THERMAL INSTABILITY OF TETRAHYMENA RIBOSOMES: EFFECTS ON PROTEIN SYNTHESIS

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Summary: The thermal denaturation curves of ribosomes from two strains of Tetrahymena pyriformis and a wild strain of Escherichia coli have been compared. The Tetrahymena ribosomes show significant denaturation at temperatures close to each strain's maximum growth temperature; both are considerably less stable than those of the bacterium. The translational efficiency of each strain was measured by determining the ratio of amino acid incorporation under optimal growth conditions and at temperatures slightly above and below the temperature supporting a maximum growth rate. The same temperature levels which initiate in vitro ribosomal melting cause significant in vivo reductions in the efficiency of messenger translation. The loss in template efficiency does not appear to be associated with any qualitative impairment of the translation of individual messengers, since the size distribution on a molecular sieve (Sephadex G-200) is unchanged.

Tetrahymena pyriformis is a ciliated protozoan which divides synchronously following release from a cyclical temperature treatment (1). A variety of high temperature ("heat-shock") regimens have been developed, each consisting of repeating cycles between the maximum growth temperature and a temperature 5 to 10 degrees higher (2). Each heat-shock causes rapid hydrolysis of unstable RNA and a reduction in protein synthesis (3). We report here the effect of temperature increases on the in vitro optical density of purified Tetrahymena ribosomes, the effect of similar temperatures on net protein synthesis in vivo, and the chromatographic properties on a molecular sieve of the proteins synthesized in vivo by normal and heat-shocked cells. The effects of temperature variation on the protein-synthesizing machinery of thermophilic bacteria have recently been reviewed in detail (11).

Tetrahymena pyriformis strains GL and WH-14 were grown in a media consisting of 1% proteose-peptone (Difco), and 0.1% K_2HPO_4, Na acetate, and bacto-dextrose (Difco). E. coli (wild type) was grown in nutrient broth (Difco). Ribosomes were prepared from early stationary phase cultures of each organism essentially as described by Pace and Campbell (ref 4; Fig. 1).

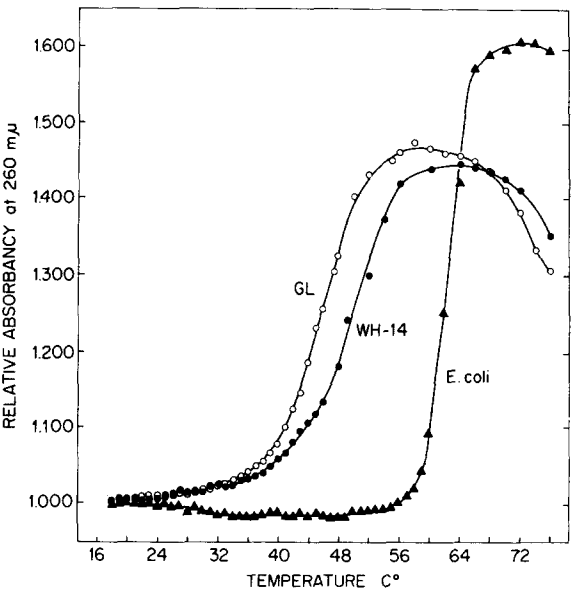

Figure 1: Thermal denaturation of ribosomes from Tetrahymena pyriformis strains GL, WH-14, and from wild-type Escherichia coli: The Tetrahymena strains were grown in a complex media containing 1% proteose-peptone (Difco), and 0.1% KH_2PO_4, Na acetate, and bacto-dextrose (Difco). E. coli was grown in nutrient broth. Ribosomes were isolated (4) from early stationary phase Tetrahymena cultures as follows: Washed and frozen cell pellets were thawed and homogenized in 9 volumes of 0.01 M Tris buffer, pH 7.3, containing 0.01 M $MgCl_2$ (TM buffer). After centrifuging for 10 minutes at 15,000 x g, the ribosomes present in the supernatant were pelleted at 100,000 x g (60 minutes) After gentle resuspension by homogenization the single ribosomes were purified on a 5-20% linear sucrose gradient. Preparation of E. coli ribosomes from sonicated cells followed essentially the same method. The pooled single ribosome peak from each organism was dialyzed overnight against TM buffer and clarified by low speed centrifugation. Thermal denaturation of the ribosome preparation was followed in a Beckman DU spectrophotometer at 260 mμ equipped with a Tamson programmed circulating water bath. The curves are expressed as relative optical density with OD 1.000 representing the average OD recorded between 18° and 23°. Tetrahymena pyriformis strain GL (-o-), strain WH-14 (-●-), E. coli (-▲-). These procedures were adapted from those described by Pace and Campbell (4).

To determine the effect of different temperatures on net protein synthesis in the absence of RNA synthesis, log phase Tetrahymena cultures

were simultaneously exposed to a uniformly labeled ^{14}C-amino acid mixture and Actinomycin D (Act D) as described in the legend to Fig. 2.

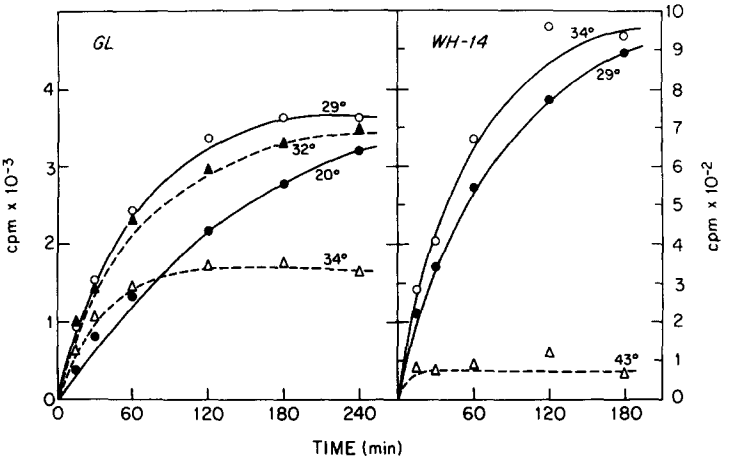

Figure 2: Effect of temperature on ^{14}C-amino acid incorporation in two strains of Tetrahymena: Act D (20 µg/ml) and uniformly labeled ^{14}C-amino acids (2 µc/ml) were added to log phase cultures of Tetrahymena pyriformis strains GL and WH-14. Following removal of zero time controls the cultures were divided and incubated at the temperatures indicated. Uptake of ^{14}C-amino acids was followed by the direct filter paper disc assay (8). Strain GL has a maximum growth rate of 29°, strain WH-14 at 34°.

The effect of a synchronizing heat-shock on the size distribution of newly synthesized proteins was determined by double-labeling the cells and chromatographing the pooled protein extracts on Sephadex G-200 (Fig. 3). Results: Both strains of Tetrahymena studied here can be temperature-synchronized but each strain has different temperature requirements. Strain GL is synchronized by shifts between 29° and 34°, strain WH-14 by shifts between 34° and 43°. In each case the lower temperature is identical with the temperature supporting a maximum growth rate[1] for that strain (2). The thermal denaturation curves of purified ribosomes from Tetrahymena pyri-

[1] Maximum growth temperature is defined here as the temperature supporting a maximum rate of increase in cell number, and is equated with the optimum growth temperature. Above the maximum growth temperature the rate of increase in cell number declines rapidly but growth and cell division are still possible to a limited degree. Hence, the term maximum growth temperature is somewhat ambiguous.

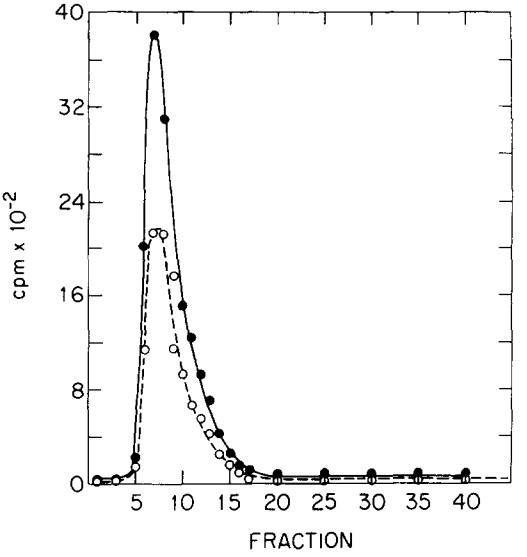

Figure 3: Sephadex G-200 chromatography of soluble proteins synthesized by Tetrahymena pyriformis GL at its maximum growth temperature (29°) and at its synchronizing temperature (34°): A 1 liter log phase Tetrahymena pyriformis GL culture was synchronized, resuspended in 100 ml of inorganic media (0.005 M, pH 7.0 phosphate buffer containing 10^{-4} M $MgCl_2$ and 0.05 M NaCl) during the last cold period, and the synchronizing treatment completed (9). Act D was added 45 minutes prior to synchronous division (20 µg/ml) and the culture was divided in two equal parts. 100 µc ^{3}H-phenylalanine was added to one half which was then incubated at 29°. 10 µc ^{14}C-leucine was added to the other half which was then incubated at 34°. After 45 minutes the cultures were pooled and the cells harvested and washed in cold 0.45% NaCl. The cell pellet was frozen, thawed, and gently homogenized in 10 ml of 1.0 M Tris buffer, pH 7.35. The soluble proteins were isolated by saturating the post-100,000 x g supernatant with solid $(NH_4)_2SO_4$. After 24 hours precipitation the proteins were obtained by centrifuging and then re-dissolved in 5 ml of the Tris buffer. The clear protein solution was chromatographed on a 0.5 cm x 25.0 cm column of Sephadex G-200, eluting with the same buffer. 5 ml fractions were collected and the proteins totally precipitated into discs of Whatman 3mm filter paper for liquid scintillation counting (8). Beyond fraction 17 radioactivity was equal to or less than background and only every fifth fraction is represented. ^{3}H, (-●-); ^{14}C, (-o-).

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formis strains GL and WH-14 are compared to that of E. coli ribosomes in Figure 1. The thermal stability of E. coli ribosomes is significantly greater than either Tetrahymena strain. In addition, the Tm for strain WH-14 is shifted 5-6° into the warmer regions in comparison to strain GL. Ribosomal melting begins in vitro at temperatures remarkably close to the maximum growth temperature of each Tetrahymena strain.

If thermal denaturation occurs in vivo one might reasonably anticipate some disturbance in protein biosynthesis. We previously reported reduced amino acid incorporation when Act D-treated Tetrahymena pyriformis GL are subjected to synchronizing temperatures; we attributed this phenomenon to a reduction in the efficiency of messenger translation (5). This observation has been studied further as shown in Figure 2. It was found that incubating Act D-treated cells from either strain at temperatures at or slightly below the maximum growth temperature had little effect on net amino acid incorporation. In each strain, however, incubation at the synchronizing temperature causes significant reductions in net amino acid incorporation. These results together with those shown in Figure 1 suggest that synchronizing temperatures may impair the binding and translation of template RNA because of in vivo thermal denaturation of ribosomes.

These experiments are consistent with a quantitative reduction in the efficiency of protein synthesis during synchronizing heat-shocks but do not give any information on whether qualitative deterioration in translation also occurs. The most obvious qualitative effect would be an impairment in the translation of messengers already initiated. For example, rupture of the messenger ribosome bonding in mid-translation could lead to release of partially completed peptide strands. This possibility was tested by labeling the newly synthesized proteins with different isotopes. One culture was labeled at the maximal growth temperature (29°) and the other at the temperature used to synchronize the cells (34°).

Conditions were chosen to maximize the possibility of detecting faulty proteins. Synchronized cells were resuspended in inorganic media to facilitate labeling and the portion of the life cycle most susceptible to arrest by a heat-shock was examined (6). After labeling, the two cultures were combined and the pooled soluble protein fractions were purified and chromatographed on Sephadex G-200 as indicated in Figure 3. So far as could be detected the two fractions had identical size distributions. If a heat-

shock led to premature release of peptide chains from the ribosomes one might expect a reduction in the average molecular length and a skewing of the downslope of the heat-shocked sample into the region containing smaller molecules. The curves appear identical.

Discussion: These observations extend the results of Mangiantini et al. (7) and Pace and Campbell (4) who have suggested that ribosomal stability may control the maximal growth temperature in bacteria. In addition, the current experiments strongly imply that exposure to temperatures slightly above the maximal growth temperature can lead to aberrations in protein biosynthesis without necessarily impairing cell viability. It should be noted that while the temperatures which induce in vitro ribosome melting appear to correspond to those which inhibit protein synthesis in vivo there is as yet no direct demonstration that this is the real mechanism of action. The strains involved differ serologically (i.e. possess immunologically demonstrable differences in their surface antigens.) In addition, strain GL is amicronucleate which precludes genetic analysis. Further in vitro studies on protein biosynthesis will therefore be required to confirm the proposal that ribosome thermal denaturation is the primary source of inhibition of protein synthesis which occurs during a heat-shock.

Similarly, qualitative changes in protein synthesis during synchronizing shifts remain to be studied in greater detail. Thus far it appears that the deterioration in translation is an all-or-none effect. Chromatography on Sephadex G-200 would be expected to fractionate proteins in the 1,000 to 200,000 molecular weight range. No evidence for a reduction in the average molecular weight was found although the loss of small oligopeptides during the ammonium sulfate concentration step cannot be ruled out.

The relevance of this type of experiment to metazoans remains to be established. Recent experiments on the slime mold Physarum have shown decreased protein synthesis and polysome breakdown at temperatures above the growth optimum of that organism (10). In addition, we have found that

mouse liver ribosomal RNA undergoes thermal denaturation at temperatures slightly above a mouse's normal body temperature. It is therefore particularly intriguing to speculate on the possible evolutionary role of homeothermy as a protection against thermal impairment of messenger RNA translation in higher forms.

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