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Biochemistry

Cycloheximide resistance can be mediated through either ribosomal subunit

[*Tetrahymena thermophila*/poly(U)-directed poly(phenylalanine) synthesis/eukaryotic ribosome]

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ABSTRACT **Two cycloheximide-resistant mutants of *Tetrahymena thermophila* were analyzed to determine the site of their cycloheximide resistance. The mutations in both strains had been previously shown to be genetically dominant and located at separate loci (denoted *Chx-A* and *Chx-B*). Strains carrying these mutations were readily distinguished by the extent to which they were resistant to the drug. The homozygous double mutant was more resistant than either single mutant. Cell-free extracts of wild type and of the three mutant strains, assayed for protein synthetic activity by both runoff of natural mRNA and poly(U)-dependent phenylalanine polymerization, demonstrated that *in vitro* the mutants were all more resistant than the wild type. Further fractionation of the cell-free systems into ribosomes and supernates localized cycloheximide resistance to the ribosome for both *Chx-A* and *Chx-B* homozygotes. Ribosome dissociation and pairwise subunit mixing in the *in vitro* system indicated that ribosome resistance was conferred by the 60S subunit from one strain whereas resistance in the other strain was mediated through the 40S subunit. This was further confirmed by reconstruction of all four cycloheximide-resistance "phenotypes" by mixing ribosomal subunits from appropriate strains. This finding suggests that the mechanisms by which these mutations confer resistance to cycloheximide are different.**

Cycloheximide is an antibiotic that inhibits translation on eukaryotic cytoplasmic ribosomes. The primary site of inhibition has been reported as initiation (1, 2), elongation (3–5), and termination (6, 7). Oleinick (8) has recently shown that all energy-dependent steps in translation are sensitive to the drug; the relative effect of the drug on the different translation steps appears to vary depending on the cycloheximide concentration. Similar concentration-dependent inhibition has been demonstrated in yeast (9). It has been suggested (7) that cycloheximide may interfere with GTP binding or hydrolysis at each of these steps, but this has yet to be shown.

Rao and Grollman (10) demonstrated that the cycloheximide resistance of *Saccharomyces fragilis* (which is naturally resistant to the drug) could be localized to the large subunit of the ribosome. This was accomplished by mixing ribosomal subunits of *S. fragilis* and *S. cerevisiae* (which is sensitive to cycloheximide) and showing that the *in vitro* resistance of the reconstituted ribosomes required the *S. fragilis* 60S subunit. The interpretation of this result by other investigators was that cycloheximide resistance or sensitivity was a property of the 60S subunit, presumably because cycloheximide bound there (11). Direct evidence of cycloheximide binding is lacking but the results of Skogerson and Wakatama (12) and Somasunduran and Skogerson (13) with yeast ribosomes are consistent with this interpretation.

Mutations altering cycloheximide sensitivity of ribosomes have been described in yeast (14–16), *Neurospora* (17), *Physarum* (18), and Chinese hamster ovary cell lines (19). Jimenez *et al.* (15) demonstrated that the cycloheximide resistance of one of their mutants was attributable to an altered 60S subunit, and it was further reported that two-dimensional gel electrophoresis of this subunit revealed an altered protein. However, in a discussion of this mutation, *cyh2*, McLaughlin (16) indicated that the molecular alteration of the 60S subunit is unknown. In this paper we describe the preliminary characterization of two different cycloheximide-resistant mutants of *Tetrahymena thermophila*. We show that both mutations confer different degrees of resistance to the ribosome and that the resistance of one mutant is large subunit-associated and that of the other is small subunit-associated. The mechanisms by which these two mutations might confer resistance to cycloheximide are discussed.

EXPERIMENTAL PROCEDURES

Strains, Media, and Culture Conditions. The mutant strains CU333, CU334, and CU335 (Cornell University stock designations) were homozygotes, derived from *T. thermophila* inbred strain B. Selection of the *Chx-B* mutation and construction of strains will be described elsewhere (M. Ares and P. Bruns). The wild-type strain used was B1868, mating type IV. For labeling of growing cells, cultures were grown at 30° in 1% Proteose peptone (Difco)/0.003% Na_4EDTA (Geigy). Cell-free extracts were prepared from cultures grown at 30° in 1% Proteose peptone/0.25% yeast extract/0.003% Na_4EDTA, to increase the span of exponential growth.

***In Vivo* Labeling.** Exponentially growing cells, at a density of 8–13 × 10^4 cells per ml, were added to incubation tubes containing $\frac{1}{10}$ vol of cycloheximide and [^{3}H]lysine (Amersham, 83 Ci/mmol) at 10 times the final concentration desired. Total reaction volumes were 220 μl. Final [^{3}H]lysine concentration was 5 μCi/ml. Reactions were incubated at 30° for 30 min, at which time duplicate 100-μl samples were spotted onto Whatman 3MM filters. Filters were precipitated in 10% trichloroacetic acid and washed in boiling 5% trichloroacetic acid, cold 5% trichloroacetic acid, 70% ethanol, and ether. Dried filters were assayed for radioactivity in a toluene-based fluor. Reactions containing cycloheximide were compared to the control to give percentage incorporation; incorporation at 2.5 mM cycloheximide (reproducibly 2% of the total) was assumed to be due to mitochondrial protein synthesis and was subtracted from each point.

Cell-Free Extracts. The crude cell-free system was devised

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Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

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by E. Palmer and extensively modified. Exponentially growing cells (3–5 × 10^5 cells per ml) were washed and pelleted. Packed cells were resuspended in 1 vol of cold 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes)/0.1 M KCl/3.5 mM $Mg(OAc)_2$/0.25 M sucrose/6 mM 2-mercaptoethanol, pH 7.6, and homogenized with a tight-fitting Dounce homogenizer (80–120 passes) until well broken. Cell debris was pelleted at 14,000 × *g* for 15 min. The supernate was adjusted to 1 mM ATP (neutralized)/0.25 mM GTP/1 mM dithiothreitol/10 mM creatine phosphate/creatine phosphokinase (50 μg/ml) and incubated at 30° for 30 min in order to allow release of ribosomes from mRNA. The reaction mixture was then chilled and passed through a Sephadex G-25 column made up in 20 mM Hepes/0.1 M KCl/4 mM $Mg(OAc)_2$/6 mM 2-mercaptoethanol, pH 7.6. The excluded volume was used in experiments to test the protein synthetic activities of unfractionated cell-free extracts. To further fractionate the extract, the excluded volume was centrifuged at 100,000 × *g* for 60 min at 4° to separate ribosomes and supernate. Ribosomes were resuspended in the column elution buffer in ½–¼ their original volume. Unfractionated extracts, supernates, and ribosomes were frozen in small portions in liquid N_2 and stored at −60° until used.

Ribosome Dissociation. To prepare ribosomal subunits, ribosome aliquots from above were thawed and layered on 15–30% (wt/vol) sucrose gradients containing 0.3 M KCl (sometimes 0.5 M)/6 mM $MgCl_2$ (or 10 mM)/10 mM Tris, pH 7.5/6 mM 2-mercaptoethanol. Gradients were spun at 20,500 rpm at 3° for 16 hr in a Spinco SW 27 rotor. These salt conditions will dissociate runoff (inactive) ribosomes but not polysomal ribosomes (C. Sutton, unpublished data). Fractions corresponding to the large or small subunits (derived from 80S runoff ribosomes) were pooled, dialyzed against a 100-fold excess of 10 mM Hepes/50 mM KCl/2 mM $Mg(OAc)_2$ for 60 min with one change, and pelleted at 95,000 × *g* for 20 hr. Pellets were resuspended in H_2O and frozen (−60°) until used. Ribosomal subunits prepared in this way gave good activity recovery upon reassociation, being 50–95% as active as undissociated ribosomes (see legend to Table 2).

Poly(U)-Directed Poly(Phe) Synthesis. Reactions were generally carried out in 220-μl volumes and incubated at 30° for 30 min. The following components were mixed on ice: 1 vol of buffered energy-generating system plus amino acids, 1 vol of poly(U) plus cycloheximide, 1 vol of supernate, and 1 vol of ribosomes (or 2 vol of unfractionated supernate). Ribosomes were added back to the supernate at a concentration equal to or less than that at which they had been recovered (usually 40–60 A_{260} units/ml of supernate). For reassociation of ribosomes, large and small subunits were added at an A_{260} ratio of 2.5:1. The final concentration of reactants was 20 mM Hepes (pH 8.0), 2 mM dithiothreitol, 1 mM ATP, 0.25 mM GTP, 10 mM creatine phosphate, 50 μg of creatine phosphokinase per ml, 11 mM $Mg(OAc)_2$, 125 mM KCl, 19 unlabeled amino acids at 25 μM each, [^{3}H]phenylalanine (New England Nuclear, 22 Ci/mmol) at 12.5–25 μCi/ml, phenylalanine to bring total phenylalanine to 5 μM, 50–75 μg of poly(U) per ml, and cycloheximide as indicated. Duplicate Whatman 3MM filters, pretreated with 10% trichloroacetic acid and 1% Casamino Acids, were spotted with 50-μl samples at intervals throughout the course of the reaction. Filters were processed as above. Total trichloroacetic acid-precipitable incorporation was calculated by using zero-time counts as background. Control reactions without ribosomes had less than 2% of the counts of complete reactions. Control reactions were run to determine the amount of contamination by whole ribosomes in the subunit preparations. In general, subunits had less than 5% of the activity of whole ribosomes. No subunit preparations with >10% activity of undissociated subunits were used in these experiments.

Maximal polymerization of phenylalanine was about 150 pmol of phenylalanine per mg of ribosomes in 30 min. The reaction was complete by this time. Only a small fraction (10–15%) of the ribosomes participate in poly(Phe) polymerization as judged by the percentage of ribosomes that were resistant to dissociation in 0.3 M KCl (results not shown). Therefore, the average extent of polymerization was six to seven phenylalanines per ribosome.

***In Vitro* Polyribosome Runoff.** Extracts were prepared in the same way as for the poly(U) reactions through preparation of the 15,000 × *g* supernate. The supernate was then passed through Sephadex G-25 in the column elution buffer, and the turbid exclusion volume fractions were pooled. Final reactant concentrations were 20 mM Hepes (pH 8.0), 2 mM dithiothreitol, 1 mM ATP, 0.25 mM GTP, 10 mM creatine phosphate, 50 μg of creatine phosphokinase per ml, 6 mM $Mg(OAc)_2$, 150 mM KCl, 25 mM each of 19 unlabeled amino acids, [^{3}H]phenylalanine (New England Nuclear, 22 Ci/mmol) at 25 μCi/ml, unlabeled phenylalanine to 5 μM, and 0.3–0.5 A_{260} unit of supernate. Duplicate 80-μl reactions were incubated at 30° for 30 min, at which time the total reaction mix was spotted on a pretreated Whatman 3MM filter. Filters were processed as before.

RESULTS

Strain Phenotypes. CU333 carries a dominant mutation (*Chx-A*) that confers resistance to cycloheximide (20). Two mutant alleles of the *Chx-A* locus are known (21). When first obtained, the basis of resistance in these mutants was presumed to be nonribosomal, due in part to the fact that both mutations at the *Chx-A* locus are strongly dominant (22). Under the assumption that the basis of resistance in *Chx-A*/*Chx-A* lines was likely a transport defect, a homozygous *Chx-A* strain was mutagenized and lines resistant to even higher concentrations of cycloheximide were selected, in the hope that a ribosomal mutant might be obtained. The resistant line recovered from this selection scheme was shown to carry a second mutation, locus designation *Chx-B*, which alone caused weak cycloheximide resistance (unpublished data). A strain homozygous for *Chx-B*, CU334, was constructed. Additionally, a homozygous double mutant, CU335, was made; the resistance phenotype of this line was identical to that of the initial isolate.

Table 1 lists the strain nomenclature used throughout the paper and the genotypes and phenotypes in cycloheximide. CU334 is weakly cycloheximide resistant, CU333 is more resistant, and the double mutant strain CU335 is more resistant than its CU333 parent. To confirm that these cycloheximide concentrations reflect inhibition of protein synthesis, we per-

Table 1. Cycloheximide resistances of the various strains

Strain	Genotype/ phenotype	Cycloheximide dose, μM	
		Selection	Min. lethal
Wild type	*ChxA*$^+$ *ChxB*$^+$/ ChxA$^+$ ChxB$^+$	—	20
CU334	*ChxA*$^+$ *ChxB*/ ChxA$^+$ ChxB	25	36
CU333	*ChxA ChxB*$^+$/ ChxACHxB$^+$	90	300
CU335	*ChxA ChxB*/ ChxA ChxB	500	640

Selection doses are cycloheximide concentrations used routinely to distinguish between clones of the four phenotypes in crosses and to allow resistant lines to grow while sensitive strains die. Minimum lethal doses were defined as the lowest cycloheximide concentrations that would yield no viable (transferable) cells after 3 days' exposure to the drug.

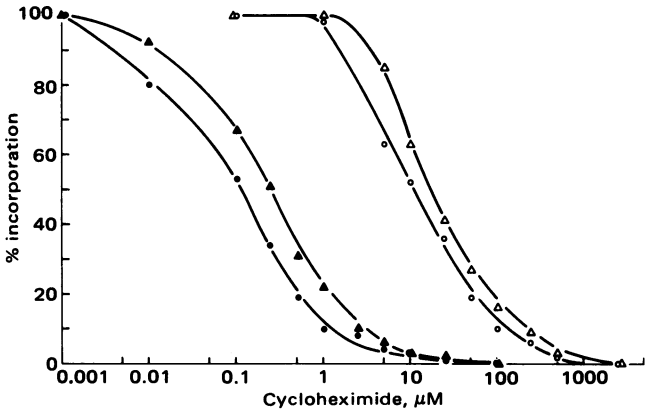

FIG. 1. Effects of varying concentrations of cycloheximide on [^{3}H]lysine incorporation in exponentially growing cells. ●, Wild type; ▲, CU334; ○, CU333; △, CU335.

formed an *in vivo* dose-response study with exponentially growing cells. Fig. 1 shows the results of one such experiment. Although there was not a perfect correspondence between minimum lethal dose and maximal inhibition of incorporation *in vivo*, the results are in relative agreement. In both instances, CU335 was about 20 times more resistant than CU334 and about 1.5 times more resistant than CU333.

***In Vitro* Cycloheximide Sensitivity.** As an assay for protein synthesis *in vitro*, poly(U)-directed poly(Phe) synthesis was measured in cell-free extracts with varying concentrations of cycloheximide. By this assay we found (Fig. 2) that all three mutant strains were more resistant to cycloheximide inhibition than was the wild type. Furthermore, CU335 extracts were more resistant than either CU333 or CU334 extracts. Although the orders of magnitude of *in vitro* resistance were not the same as the *in vivo* resistances (discussed below) the results were reproducible (three experiments) and suggested that decreased uptake was not the cause of resistance.

To identify the resistant cell component we fractionated the crude extract. Ribosomes were pelleted from each of the cell-free extracts, and cycloheximide sensitivity of wild-type ribosomes was tested in each of the four ribosome-free supernates. In addition, ribosomes from the four strains were assayed for cycloheximide resistance in wild-type supernate. These mixing experiments should (*i*) confirm the cytoplasmic location of the resistance and (*ii*) localize the resistance of the extracts to either the ribosome fraction or to the postribosomal supernate. When wild-type ribosomes were tested in the four ribosome-free extracts, all four combinations showed equivalent dose responses

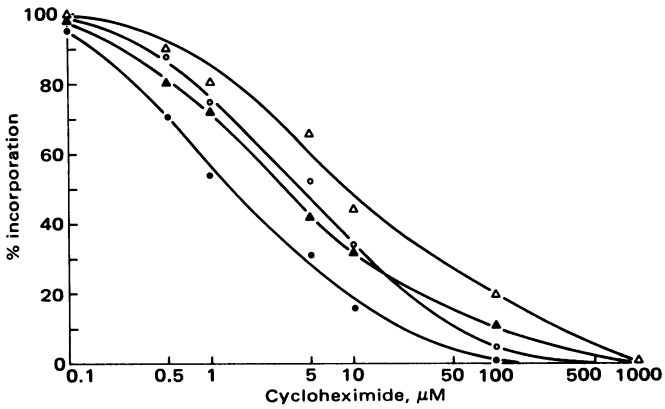

FIG. 2. Effects of varying concentrations of cycloheximide on poly(U)-directed poly([^{3}H]Phe) synthesis in unfractionated cell-free extracts. ●, Wild type; ▲, CU334; ○, CU333; △, CU335.

(Fig. 3 *left*). However, the reciprocal mixing experiment demonstrated that the resistance seen in the crude cell-free extracts (Fig. 3 *right*) could be generated by ribosomes from the three mutant strains. Comparison of Figs. 2 and 3 *right* shows that the dose-response curves are nearly superimposable; thus, both quantitatively and qualitatively, the ribosomes possess the cycloheximide resistance demonstrable in the poly(U) system.

Validity of the Assay System. Whereas the *in vivo* cycloheximide responses of the four strains were maximally separated by two orders of magnitude of cycloheximide concentration (Fig. 1), the maximal resistance *in vitro* was found only at 10 times the concentration of cycloheximide necessary to inhibit wild-type extracts (Figs. 2 and 3 *right*). However, a poly(U) system cannot assay effects on any step in protein synthesis except elongation (23, 24). The measurement of *in vivo* inhibition is the result of inhibition of all steps in protein synthesis. If elongation in wild-type cells is less sensitive to cycloheximide than is either initiation or termination, as has been shown in Chinese hamster ovary cells (8), then the *in vitro* results need not agree quantitatively with the *in vivo* measurements. Furthermore, the poly(U)-directed poly(Phe) incorporations are not carried out under optimal conditions for natural mRNA translation.

To assay the inhibition of elongation under more "natural" conditions, we measured inhibition of incorporation in a cell-free polyribosome runoff system. With this system as our estimate of inhibition of elongation, the mutant extracts were more resistant than the wild-type extract (Fig. 4). The resistance of CU335 was 10 times greater than that of the wild type; the values of CU333 and CU334 again fell between those for the wild type and the double mutant (results not shown). The actual levels of resistance in the runoff system were approximately 5 times greater than in the poly(U) system. Although natural initiation does not occur in the latter system (24), the ribosomes must attach to the poly(U) in some manner that may be sensitive to cycloheximide. This could affect the absolute levels of resistance. However, because the relative differences were the same, we concluded that both *in vitro* systems measured similar or identical steps of protein synthesis. Even though we cannot account for the relative differences seen when comparing the *in vitro* and *in vivo* dose responses, our data show that the ribosomes from the four strains differ in their cycloheximide sensitivity at least one step in the protein synthetic pathway.

Ribosomal Subunit Localization of Resistance. Further analysis of the mutants was done with the poly(U) assay system to determine which ribosomal subunit was required to confer cycloheximide resistance to the ribosome. Ribosomes from CU333, CU334, CU335, and wild-type extracts were dissociated and reassociated in all pairwise combinations and tested for activity at 10 μM cycloheximide, a concentration that should discriminate among all possible ribosomal phenotypes (see Fig. 2). Tables 2–4 give the results of several experiments. First, cycloheximide resistances of homologous reassociations were compared to the resistance of the undissociated ribosomes from which they were derived. Table 2 indicates that (*i*) dissociation and reassociation does not cause a large loss in overall protein synthetic activity of the ribosome and (*ii*) the *in vitro* cycloheximide resistance is retained (in fact it is slightly enhanced). Thus, at 10 μM cycloheximide, the four "phenotypes" are readily distinguishable from one another. The cycloheximide sensitivity of hybrid ribosomes composed of one wild-type and one mutant subunit clearly suggested (Table 3) that resistance in CU333 was mediated through the large subunit and resistance in CU334 was conferred by the small subunit. This finding predicts that ribosomes with *in vitro* phenotypes of either single mutant may be reconstructed from double mu-

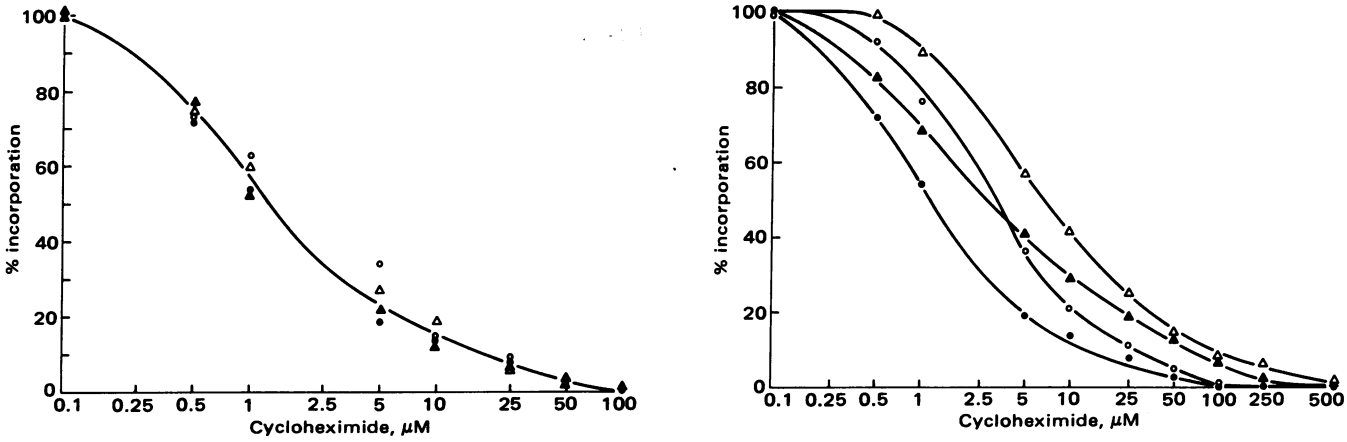

FIG. 3. Effects of varying concentrations of cycloheximide on poly(U)-directed poly([^{3}H]Phe) synthesis in reconstructed cell-free extracts. (*Left*) Wild-type ribosomes combined with ribosome-free extracts of wild type (●), CU334 (▲), CU333 (○), and CU335 (△) cells. The incorporation values taken as 100% (in cpm/OD ribosomes) in the various reactions were: wild-type, 23,829; CU334, 23,039; CU333, 18,446; CU335, 28,754. (*Right*) Wild-type ribosome-free extracts combined with ribosomes of wild type (●), CU334 (▲), CU333 (○), and CU335 (△) cells. The incorporation values taken as 100% (in cpm/OD ribosomes) in the various reactions were: wild type, 23,829; CU334, 24,451; CU333, 24,504; CU335, 24,492.

tant–wild-type subunit combinations and that phenotypically double mutant and wild-type ribosomes may be formed by using CU333 and CU334 subunit combinations.

These hybrid combinations, their predicted levels of resistance, and the actual resistances found are shown in Table 4. Except for the third combination set (CU334 + CU335 subunits), which gave higher resistances than predicted (although relative resistances are still consistent), there is good agreement between actual and predicted resistances. We conclude that CU334 resistance *in vitro* requires the CU334 40S subunit, CU333 ribosomal resistance is conferred by its 60S subunit, and CU335 *in vitro* resistance is due to combined effects of the two subunits.

DISCUSSION

Two cycloheximide-resistance mutants of *T. thermophila* have been shown to have resistant ribosomes *in vitro*. Resistance of CU333 ribosomes requires the presence of the 60S subunit. Given the current information on the probable cycloheximide binding site (or sites) (12, 13) and the ribosomal subunit location of other cycloheximide-resistant mutants (10, 15), this result is not surprising. CU334 resistance, however, is conferred by the 40S subunit. If there is not a cycloheximide binding site on the small ribosomal subunit, then another means of conferring *in vitro* resistance must be invoked. We have evidence that CU334 ribosomes are conformationally altered relative to wild type (25). Assuming that the CU334 40S ribosomal subunit is changed in structure, one can imagine that the affinity of cycloheximide for the ribosome is also different. This could occur either by changing the conformation of the entire ribosome or by masking the cycloheximide binding site or both.

Is the *in vivo* resistance of the two strains due entirely to the demonstrated *in vitro* ribosomal resistance? Genetically, each mutant behaves as if it were the result of a mutation at a single locus; typical Mendelian ratios are found in crosses with these strains (unpublished data). Although pleiotropic mutations that affect ribosomal resistance to antibiotics and other physiological functions at the cell membrane exist in bacteria (26), they are uncommon. It seems unlikely that we have pleiotropic mutations like the above. However, until cycloheximide uptake and internal concentration can be measured in our strains we cannot rule out the possibility of the exsistence of closely linked mutations that alter cycloheximide transport (or toxicity) in intact cells.

However, assuming that the ribosomal resistance detected

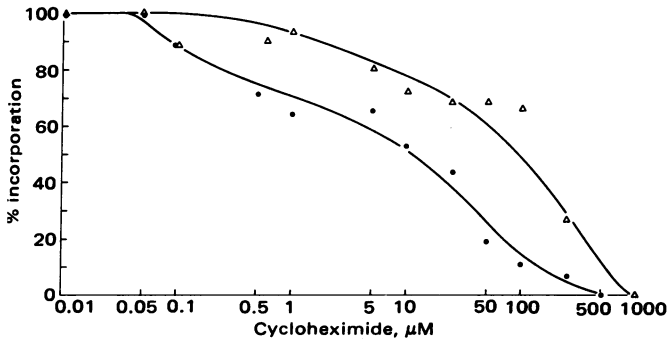

FIG. 4. Effects of varying concentrations of cycloheximide on [^{3}H]phenylalanine incorporation into polypeptides synthesized *in vitro* by polyribosome runoff. Polyribosomes were from wild-type (●) and CU335 (△) cells.

Table 2. *In vitro* assays for cycloheximide resistance: Maintenance of resistance in reassociated subunits*

Strain phenotype†	Activity in 10 μM cycloheximide, % of control: Undissociated ribosomes	Reassociated subunits‡
++	15.4 ± 7.4 (n = 7)	22.0 ± 6.4 (n = 10)
+B	31.2 ± 6.7 (n = 6)	36.0 ± 7.9 (n = 9)
A+	33.0 ± 18 (n = 3)	48.3 ± 7.4 (n = 3)
AB	53.2 ± 7.7 (n = 5)	69.1 ± 8.5 (n = 3)

* Undissociated runoff ribosomes and purified ribosomal subunits were used in poly(U)-directed poly(Phe) synthesis reactions with (10 μM) and without (control) cycloheximide. Typical control reactions with undissociated ribosomes gave 80–120 pmol of phenylalanine incorporation per mg of ribosomes in a 30-min incubation. All values are means ± SD obtained from independent ribosome isolations.

† ++, wild type; +B, CU334; A+, CU333; AB, CU335.

‡ In 20 direct comparisons of reassociated subunits with the preparations of undissociated ribosomes from which they came, we recovered 71.3 ± 11.2% of the protein synthetic activity in the reassociated subunits relative to the undissociated ribosomes in reactions without cycloheximide; the range was 57–90%.

Table 3. *In vitro* assays for cycloheximide resistance: Localization of cycloheximide resistance by subunit mixing

Subunit source* (strain phenotype)		Activity in 10 μM cycloheximide,
Large	Small	% of control
+B	++	24.2 ± 8.0 (n = 9)
++	+B	41.2 ± 6.5 (n = 6)
A+	++	59.0 ± 2.8 (n = 2)
++	A+	33.5 ± 0.5 (n = 2)

* As in Table 2 footnote †.

in vitro is responsible for resistance in whole cells, clearly our assay method is not optimal. CU334 and CU333 have different phenotypes *in vivo*; their behavior *in vitro* is similar in terms of relative cycloheximide resistance. Because cycloheximide is not primarily an inhibitor of elongation in other organisms (see ref. 8), it is likely that the two mutants respond differently to the drug at either initiation or peptide chain release. Unfortunately, we currently have no assay system for these steps.

Prior to these experiments, the *Chx-A* locus was considered to be an unlikely candidate for a ribosomal function because mutations at the locus are dominant. Ribosomal drug resistance in prokaryotes is recessive (27). Recessive ribosomal drug resistance has also been shown for Chinese hamster ovary cell lines (28) and for yeast (29). Interestingly, the cycloheximide-resistant ribosomal mutations described in yeast (15), *Neurospora* (17), and *Physarum* (18) do not behave as true recessives, either in yeast diploids or in heterokaryons of *Neurospora* and of *Physarum*. A trivial explanation for semidominance in the latter two organisms involves unequal nuclear ratios. However, a possible explanation for the semidominance of cycloheximide resistance may be the site of action of the drug. If inhibition occurs prior to the formation of the mRNA–ribosome initiation complex, then sensitive ribosomes will be selectively excluded from polyribosomes. Thus, resistant ribosomes will preferentially translate mRNA, and heterozygotes will still have functioning protein synthetic machinery. However, until ribosomal cycloheximide-resistant mutants that have clear alterations in ribosomal structural proteins are found, this hypothesis cannot be tested.

Table 4. *In vitro* assays for cycloheximide resistance: Reconstruction of ribosome phenotypes by subunit mixing

Subunit source* (strain phenotype)		Activity in 10 μM cycloheximide, % of control	
Large	Small	Predicted†	Found
+B	A+	22	28.0 ± 12.5 (n = 3)
A+	+B	69	60.3 ± 2.3 (n = 3)
++	AB	36	40 (n = 1)
AB	++	48	50 (n = 1)
+B	AB	36	59 (n = 1)
AB	+B	69	79 (n = 1)
A+	AB	69	64 (n = 1)
AB	A+	48	47 (n = 1)

* As in Table 2 footnote †.

† Assuming the CU334 small subunit and the CU333 large subunit to be the mediators of cycloheximide resistance, one can predict the "phenotype" of a ribosome reconstructed from two different ribosomes. The level of resistance found in Table 2 for reassociated subunits in 10 μM cycloheximide was taken as the "phenotype" expected—+ = 22%; CU334 = 36%; CU333 = 48%; CU335 = 69%.

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