unstable and cosegregate with Leu$^+$ after growth in rich medium. To test this prediction, cells were grown for about 10 generations in rich medium and then were plated and tested by replica plating onto minimal medium to determine their Leu$^+$ phenotype and onto rich medium containing trichodermin to determine their resistance phenotype. [Controls demonstrated that all cells maintained on SD (selective) medium were Tcm^R (data not shown).] An example of the segregation pattern for one transformant is shown in Table 1. The top two lines show that, of cells carrying the resistance trait, 95% had lost their plasmid (5% Leu$^+$) after nonselective growth. Nevertheless, those cells that retained their Leu$^+$ phenotype were also Tcm^R and those that retained their resistance phenotype were also Leu$^+$. Selection for Tcm^R was as effective in maintaining the Leu$^+$ phenotype as was growth in SD

Table 2. Antibiotic resistance of strains used in this study

Strain	Trichodermin genotype*	Resistance *in vivo*†				Resistance *in vitro*‡
		Trichodermin	Verrucarin A	Anisomycin	Cycloheximide	% amino acid incorporation in trichodermin (10 μg/ml)
JC27 [YEp13]	+	−	−	−	−	37 ± 4
HF - T1	*tcm1*	+	+	+	−	77 ± 12
HF - D100	+/*tcm1*	+	+	+	−	56 ± 8
JC27 [pTCM]	+/[*tcm*^{r}]	+	+	+	−	55 ± 2

* + indicates the wild-type (sensitive) allele of *tcm1*.

† The strains were grown in SD medium containing histidine and diluted to give about 10^3 single colonies on SD + His plates. The colonies were then replica plated to SD + His plates containing trichodermin (10 μg/ml), verrucarin A (5 μg/ml), anisomycin (100 μg/ml), or cycloheximide (2 μg/ml). +, Growth; −, failure to grow. Although both the diploid and the pTCM transformant grew on all the drug-containing plates (except cycloheximide), both strains grew slowly (compared to HF-T1) and no difference in growth rate could be discerned.

‡ Cell extracts were prepared essentially as described by Gallis and Young (15). Incubation without added template was carried out at 30°C. At 2-min intervals, 10-μl samples were removed to filters which were processed to determine hot-acid precipitable radioactivity. The incorporation was linear for 6 min and plateaued by 8–10 min which was identical behavior to that reported previously (15). The initial rate of incorporation was determined by least squares analysis and ranged from 8000 to 25,000 cpm/min in the absence of trichodermin.

medium. Despite the fact that their plating efficiency on trichodermin was halved, 100% of the cells grown in rich medium containing trichodermin were found to be Leu$^+$. These results demonstrate that both the Leu$^+$ and Tcm^R phenotypes cosegregate and are mitotically unstable, suggesting that the resistance trait of the transformants is plasmid associated. On the other hand, attempts to demonstrate cosegregation of the traits during meiosis were frustrated by the fact that virtually all the meiotic products recovered were Leu$^-$ and Tcm^S. This failure may be due to the high degree of instability of the Tcm^R plasmid (compare lines 1 and 5 in Table 1).

To examine the plasmids present in Tcm^R transformants, DNA was extracted from three resistant yeast transformants and used to transform *E. coli* to ampicillin resistance. Plasmid DNA was then isolated from a single ampicillin-resistant bacterial transformant for each of the three original yeast transformants. Digestion of these plasmids with *Bam*HI revealed that all three consisted of the same two fragments, one corresponding to the YEp13 vector (10.7 kb) and another of ≈13.5 kb. When one of these plasmids, denoted pTCM, was reintroduced into strain JC27, 100% of the Leu$^+$ transformants tested were also Tcm^R. These results support further the conclusion that the Tcm^R trait is plasmid associated. On the other hand, no transformants were observed when JC27 was transformed with pTCM and the cells were plated directly in minimal medium containing trichodermin at 2, 5, or 10 μg/ml. This result is consistent with the suggestion that the Tcm^R gene of the pTCM encodes a ribosomal protein because challenging transformants immediately with the drug did not allow the sensitive ribosomes to be diluted by resistant ribosomes.

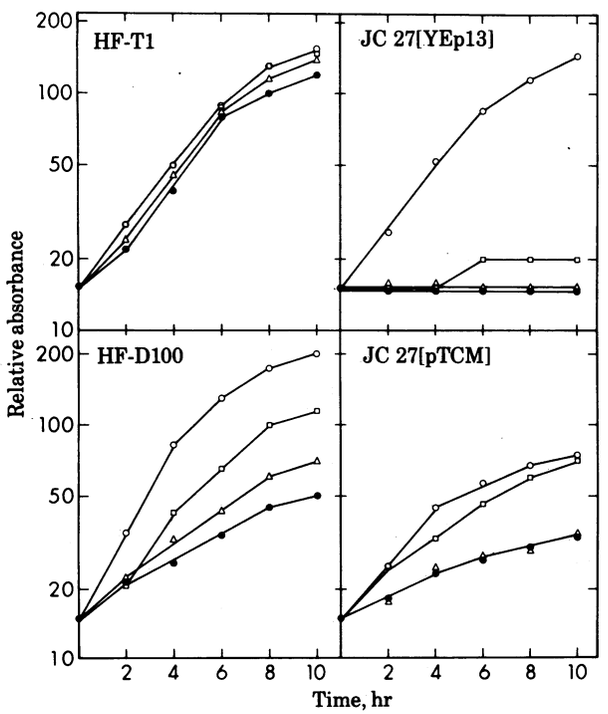

FIG. 1. Growth of strains in the presence of trichodermin. Stock cultures of each strain described in Table 2 were diluted into SD + His and grown overnight to midlogarithmic phase. Each culture was then diluted to an initial density of ≈2 × 10^6 cells per ml (Klett = 10) and allowed to grow to Klett = 15. Each culture was then divided into four aliquots to which were added 0, 2, 5, and 10 μg of trichodermin per ml. The cell density of each was then monitored as a function of time. ○, No drug; □, trichodermin at 2 μg/ml; △, trichodermin at 5 μg/ml; ●, trichodermin at 10 μg/ml.

Evidence That pTCM Carries the *tcm1* Gene. In order to establish that the Tcm^R trait carried on pTCM is actually the *tcm1* locus described previously, we compared several physiological aspects of the Tcm^R transformants to those of strains having a chromosomal copy of *tcm1*.

Grant *et al.* (5) reported that mutants carrying the *tcm1* gene are cross-resistant to other trichothecene antibiotics as well as to the unrelated drug anisomycin. The Tcm^R transformants were resistant to the trichothecene derivative verrucarin A as well as to anisomycin, suggesting that pTCM specifies the same gene product as *tcm1* (Table 2). The transformants were not resistant to cycloheximide, an unrelated inhibitor of 60S ribosome function.

As a second test that pTCM carries *tcm1*, we measured the effect of various concentrations of trichodermin on the growth of the same strains described in Table 2. Both the diploid het-

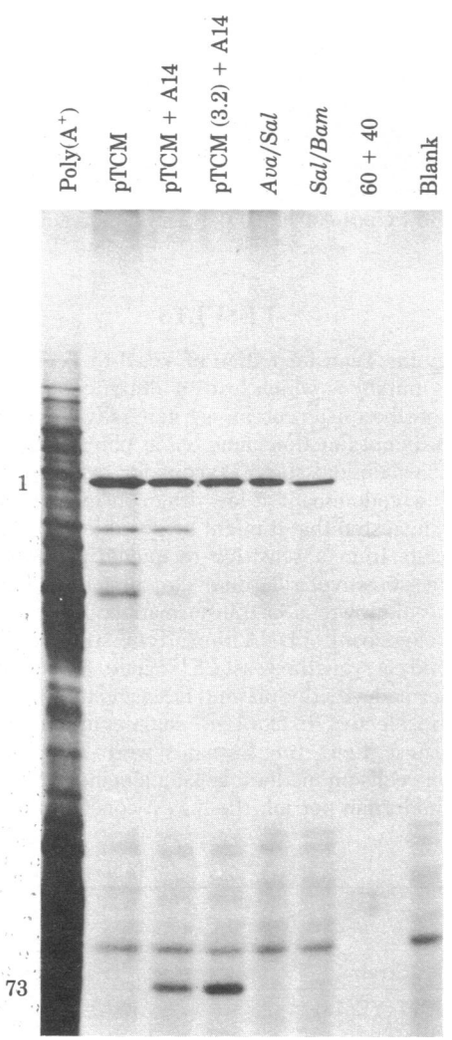

FIG. 2. Analysis of translation products of mRNA hybridized to various plasmids. Total yeast mRNA was R-looped to various plasmids, recovered, and translated in a wheat germ extract. The translation product was extracted and analyzed on a one-dimensional 10–15% polyacrylamide gradient gel containing $NaDodSO_4$ (17). The major product of pTCM and the only product of the subclone pTCM (3.2), labeled 1, has the same mobility as the largest protein of the purified ribosomal proteins (60S + 40S). Plasmid A14, which contains the gene for ribosomal protein 73, was added to two hybridizations as a positive control. *Ava/Sal* and *Sal/Bam* represent the two halves of pTCM (3.2) cut with *Sal* I and *Pst* (see Fig. 4 and text). "Poly(A)$^+$" and "Blank" represent translation products with or without the addition of total mRNA, respectively.

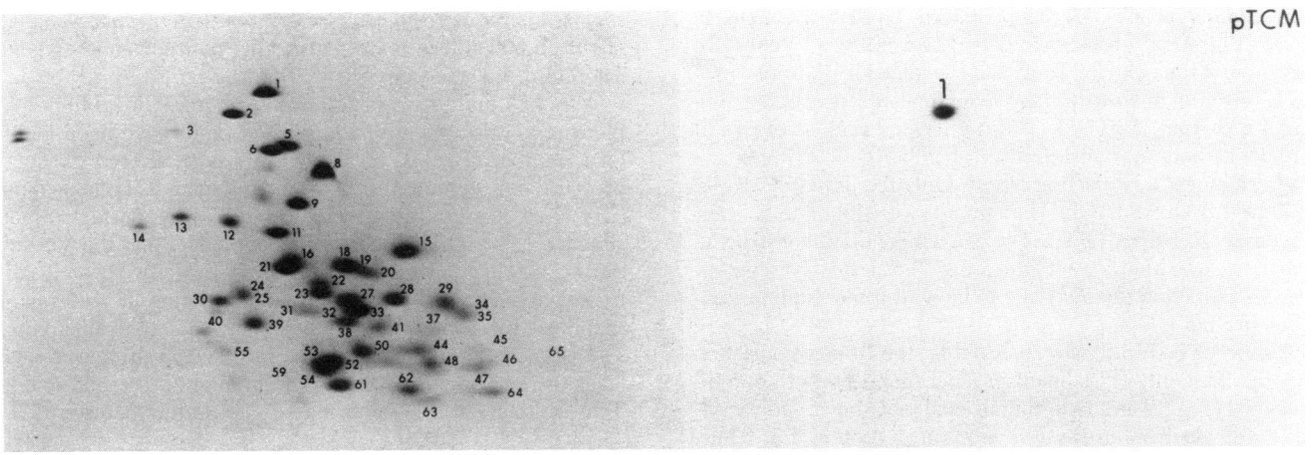

FIG. 3. Two-dimensional electrophoresis of translation products of mRNA hybridized to pTCM. A portion of the translation product from pTCM (Fig. 2) was analyzed on a two-dimensional gel [(pH 5, 8 M urea/NaDodSO$_4$ (11)] (*Right*). The major product is indicated by "1" as it migrates directly over spot 1 of purified ribosomal proteins (*Left*).

erozygous for *tcm1* and the transformant containing pTCM are able to grow in drug concentrations at least as high as 10 μg/ml, although neither strain grew as well as the resistant haploid at the same concentration (Fig. 1). The similarity of the effect of trichodermin on the growth of the transformant and of a heterozygous diploid suggests again that the pTCM specifies the same gene product as *tcm1*. However, at all concentrations of trichodermin, the transformant grew slightly faster than the heterozygous diploid, despite the fact that the transformant grew slower in the absence of the drug. This result suggests that the transformant contains a greater proportion of resistant gene products than does the heterozygote.

To demonstrate that the Tcm^R of the transformants was not due to the development of a permeability barrier, we determined the effect of trichodermin on amino acid incorporation by cell extracts (Table 2). These assay conditions measure run-off from endogenous polysomes (15). Although a given concentration of trichodermin is far less effective in inhibiting protein synthesis *in vitro* than it is in slowing cell growth (16), it is apparent that *in vitro* the resistance of both the transformant and a diploid heterozygous for *tcm1* is intermediate between the wild type and the homozygous resistant strain. These data are consistent with the transformant containing a mixture of resistant and sensitive ribosomes, suggesting that pTCM carries the gene for a component of the 60S ribosome.

Evidence That pTCM Encodes Ribosomal Protein L3. Because the gene product of the *tcm1* locus was unknown, we used a positive translation assay (3) to determine if pTCM carries a ribosomal protein gene. Yeast poly(A)$^+$ RNA was incubated with pTCM plasmid DNA under conditions that allow the formation of DNA·RNA hybrids (R loops). The hybridized RNA was recovered from the R loops and translated in the presence of [^{35}S]methionine in a wheat germ cell-free translation assay. A one-dimensional NaDodSO$_4$/polyacrylamide gel displaying the ^{35}S-labeled products showed a prominent band, corresponding in mobility to the largest ribosomal protein (Fig. 2). The three less-intense bands also were seen with other clones of YEp13 and probably represent genes on the vector. Analysis with a two-dimensional polyacrylamide gel system that displays basic proteins demonstrated a prominent radioactive spot comigrating with ribosomal protein 1 (Fig. 3). Because we have shown that this protein can be faithfully translated *in vitro* (17) and because no other yeast protein migrates in this position, we suggest that the product of the *tcm1* locus in yeast is ribosomal protein 1. This assignment has been verified by analyzing the translation product on a different, two-dimensional gel system (18) in which a single spot appeared over a protein called L3 in the standard nomenclature (19, 20). L3 and 1 have been shown to be identical by peptide analysis (21).

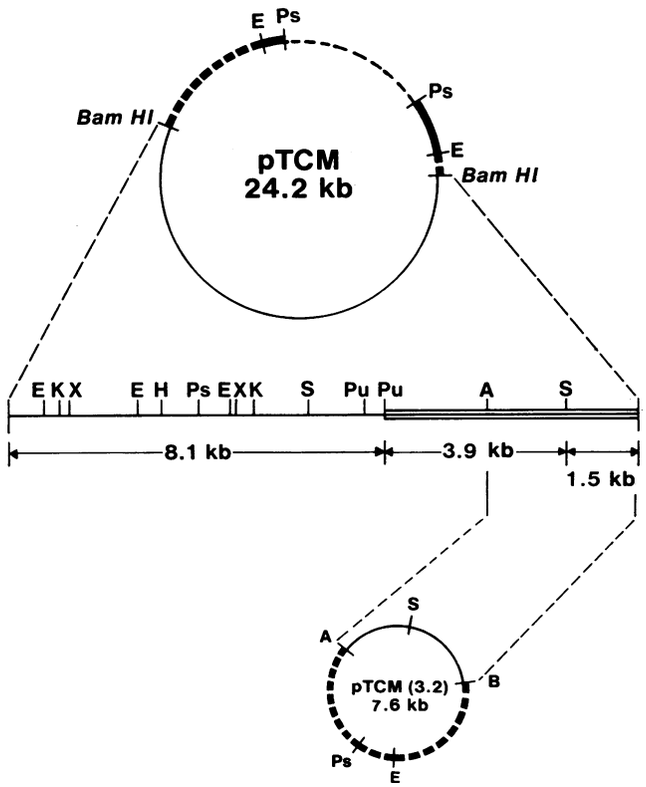

FIG. 4. Restriction map of pTCM and its subclone pTCM (3.2). Restriction sites that delineate the various portions of the cloning vector YEp13 are shown in circular figure at top. Heavy broken line, pBR322 sequences; heavy continuous line, 2-μm DNA sequences; light broken line, LEU2 structural gene. The map, drawn roughly to scale, of the 13.5-kb *Bam*HI fragment was determined by a series of single and double restriction digests of either the intact plasmid or of the *Bam*HI fragment purified from a preparative agarose gel. E, *Eco*RI; K, *Kpn* I; X, *Xho* I; H, *Hin*dIII; Ps, *Pst* I; Pv, *Pvu* I; S, *Sal* I; A, *Ava* I. Two additional *Sal* I and *Ava* I sites in the left half of the *Bam*HI fragment have been omitted for clarity. The fragments that hybridize with mRNA are shown with a double line. The figure below this fragment shows the 3.2-kb *Ava* I/*Bam*HI segment subcloned in pBR325.

Subcloning of pTCM. The 13.5-kb *Bam*HI fragment of pTCM (Fig. 4) is sufficient to encode several yeast genes. To determine if the gene for Tcm^R and the gene for ribosomal protein L3 are contained within the same smaller segment, we subcloned a portion of pTCM. The transcribed regions of pTCM were identified by hybridization of poly(A)$^+$ [^{32}P]RNA to a Southern blot of a restriction digest. Only the 3.9-kb *Pvu* I-*Sal* I and the 1.5-kb *Sal* I/*Bam*HI fragments were found to be complementary to poly(A)$^+$RNA. For convenience, we constructed a subclone, pTCM (3.2), with the 3.2-kb *Ava* I/*Bam*HI segment (Fig. 4), expecting the *Sal* I site to lie within the sequence encoding protein L3.

Plasmid pTCM (3.2) was analyzed by the R-loop translation assay (3). The only ^{35}S-labeled product found by both one-dimensional (Fig. 2) and two-dimensional (not shown) polyacrylamide gel electrophoresis was ribosomal protein L3. Thus, pTCM (3.2) is indeed a subclone containing the gene for protein L3. To ascertain that the *Sal* I site of pTCM (3.2) is within the gene, we digested the plasmid with *Sal* I and *Pst* I, separated the fragments on a 0.8% agarose gel, recovered the DNA from the gel, and analyzed the two halves of the plasmid by R-loop translation. mRNA for ribosomal protein L3 was found to be complementary to both segments (Fig. 2), confirming that the *Sal* I site is within the mRNA coding for L3.

Finally, to determine if pTCM (3.2) retains the *tcm1* gene, we digested the plasmid with *Bam*HI and ligated it to a 1.7-kb *Bam*HI fragment containing the HIS3 gene of yeast. This HIS3 derivative of pTCM (3.2) was introduced into DBY703, a his3,Tcm^S strain. Because His^+ transformants of DBY703 were found to be partially Tcm^R, pTCM (3.2) must contain the *tcm1* gene.

DISCUSSION

The data presented above suggest that the plasmid pTCM contains the gene responsible for Tcm^R and that this gene specifies ribosomal protein 1 (L3). When transformed with pTCM, Leu^-,Tcm^S cells become Leu^+ and can grow in the presence of trichodermin at 10 μg/ml. As has been observed in other transformants (12–14), growth in nonselective medium frequently leads to loss of the plasmid. Loss of pTCM leads to simultaneous loss of both the Leu^+ and Tcm^R phenotypes. The plasmid is maintained by growth either in the absence of leucine or in the presence of trichodermin. Therefore, pTCM contains a gene conferring Tcm^R.

Several lines of evidence suggest that the Tcm^R gene on pTCM is the same as the *tcm1* gene from the donor strain. Transformants as well as the donor are cross-resistant to other trichothecenes as well as to the structurally unrelated drug anisomycin. Extracts of a transformant show about the same resistance, *in vitro*, as do extracts of a strain heterozygous for *tcm1*. Although, *in vivo*, transformants are somewhat more resistant to trichodermin than are cells heterozygous for *tcm1*, they are far less resistant than the homozygous donor strain.

Several lines of evidence suggest that the Tcm^R gene on pTCM specifies ribosomal protein 1 (L3). The intermediate resistance of the transformants as well as of the heterozygote, both *in vivo* and *in vitro*, suggests that the gene specifies a ribosomal component rather than a modifying enzyme. Failure to select directly for Tcm^R suggests that sensitive products (i.e., ribosomes) must be diluted out. Finally, both the resistance gene and the gene for the 60S protein L3 are contained on a 3.2-kb DNA segment. Because at least 1.5 kb from the middle of that segment is required to code for L3, whose molecular weight is about 50,000, there is not sufficient room for another gene. Therefore, L3 is responsible for Tcm^R.

Although mutants resistant at the ribosome level to cryptopleurine, cycloheximide, and trichodermin have been known for a number of years, only recently has an altered ribosomal protein been detected, in a strain resistant to cycloheximide (22). Thus, it is gratifying to identify L3 as the protein responsible for Tcm^R. Of further interest is the fact that the 60S subunits of all eukaryotic ribosomes studied have two proteins distinctly larger than all other ribosomal proteins. It seems likely that these two proteins are homologous among the eukaryotes. The fact that an alteration in the largest 60S protein of yeast leads to Tcm^R suggests which protein might be responsible for Tcm^R mutants of other species—e.g., Chinese hamster (23).

Note Added in Proof. Transformation of yeast with a non-autonomously replicating plasmid, which occurs by recombination between homologous segments of the plasmid and host chromosomal DNA (7), followed by genetic mapping of the integrated plasmid confirmed that the 13.5-kb Tcm^R fragment is derived from the *tcm1* locus.

The authors thank Dr. Joe Cohen, Dr. Nancy Pearson, Chung H. Kim, and Barbara Buchferer for their valuable discussions and technical assistance. This work was supported by grants from the National Institutes of Health (5R01 GM 25532 and 1F32 GM 07165) and the American Cancer Society (NP 72J).

1. Nomura, M., Morgan, E. A. & Jaskunas, S. R. (1977) *Annu. Rev. Genet.* **11**, 297–347.
2. Petes, T. D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 410–414.
3. Woolford, J. L., Hereford, L. M. & Rosbash, M. (1979) *Cell* **18**, 1247–1260.
4. Vazquez, D. (1979) *Inhibitors of Protein Biosynthesis* (Springer, Berlin), pp. 138–144.
5. Grant, P. G., Schindler, D. & Davies, J. E. (1976) *Genetics* **83**, 667–673.
6. Schindler, D., Grant, P. & Davies, J. (1974) *Nature (London)* **248**, 535–536.
7. Hinnen, A., Hicks, J. B. & Fink, G. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1929–1933.
8. Broach, J. R., Strathern, J. N. & Hicks, N. B. (1979) *Gene* **8**, 121–133.
9. Cryer, D. R., Eccleshall, R. & Marmur, J. (1975) in *Methods in Cell Biology*, ed. Prescott, D. M. (Academic, New York), pp. 39–44.
10. Blin, N., Gabain, A. V. & Bujard, H. (1975) *FEBS Lett.* **53**, 84–86.
11. Gorenstein, C. G. & Warner, J. R. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1547–1551.
12. Beggs, J. D. (1978) *Nature (London)* **275**, 104–109.
13. Struhl, K., Stinchcomb, D. T., Schetler, S. & Davis, R. W. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1035–1039.
14. Gerband, C., Fournier, P., Blank, H., Aigle, M., Hescot, H. & Guerineau, M. (1979) *Gene* **5**, 233–253.
15. Gallis, B. M. & Young, E. T. (1975) *J. Bacteriol.* **122**, 719–726.
16. Carrasco, L., Barbacid, M. & Vazquez, D. (1973) *Biochim. Biophys. Acta* **312**, 368–376.
17. Warner, J. R. & Gorenstein, C. G. (1977) *Cell* **11**, 201–212.
18. Kaltschmidt, E. & Wittman, H. G. (1970) *Anal. Biochem.* **36**, 401–407.
19. Kruiswijk, T. & Planta, R. J. (1974) *Mol. Biol. Rep.* **1**, 409–415.
20. Warner, J. R. (1981) in *Molecular Genetics of Yeast*, eds. Broach, J., Jones, E. & Strathern, J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), in press.
21. Warner, J. R. & Gorenstein, C. G. (1978) in *Methods in Cell Biology*, ed. Prescott, D. (Academic, New York), Vol. 20, pp. 45–60.
22. Stocklein, W. & Piepersberg, W. (1980) *Curr. Genet.* **1**, 177–183.
23. Gupta, R. S. & Siminovitch, L. (1978) *Somatic Cell Genet.* **4**, 355–374.