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Characterization of a Cycloheximide-Resistant *Tetrahymena thermophila* Mutant Which Also Displays Altered Growth Properties

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A cycloheximide-resistant strain of *Tetrahymena thermophila*, expressing a mutant *chx-B* gene (Ares and Bruns, Genetics **90**:463–474, 1978), displayed very different temperature-dependent growth characteristics than either wild-type cells or another cycloheximide-resistant strain expressing a different mutant gene. Whereas wild-type cells showed an immediate decline in ribosome translocation rates when shifted from 30 to 38 or 40°C, this mutant strain (X-8) showed no such decline. These results directly correlated with the growth rate differences we found for these cells at these temperatures. By genetic analysis, we showed that the phenotype of cycloheximide resistance cosegregated with the ability to grow rapidly at 40°C. Analyses, both direct and indirect, suggested that a number of functional and structural characteristics of the ribosomes from strain X-8 cells are most likely conformationally different from those of wild-type ribosomes.

A large proportion of the procaryotic and eucaryotic ribosome structural mutants, both presumed and proven, found to date were isolated by selection for resistance to some antibiotic that inhibited a step(s) in protein synthesis. Although there are now many documented examples for procaryotes (reviewed by Cundliffe [4]) of altered ribosomal proteins correlating with a change in the drug sensitivity of the ribosome, only recently have similar conclusions been possible for eucaryotes (2, 6, 18). A change in the secondary and tertiary structure of a ribosomal protein that could alter its affinity for an antibiotic might be expected, in some cases, to also change its affinity for one or more of the neighboring ribosomal proteins. Such a change could have a profound effect on the structure of the ribosome as a whole, producing an array of cellular pleiotropic responses. Indeed, in bacteria many such secondary effects of antibiotic resistance ribosome mutations have been noted (reviewed by Isono [11] and Chambliss [3]), e.g., antibiotic dependence, temperature-sensitive growth, cold lethality, and sporulation defects.

Recently, two mutant strains of the ciliate organism *Tetrahymena thermophila* were characterized that were more resistant than the wild type to the inhibitory effects of the antibiotic cycloheximide and were shown to express this resistance through altered ribosomes (21). All the available evidence from other systems suggested that cycloheximide interacted with the large ribosomal subunit (it has now been unequivocally shown [19]), and it was expected that cycloheximide resistance in those two strains would also be the result of an alteration of some large subunit protein. One of the mutants (CU-333, a strain expressing a mutation of the *Chx-A* locus) behaved as expected; that is, it could be shown in vitro that its cycloheximide resistance phenotype was mediated through a modified large subunit. The other mutant (CU-334, a strain expressing a mutation of the *chx-B* locus), however, gave an unexpected result: the small subunit of its ribosomes gave the cell its cycloheximide resistance. At that time it was argued that even if cycloheximide interacted with the large ribosomal subunit, this result could be rationalized by assuming one of two possibilities (there were others as well). First, the cycloheximide binding site(s) is near the large subunit-small subunit interface, and an altered small subunit protein in strain CU-334 interferes with the binding site on the large subunit. Second, the small ribosomal subunit in strain CU-334 is altered so that after combining with the large subunit the ribosome assumes an abnormal conformation, which then changes the cycloheximide binding site on the large subunit. Either of these alternatives allowed an explanation of the fact that the double mutant had more than additive resistance to cycloheximide. The difference between these two possible explanations is that in the first only a localized change in ribosome structure is invoked, whereas in the

second changes in many sites on the ribosome would be predicted. Were the second possibility correct, then a variety of ribosome functions would probably be affected.

In this paper we examined a number of cell properties that we thought might be secondarily affected by a change in ribosome structure. The results reported here support the conclusion that the ribosomes in strains expressing the *chx-B* mutation are altered as described by the second possibility mentioned above.

MATERIALS AND METHODS

Cells strains, media, and chemicals. Our wild-type *T. thermophila* strain was designated BIV1868. Strains CU-333 and CU-335 (Cornell University stocks) were homozygotes derived from *T. thermophila* inbred strain B (1). These three strains were obtained from P. Bruns, Cornell University, Ithaca, N.Y. Strain CU-333 has a dominant mutation at the *Chx-A* locus that confers resistance to cycloheximide (up to 25 μg/ml) (wild type will not grow at concentrations above 1 μg/ml). Strain X-8 is a homozygous strain derived from strain CU-334 (1) that carries a recessive mutation at the *chx-B* locus that also confers resistance to cycloheximide. Strain X-8 grows at cycloheximide concentrations of up to 10 μg/ml. Strain CU-335 is homozygous for these two mutant genes and grows with 75 μg of cycloheximide per ml.

Cells were normally grown at 30°C in 1% proteose peptone (Difco Laboratories), 0.003% Sequestrene (Geigy) on a gyratory shaker at 90 rpm. Cells were starved in 50 mM Tris (pH 7.4) at 30°C. All in vivo labeling of cells was done directly in these media with [^{3}H]lysine (95 Ci/mmol) or [^{3}H]uridine (40 Ci/mmol) (Amersham Corp.) at 1 to 10 μCi/ml. The procedures we followed to induce mating and isolate conjugant pairs are those described by Orias and Bruns (16).

Growth rates were measured by removing 0.5-ml samples from cell cultures at various intervals and fixing the cells with 2% Formalin. Cells were then counted in an automatic cell counter.

The antibiotics cycloheximide, tetracycline, and emetine were purchased from Sigma Chemical Co. Paromomycin was a gift from H. Machamer, Rohm and Haas Co. Anisomycin was a gift from N. Belcher, Pfizer Inc.

Dose-response curve measurements. The details for dose-response curve measurements for both growing and starved cells were described previously (10). The in vitro responses of ribosomes in polyuridylate-directed polyphenylalanine synthesis to increasing concentrations of cycloheximide were determined as previously described (21).

Elongation rate measurements. The method of Fan and Penman (5) for estimating the average half-transit times ($\bar{t}/2$) of ribosomes on mRNAs was used to determine polypeptide elongation rates. To cultures (10 to 12 ml, 3×10^4 to 5×10^4 cells per ml) was added [^{3}H]lysine (95 Ci/mmol) at 20 μCi/ml. At intervals after the label was added, 0.5-ml portions were pipetted into tubes containing 1.5 ml of ice-cold 0.1 M KCl–0.01 M Tris (pH 7.5)–0.01 M MgCl–1.5% Triton X-100–0.75% deoxycholate, and the tubes were agitated on a Vortex mixer. Triplicate 0.1-ml samples were removed, precipitated with trichloroacetic acid (TCA), and counted to determine the overall rate of incorporation into new proteins. The remaining solution was centrifuged for 90 min at 50,000 rpm and 3°C in a Spinco 65 Ti rotor. Triplicate 0.1-ml samples of the resulting supernatant were removed, TCA precipitated, and counted to determine the incorporation rate into released polypeptide chains. After the average values of the data obtained were plotted (see Fig. 5 and 6), the horizontal distance between the curves was taken as the average half-transit time ($\bar{t}/2$).

Ribosome melting curves. The method of determining the melting temperatures of ribosomes from wild-type and strain X-8 cells was described previously (10). Before preparing ribosomes from log-phase cells, we placed our cultures on ice for 15 min to ensure complete polysomal run-off.

rRNA degradation rates. The details for rRNA degradation rate measurement have been described (22). In determining the degradation rate, we measured the loss of TCA-insoluble counts in total radioactive cellular RNA. This was possible because, after cells had been labeled for two generations in growth medium containing [^{3}H]uridine, followed by two to three generations of growth in nonradioactive medium, 90% of the TCA-insoluble counts in the cell were in the rRNA, and none were nonalkaline labile. Furthermore, when cells are washed into starvation medium and turnover of RNA is induced, the percentage of RNA remaining TCA insoluble as rRNA remains constant for the first 24 h of starvation (22).

RESULTS

Effects of other protein synthesis inhibitors on strains X-8 and CU-333. We determined the dose-response sensitivities of log-phase strain X-8 (a cell line homozygous in both its macronucleus and micronucleus for the mutant *chx-B* gene expressed in strain CU-334) and CU-333 cells to other antibiotics that inhibit protein synthesis and are known or thought to interact with either the large or small ribosomal subunit (Table 1). The response of the growing strain CU-333 cells was straightforward: the only drug to which this strain showed any sensitivity different from the wild type was cycloheximide, the drug it had been selected against. On the other hand, the growing strain X-8 cells showed a number of differences relative to the wild type: resistance to anisomycin and sensitivity to paromomycin were both increased. That the mutation in this strain affected the action of other protein synthesis inhibitors was not particularly unusual, except that these three drugs are not structurally related to each other, and they do not inhibit the same steps in protein synthesis (24), nor do they share overlapping ribosome binding sites (19). Furthermore, paromomycin is thought to act on the small subunit, whereas anisomycin is thought to act on the large subunit (24). No other cycloheximide-resistant strain isolated to date (six strains) showed cross-resistance to any other drug.

Starvation elicits a change in the sensitivity of

TABLE 1. Sensitivity of growing and starved cells to inhibition of protein synthesis by various antibiotics

Drug	50% inhibiting concn (μg/ml) (growing cells/starved cells)[a] for strain:		
	Wild type	CU-333	X-8
Cycloheximide	0.07/0.14 (+)	3.5/4.2 (+)	0.54/0.14 (−)
Emetine	0.5/1.2 (+)	0.47/1.3 (+)	0.5/0.3 (−)
Paromomycin	350/145 (−)	350/145 (−)	260/530 (+)
Anisomycin	2.2/1.2 (−)	2.3/1.2 (−)	5.1/1.8 (−)
Tetracycline	275/690 (+)	275/650 (+)	290/675 (+)

[a] Whether starved cells were more resistant (+) or more sensitive(−) than growing cells is indicated in parentheses.

wild-type cells to a number of protein synthesis inhibitors, and at least some of these changes are directly attributable to a conformational change in the ribosome associated with a specific ribosomal protein phosphorylation (10). Knowing this, we also tested the effects of starvation on the resistance of strains X-8 and CU-333 to the same array of antibiotics used on log-phase cells. Whereas wild-type cells became more resistant to cycloheximide when starved, X-8 cells became more sensitive (Fig. 1). However, the timing of the changes in drug sensitivity and extent of phosphorylation of the small subunit protein was the same for both strains (data not shown). Conversely, when wild-type cells were starved they become more sensitive to paromomycin, whereas the X-8 cells become more resistant (Fig. 2). In examining the effects of starvation on the sensitivity of the strains to all the drugs (Table 1), we noted two things. In every case strain CU-333 responded identically to starvation as did the wild-type strain (Fig. 2), but strain X-8 showed a variety of responses. For example, when wild-type cells became more resistant, X-8 cells could either become more resistant (e.g., tetracycline) or more sensitive (e.g., cycloheximide); and when wild-type cells became more sensitive, X-8 cells might do the same (e.g., anisomycin) or the opposite (e.g., paromomycin).

Effects of the mutant *chx-B* gene on cell growth. One explanation for these results was that the strains expressing the mutant *chx-B* gene have their ribosomes changed in such a way that a variety of drug-ribosome interactions is affected. Assuming this was so, we predicted that other ribosome functions, namely those requiring ribosome-translational factor interactions, might also be altered, thereby affecting the growth properties of these strains. Accordingly, we measured the growth rates of both strains at 30°C and found that wild-type and X-8 cell growth rates were indistinguishable (Fig. 3a). When cells growing at 30°C were shifted to 38°C, both strains continued to divide at the same rate for one to two cell generations, but after that the wild-type strain showed a decreased division rate (Fig. 3b); strain X-8 showed no such decrease. We allowed the cells to grow overnight at 38°C and then measured their growth rates; we found that the wild type grew with a doubling time of 4.5 h, whereas strain X-8 divided every 2

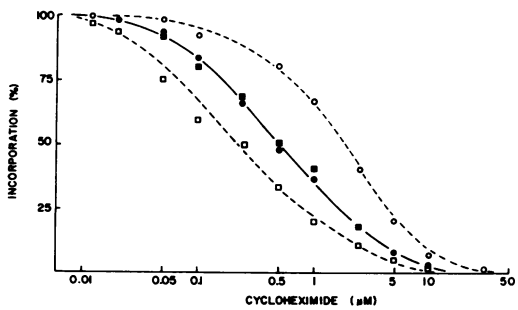

FIG. 1. Effect of increasing concentrations of cycloheximide on the relative rate of amino acid incorporation in strain X-8 (○, ●) and wild-type (□, ■) cells. Measurements were made on early log-phase cells (○, □) and on cells starved for 18 h in a dilute salt solution (●, ■). The results are plotted as the percentage of incorporation relative to non-drug-treated controls.

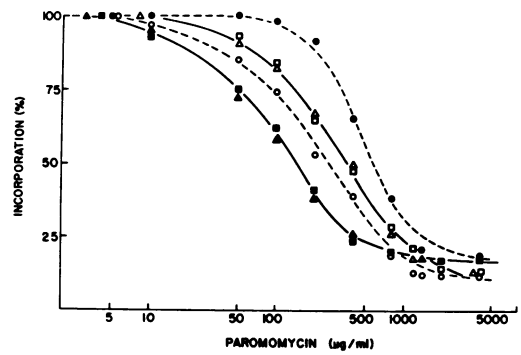

FIG. 2. Effect of increasing concentrations of paromomycin on the relative rate of amino acid incorporation in strain X-8 (○, ●), wild-type (□, ■, and strain CU-333 (△, ▲) cells. Measurements were made on early log-phase cells (open symbols) and on cells starved for 18 h in a dilute salt solution (solid symbols). The data were plotted as described for Fig. 1.

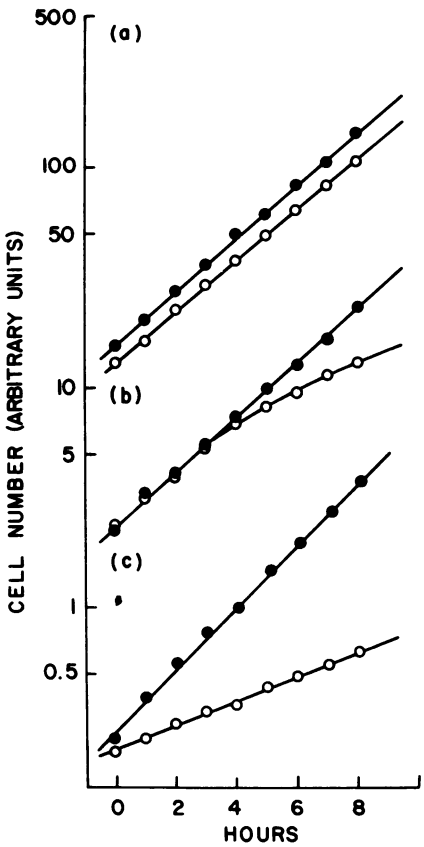

FIG. 3. Cell division rates of strain X-8 (●) and wild-type (○) cells at 30 and 38°C. Increase in cell number for (a) cells in early log-phase growth (<30,000 cells per ml) at 30°C and (b) cells that had been in early log-phase growth at 30°C and were then shifted to 38°C (time zero is the time of the temperature shift); (c) cells (<1,000 cells per ml) were placed at 38°C and allowed to grow for 12 to 14 h overnight, and their division rate was measured the next morning.

h, a faster rate than it had shown at 30°C (Fig. 4). We adapted the cells to overnight growth at a variety of temperatures and determined their growth rates. Between 30 and 34°C, both strains grew at about the same rate (Fig. 4). Above this temperature range, however, the wild type showed a steady decline, unitl at 41°C there was no further cell division (cells kept at this temperature for 24 h showed no decrease in viability). In contrast, strain X-8 showed no such decrease in growth rate up to 39°C, after which there was an abrupt and dramatic decline. Strain X-8 grew at 41°C, unlike the wild type, but at 42°C showed no growth. Both strains displayed loss of viability when maintained for 24 h at 42°C. Strain CU-333 had a division time identical to that of the wild type at 30, 38, and 40°C.

We showed that strain X-8 had altered growth characteristics, but to determine whether this was directly attributable to the effects of the *chx-B* mutation on the ribosomes we examined the coselectability of the drug resistance and growth rate phenotypes. This was done in two ways. We first crossed our wild-type strain (*chx-B*$^+$/*chx-B*$^+$, cycloheximide sensitive, slow growing) with a strain that expressed the mutant Chx-B phenotype in its macronucleus (that is, it grew with 7 μg of cycloheximide per ml; the wild type will not grow at concentrations above 1 μg/ml) and was homozygous for the mutant *chx-B* gene in its micronucleus (*chx-B*/*chx-B*, cycloheximide resistant, fast growing). After mating was induced, pairs were isolated and allowed to grow up, and cycloheximide-sensitive clones were identified (true exconjugants). Each of these 67 clones was then tested for growth at 40°C and found to divide with a doubling time of >10 h. After allowing at least 40 cell divisions to take place (at 30°C), we screened all these clones for those that had assorted to the recessive Chx-B phenotype (14), i.e., that now grew with 7 μg of cycloheximide per ml. All 23 such phenotypic assorters grew with a doubling time of about 2.5 h at 40°C. Another 25 clones that had not assorted cycloheximide resistance were also tested, and all of these grew at the slow doubling rate.

The second test measured the cosegregation of the two phenotypes when cycloheximide-sensitive, slow-growing *chx-B*/+ micronuclear heterozygotes were selfed. After the mating, 75 pairs were isolated and allowed to grow up, and then each clone was tested for cycloheximide resistance. Altogether, 17 (22%) expressed the

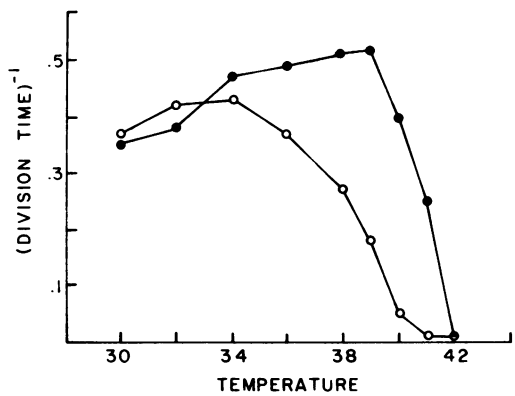

FIG. 4. Temperature dependence of cell division rates for wild-type (○) and strain X-8 (●) cells. Cells were adapted to various temperatures as described in the legend to Fig. 3. Their division times were then measured after 12 to 18 h of equilibration at a given temperature. The reciprocal of the division time (h) is plotted on the ordinate.

original resistant Chx-B phenotype. All 17 grew at the fast doubling time at 40°C, whereas all 30 of the sensitive strains tested showed the slow growth rate. Thus, in all 95 cases tested, cycloheximide-resistant cells grew rapidly and -sensitive cells grew slowly. At this point we concluded that either the mutant gene confering cycloheximide resistance also determines the faster growth rate or there must be two closely linked mutant genes.

Effect of *chx-B* on protein synthesis rates. There is evidence (17) that under some circumstances the polypeptide elongation step can be the rate-limiting factor in determining the overall rate of protein synthesis. To see whether and how elongation rates varied in our cells as a function of temperature, we used the method of Fan and Penman (5) to measure the average translocation rates of ribosomes (i.e., the elongation rates of new polypeptides) in wild-type and strain X-8 cells growing at 30 and 38°C (Fig. 5). Where there were no growth rate differences, we found that the translocation rates were identical. Where there was a difference in growth rate, we found a proportional ribosome translocation rate difference. However, if the differences in ribosome translocation rates were actually directly involved in determining the growth rate differences, then it was not obvious why wild-type cells growing at 30°C showed such a long lag in establishing a new fission rate when shifted to 38°C (Fig. 3b). Visual inspection of a wild-type culture shifted from 30 to 38°C gave an answer: the cells continued to divide but became progressively smaller. Cell fission and cell growth (i.e., protein synthesis) were not equally temperature sensitive. This was confirmed by measuring translocation rates in the wild-type and X-8 strains immediately (<10 min) after a shift from 30 to 38°C (Fig. 5e and f). After the temperature shift, strain X-8 showed no change, whereas within 10 min the wild-type strain already manifested a decreased translocation (and overall incorporation) rate which approached the steady-state rate they would eventually attain. The results for cells shifted from 30 to 40°C confirmed this observation and showed that wild-type cells were depressed even further in elongation and incorporation rates by that shift (Fig. 6).

There are a number of possible interpretations for these data. The relative roles played by initiation, elongation, and termination cannot be deduced from these data alone. We ruled out premature termination as a factor because the average size of the polypeptides synthesized was the same in all cells tested (data not shown). Whatever the cause, one or more steps in protein synthesis appear to be less temperature sensitive in strain X-8 than in the wild type.

Evidence that strain X-8 ribosomes differ physically from wild-type ribosomes. Ribosomes from growing and starved cells are differentially phosphorylated on a small subunit protein and coincidentally display other differences that were interpreted as being due to conformational alterations in ribosome structure (10). One manifestation of this conformation difference was in vitro thermal stability: the ribosome melting temperatures differed by 2.5°C. We reasoned that if ribosomes from strain X-8 differed conformationally from wild-type ribosomes isolated

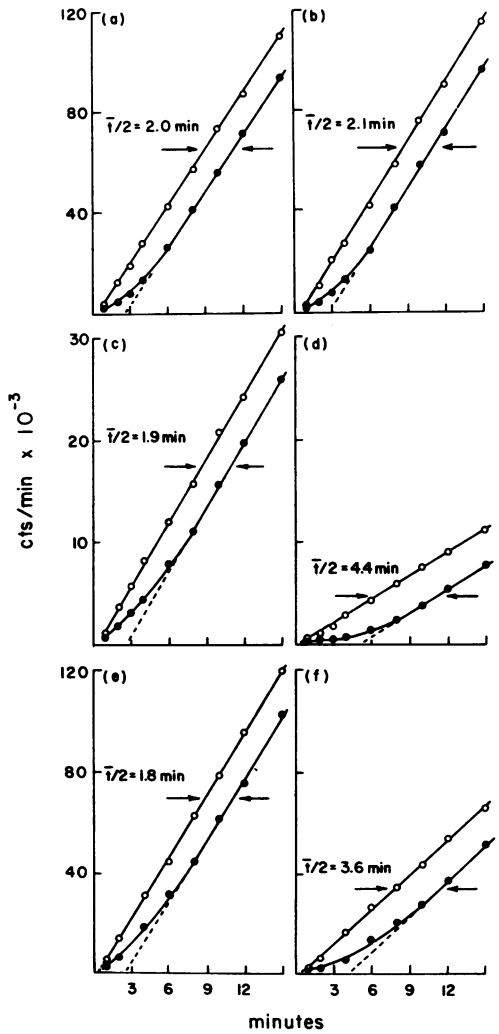

FIG. 5. Ribosome half-transit time (t/2) measurements for strain X-8 (a, c, and e) and wild-type (b, d, and f) cells growing at 30 or 38°C. Measurements were made on cells in early log phase at 30 (a, b) and 38°C (c, d) and on cells at 38°C 10 min after a shift from early log-phase growth at 30°C (e, f). Symbols: O, total radioactive amino acid incorporation; ●, incorporation into released polypeptides.

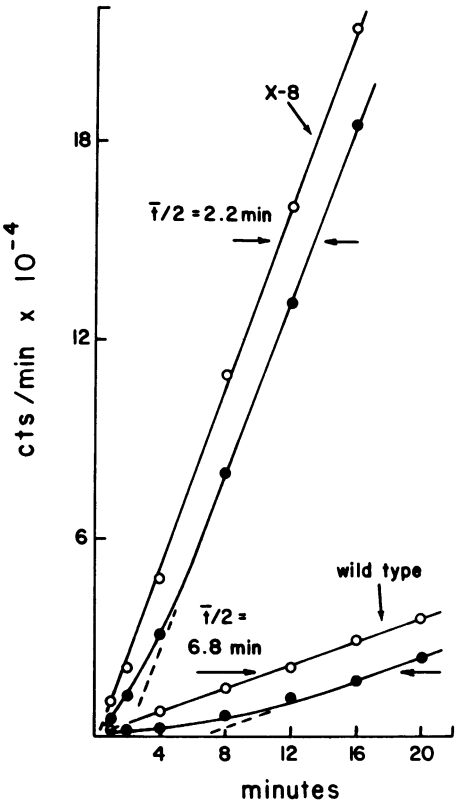

FIG. 6. Ribosome half-transit time ($\bar{t}/2$) measurements for strain X-8 and wild-type cells shifted from 30 to 40°C. Cells in early log-phase at 30°C were shifted to 40°C, and after 10 min transit time measurements were made. Symbols: ○, total radioactive amino acid incorporation; ●, incorporation into released polypeptides.

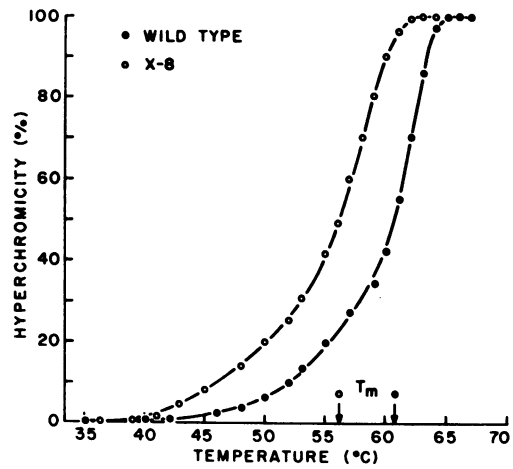

FIG. 7. In vitro thermal stability of ribosomes isolated from strain X-8 (○) and wild-type (●) cells. Ribosomes were subjected to thermal denaturation (10). The half-melting temperature (T_m) for each strain is indicated by arrows.

from cells in the same state of growth, they might display different thermal stabilities. This proved to be so (Fig. 7). The melting temperature of ribosomes from log-phase cells (totally nonphosphorylated) of the two strains differed by almost 5°C. Ribosomes from long-starved cells (fully phosphorylated) of both strains showed increased thermal stability for up to 2.5°C in each case (data not shown), thus retaining their 5°C melting temperature differential.

Another indication that ribosomes from strain X-8 were physically different from those of the wild type came from examining their inhibition by cycloheximide in an in vitro, polyuridylate-directed polyphenylalanine synthesizing system (Fig. 8). The inhibition of wild-type and strain CU-333 ribosomes displayed single-hit inactivation kinetics (this agreed with the finding of a single strong binding site per ribosome [19]), the only difference being that a higher concentration of cycloheximide was required to inhibit the strain CU-333 ribosomes. However, the ribosomes from strain X-8 showed a more complex pattern of inactivation. The molecular interpretation of this inhibition is not clear, but it is certain that strain X-8 ribosomes must be re-

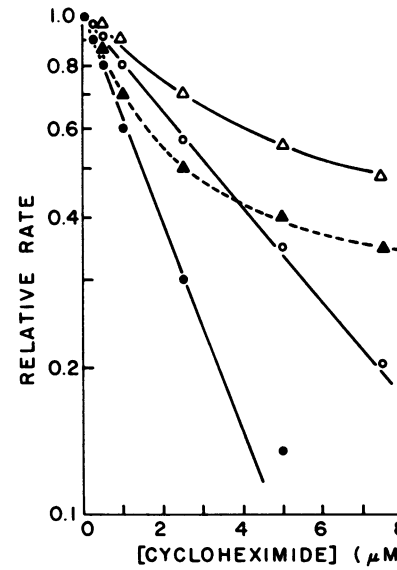

FIG. 8. In vitro effect of cycloheximide on the rate of amino acid polymerization directed by wild-type (●), strain CU-333 (○), strain X-8 (▲), and strain CU-335 (△) ribosomes. Ribosomes were prepared from exponentially growing cells and tested for their ability to function in polyuridylate-directed [^{3}H]polyphenylalanine polymerization in the presence of increasing concentrations of cycloheximide (see the text for details).

sponding differently in their cycloheximide interaction. The inhibition of ribosomes from a strain expressing both mutant genes (CU-335; see above) indicated that the effects were qualitatively and quantitatively additive.

We found a final dissimilarity between the two strains that could be interpreted as resulting from a physical difference in ribosome structure: the turnover rate of rRNA in mature ribosomes of starved cells. rRNA does not turn over in cells in early exponential phase, but after cells are transferred to starvation conditions cellular degradation of rRNA is induced (22). This degradation is biphasic; there is a rapid initial turnover rate that lasts for about 6 h, after which a slower rate ensues. This change in turnover rate and its cessation at refeeding correlated with all the other changes in ribosome structure and function noted above that we presumed to be the result of a change in the conformational state of the ribosome (10). Figure 9 shows the kinetics of degradation of rRNA in newly starved strain X-8 cells and compares it with the data presented previously for wild-type cells (22). Although the slow turnover rates were the same for the two strains, there was clear-cut difference between the initial rates of rRNA degradation. In themselves, these data do not necessarily suggest a physical difference between the ribosomes, but in light of the other data presented we argue that a ribosome with an altered conformation might well have a different susceptiblity to the degradative enzymes involved in its catabolism.

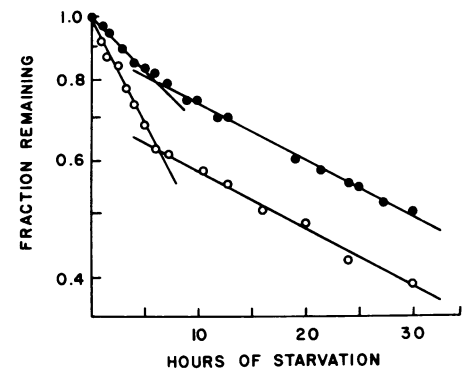

FIG. 9. Degradation of rRNA in starved strain X-8 cells. The cells were labeled with [^{3}H]uridine for two generations in early log-phase growth and then chased in fresh nonradioactive medium for an additional two to three generations. They were then collected, washed, and suspended in starvation medium. Samples were collected at various times during starvation, and their radioactive TCA-insoluble uridine counts were determined. The average of three assays at each time point was normalized to the zero-time value and plotted (●). The other data presented (○) are taken from an earlier experiment (22), in which wild-type cells were treated and analyzed in a like manner.

DISCUSSION

Our conclusion from these studies is that the gene product of the mutant *chx-B* gene probably causes the cells expressing it to have conformationally altered ribosomes. This alteration in ribosome structure then produces a variety of pleiotropic effects. Whether the modified *chx-B* gene in strain X-8 codes for a mutant ribosomal structural protein, a ribosomal protein-modifying enzyme (25), a nucleolar ribosome assembly protein (12), an rRNA-modifying enzyme (13), or something else, we cannot tell. We examined a number of different two-dimensional electropherograms of both the large and small ribosomal subunit proteins from the wild-type and X-8 strains and saw no difference in their electrophoretic mobilities. This, of course, does not mean that there was no mutant ribosomal protein present. However, discovering what the actual mutant gene product is or does requires further experiments. If there is an aberrant ribosomal protein, isolating a partial revertant of cells expressing the mutant Chx-B phenotype (either in growth or drug sensitivity) might allow us to find an electrophoretically altered ribosomal protein if we were to obtain an intragenic suppressor. Intergenic suppressors might also produce an array of different, electrophoretically altered ribosomal proteins. Such genetic analyses are also necessary to clarify completely whether the mutant gene in strain X-8 that confers cycloheximide resistance also imparts the altered growth properties of the strain.

The suggestion that ribosome modifications play a role in translational regulation has recently become a likely possibility (7, 15, 20, 23). If this is so in cells of *T. thermophila*, then we would predict that strain X-8 might well be more or less sensitive to physiologically induced changes in protein synthesis (such as that brought about by hear shock [8] or a change in nutrient conditions [9]) that might require translational regulation. Furthermore, we also predict that the mutant *chx-B* gene in strain X-8 should, in some cases, either suppress or augment other ribosome mutations. We are currently examining these possibilities.

The decline in growth rate for wild-type cells above 34°C could very well be due to a natural temperature sensitivity of some post-initiation step in protein synthesis. This is certainly consistent with our finding that such a rapid decrease in ribosome translocation rates occurred in temperature-shifted wild-type cells, especially if elongation is the rate-limiting step in protein synthesis. Strain X-8 showed neither the decline in growth rate nor the decrease in translocation rate. The reason may be that the temperature

sensitivity of some ribosome-protein synthesis factor interaction is altered in these cells. Once we or others have developed a properly functioning in vitro protein synthesis system derived from *T. thermophila*, this possibility can be directly tested.

Finally, the results presented in this paper indicate that when functional ribosome mutants are desired, selecting for strains with mutations that augment or suppress known antibiotic resistance mutations can produce them. Such strains will be invaluable tools in helping to answer questions on the role of ribosomes in translational control.

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