The ease with which chick PGC's can be obtained and identified in blood smears now affords a valuable means for further characterization of these cells.

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Temperature Effect on Protein Synthesis in a Heat-Synchronized Protozoan Treated with Actinomycin D

Abstract. *Protein synthesis was studied in the ciliated protozoan* Tetrahymena pyriformis *GL after actinomycin D was added to the culture medium. When the temperature rose above that optimum for growth, there were significant reductions in protein synthesis. Lipid biosynthesis under the same conditions was slightly stimulated, an indication that the effect was not due to an underlying reduction in energy sources. The phenomenon appears to be unique to the protein synthesizing system. Correlation with previous data suggests that it is due to nontranslational destruction of template RNA.*

To a striking degree, the stability of labeled RNA depends upon temperature in *Tetrahymena pyriformis* GL (*1*), a ciliated protozoan that can be brought into division synchrony by a cyclical heat treatment (*2*). Because the exact rate of RNA degradation could not be unambiguously determined at the optimum growth temperature (29°C) we have studied protein synthesis after the addition of actinomycin D in an effort to explore this problem further. When actinomycin D (50 μg/ml) is added to a *Tetrahymena* culture, uptake of uracil stops—and the rate of protein synthesis falls gradually to zero (*1*). Since both RNA (*3*) and protein (*4*) synthesis are required for synchronous division of these organisms, it was important to ascertain whether the unstable RNA fractions included template RNA. Further, it was of interest to see whether the increased rate of degradation was accompanied by a commensurate stimulation of translation. In other words, do temperatures above the growth optimum stimulate the processes of mRNA translation and degradation equally? Our data suggest that the RNA being degraded does in fact include template RNA and that synchronizing temperatures reduce the functional lifetime of these templates (as measured by net protein synthesis). If one defines, for the purpose of comparison, the efficiency of a messenger RNA (mRNA) pool as the net protein synthesized under a given experimental situation (ideal growth conditions yielding maximum translation and maximum efficiency) it would appear that temperatures above the optimum decrease template efficiency in *Tetrahymena*. Implicit in this interpretation is the assumption that actinomycin D acts uniquely to inhibit RNA polymerase (*5*). Parallel experiments with lipid biosynthesis offer some assurance that this is so, since lipid synthesis is increased during similar temperature elevations and this stimulation is unaffected by the presence of actinomycin D. This would seem to rule out some nonspecific effect of the antibiotic but does not rigorously exclude some undefined deleterious effect apart from polymerase inhibition.

For our experiments *Tetrahymena pyriformis* GL was cultivated in a medium consisting of 0.25 percent proteose-peptone (Difco), 0.1 percent sodium acetate, 0.1 percent dibasic potassium phosphate, and 0.1 percent yeast extract (Difco). The generation time of well-aerated cells in this medium is 6 hours at 29°C. The extent of labeling was assayed by the direct filter-paper-disc method (*6*). Protein synthesis was assayed by the addition of an amino acid mixture uniformly labeled with C^{14} to a logarithmically growing culture (final concentration 3 μc/ml). Lipid synthesis was determined by a slightly modified filter-paper-disc procedure (*7*) with C^{14}-glycerol as the substrate. Glycerol enters almost exclusively into lipids soluble in a mixture of chloroform and methanol under these conditions (*7*). In all cases, the actual assays were done by removing 75-μl portions from the culture (in duplicate) at time zero and at regular intervals thereafter. The cells were placed directly on 2.5-cm discs of Whatman 3MM filter paper, the discs were dropped, while still wet, into cold trichloroacetic acid (5 percent for lipid, 10 percent for protein), and the accumulated discs were washed and processed for scintillation counting (*6*, *7*). Where indicated, actinomycin D was added to a final concentration of 50 μg/ml, approximately twice the concentration needed to suppress uracil incorporation in these cells (*1*). To study the effect of altering the incubation temperature, the cultures were divided into equal portions after addition of the label or antibiotic (or both), and then they were incubated at the temperatures cited.

The effect on protein synthesis of varying the incubation temperature after the addition of actinomycin D is shown in Fig. 1. The strain used here (GL) grows most rapidly at 29°C. It can be brought into synchronous division by cycling the temperature to 34°C

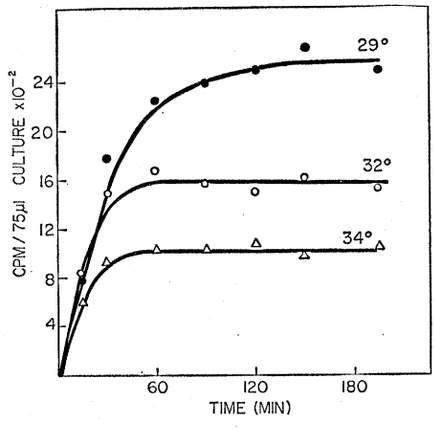

Fig. 1. Incorporation of a C^{14}-amino acid mixture (3 μc/ml) after addition of actinomycin D (50 μg/ml) to a culture of *Tetrahymena pyriformis* GL. Label and antibiotic were added at time zero, after which the culture was divided, and portions were incubated at 29°, 32°, and 34°C. A significant reduction in synthetic capacity occurs as the temperature rises above that optimum for growth (29°C).

for a series of seven temperature shifts (*2*). The amount of protein synthesized after suppression of RNA synthesis varies inversely with the culture temperature for values (32° and 34°C) above the growth optimum (29°C). Not included are curves obtained from temperatures (25° and 27°C) below the growth optimum which attained almost identical asymptotic values as that reached at 29°C. Since these cells necessarily are incubated from the start with identical amounts of template RNA and since there is a great increase in RNA degradation rate above the optimum growth temperature (*1*), it seems reasonable to assume that at least part of this loss in protein-synthesizing capacity is due to loss of mRNA. Messenger destruction may then be occurring without coincident protein synthesis.

In that an extraneous factor might be involved in this net protein loss (such as depletion of energy sources or a general cytotoxicity due to the presence of actinomycin D), we then studied a completely different biosynthetic system. We compared the effect of temperature on the rate of incorporation of glycerol (and acetate) into structural lipids. Initial experiments (not shown) indicated that actinomycin D had no effect on incorporation of either of these materials for any of the temperatures under consideration. We therefore avoided the use of actinomycin D in order to rule out some feature that may have been introduced by its presence. To do this, a culture was divided into two parts, and C^{14}-glycerol was added to one half (5 μc/ml), while the other received the amino acid mixture as before. No antibiotic was added. Each culture was again divided into two equal portions, and one half was incubated at 29°C, the other at 34°C (Fig. 2). The effect on protein synthesis is similar to that seen when actinomycin D is present (that is, net reduction in synthesis is found). The effect on lipid synthesis is the opposite, namely, a slight stimulation occurs. Similar results have been obtained with both labeled acetate and ethanolamine.

The suppression of protein synthesis during synchronizing shifts (*8*) may therefore be due to destruction of template RNA by a process that is not linked to protein synthesis. Fan *et al.* have studied the effect of temperature on mRNA and protein metabolism in *Bacillus subtilis* (*9*) and have found that, for a wide range of temperatures,

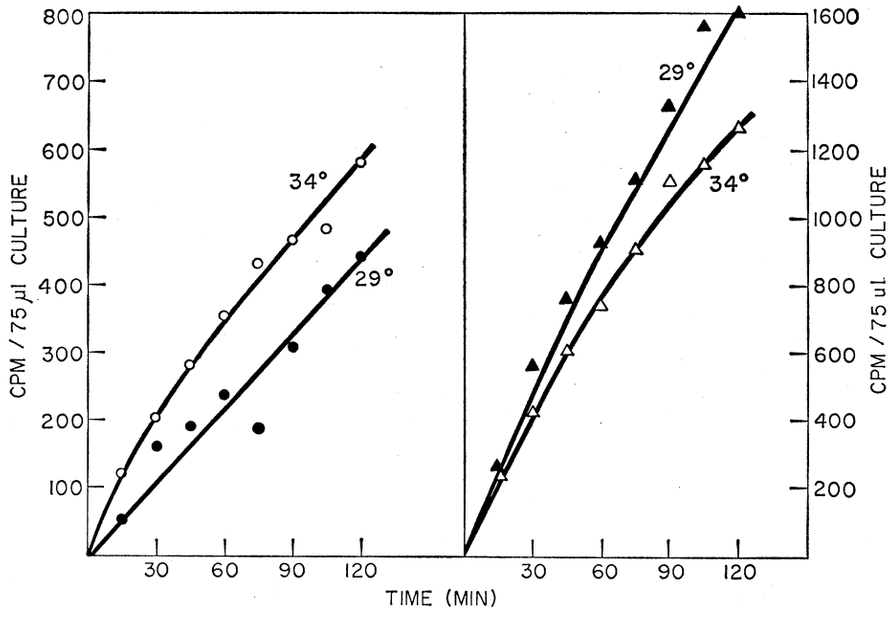

Fig. 2. Incorporation of C^{14}-glycerol (5 μc/ml) at 29°C (-●-) and 34°C (-○-) and of C^{14}-amino acids (3 μc/ml) at 29°C (-▲-) and 34°C (-△-). No antibiotic was included. Protein synthesis is reduced at the higher temperature, while lipid synthesis is slightly stimulated.

a relatively constant relation between the rate of mRNA degradation and protein synthesis existed. They determined that, although the rate of mRNA degradation was greatly influenced by the cultivation temperature, a close relation to (but not dependence on) protein synthesis prevailed. Their data suggested that the "efficiency" of template translation (molecules of protein synthesized during the lifetime of the template molecules) remained remarkably constant. Efficiency used in this sense refers only to net synthesis and is not meant to imply that variations in the number of times individual template molecules may be translated do not exist. Fan *et al.* also noted that, for temperatures above the optimum for growth, some dissociation of mRNA degradation from protein synthesis could be demonstrated.

The importance of this dissociation in *Tetrahymena* is related primarily to the phenomenon of division synchrony. These cells can be brought into good synchrony by cycling a mass culture between the optimum growth temperature and one several degrees higher (*2, 10*). To determine the relevance of this efficiency phenomenon to *Tetrahymena* synchrony in general, we performed similar experiments on strain WH-14. This strain grows best at 34°C (the synchronizing temperature for GL) and can be synchronized by shifts to 43°C. Strain WH-14 had the same characteristics; the efficiency of net mRNA translation was reduced at the temperature required for synchronization, but was not affected by temperatures below the growth optimum. Since an arrest of morphogenesis (*10*) is closely linked to the induction of synchronization, and since both RNA (*3*) and protein (*4*) synthesis are required prior to synchronous division in all strains, it seems likely that destruction of templates without translation is directly relevant to temperature synchronization in these forms.

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