The Effect of Amino Acid Starvation on Nucleoside Uptake and RNA Synthesis in Tetrahymena*

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The uptake of nucleosides and the synthesis of RNA in Tetrahymena thermophila were examined following amino acid starvation. Omission of leucine, phenylalanine, or arginine from the medium resulted in a rapid decrease in the incorporation of [3H]uridine into the acid-soluble pool and acid-insoluble material (RNA). Amino acid starvation inhibited the uptake of all ribo- and deoxyribonucleosides tested but did not affect the uptake of amino acids or glucose. In addition, under the conditions used, the omission of an amino acid did not result in a large decrease in amino acid incorporation into total protein. Treatment of cells with cycloheximide or emetine gave results similar to the effects of amino acid starvation, but in these experiments the inhibition of protein synthesis was essentially complete. Nucleotide pool sizes were also measured following amino acid starvation. ATP and UTP levels were essentially unchanged, but the dTTP pool size was decreased by 40%.

The decrease in RNA synthesis in vivo in the absence of an essential amino acid was reflected in the endogenous RNA synthetic activity of isolated nuclei. However, when solubilized RNA polymerase activity was measured with calf thymus DNA as template, no significant difference was observed between control and amino acid-starved cells.

In prokaryotes the mechanism of amino acid control of RNA synthesis is well understood. Starvation for an amino acid causes a rapid inhibition of synthesis of both stable RNA and the mRNA for ribosomal proteins and translation factors (1-7). This "stringent response" is believed to be mediated through the formation of guanosine-5'-diphosphate-3'-diphosphate (ppGpp) which accumulates during amino acid starvation and regulates transcription of specific genes (1-8).

In eukaryotic cells the "stringent" phenomenon is not as well established. A decrease in RNA synthesis upon amino acid starvation has been reported in a number of cell types (9-15). In yeast cells, amino acid starvation has also been shown to cause specific inhibition of the synthesis of mRNA for ribosomal proteins (16). A continuing search for low molecular weight effectors such as ppGpp has remained inconclusive (17). It has been suggested that the inhibition of protein synthesis caused by amino acid starvation prevents the formation of a rapidly turning over factor that regulates RNA polymerase activity (8, 14, 18, 19) or that the level of deacylated tRNA regulates the metabolic changes that occur upon amino acid starvation (12, 20). In addition, a striking effect on the uptake of nucleosides has been observed during amino acid starvation of several eukaryotic cells (10, 15, 20). However, despite all the previous studies, the mechanism by which amino acid starvation results in a decrease in RNA synthesis in eukaryotic cells is basically unknown.

The protozoan Tetrahymena appears ideally suited for the study of the stringent response in a eukaryotic cell, i.e. the relationship between amino acid availability and RNA synthesis. In this organism, ribosomal DNA (rDNA) is greatly amplified relative to the rest of the genome (21), and, under certain conditions, as much as 40% of the proteins synthesized are ribosomal proteins (22). The present report describes studies on the effect of amino acid starvation on nucleic acid synthesis and the uptake of nucleic acid precursors in Tetrahymena.

EXPERIMENTAL PROCEDURES

Materials

The following isotopes were used: [5-3H]uridine (28 Ci/mmol), [8-3H]guanosine (5.7 Ci/mmol), [methyl-3H]thymidine (6 Ci/mmol), [8-3H]deoxyguanosine (6.5 Ci/mmol), [8-3H]ATP (7 Ci/mmol), and [carboxyl-14C]valine (36 mCi/mmol) from Schwarz/Mann; [U-14C]glucose (291 mCi/mmol), [8-3H]ATP (21 Ci/mmol), and [U-14C]leucine (354 mCi/mmol) from Amersham Corp.; and [7-14C]nicotinic acid (513 mCi/mmol) and [5,6-3H]UTP (37.5 Ci/mmol) from New England Nuclear.

Methods

Cell Culture—Tetrahymena thermophila, strain B, mating type IV, was cultured in the chemically defined medium described by Rasmussen and Modeweg-Hansen (23) except that adenosine and cytidine were omitted, and the concentrations of guanosine and uridine were raised to 0.16 mM and 0.15 mM, respectively. Cells grown in this medium at 30°C with rotary shaking at 90 rpm had a generation time of approximately 3 hr. For experimental purposes cells were grown to a density of 5 to 10 x 10^9 cells/ml and washed with at least 250 volumes of defined medium minus leucine (except where noted). The cells were resuspended in the same medium at a density of 5 to 10 x 10^9 cells/ml, and one set of cultures (controls) received leucine (1.5 mM final concentration). The cells were then incubated at 30°C with agitation.

In Vivo Radiolabeling—The radiolabels were added to the cultures at the beginning of the incubation period. At the indicated times samples were prepared by a procedure similar to that described by Chen and Jones (24). One-milliliter aliquots were layered over 0.4 ml of n-butylphthalate (Fisher) and rapidly centrifuged in an Eppendorf microcentrifuge model 5412 (Brinkmann) into a 50-μl bed containing 15% sucrose and 0.5% sodium dodecyl sulfate. Within 1 min this procedure washes and lyses the cells. The aqueous and n-butylphthalate layers were removed, and the sides of the tubes were carefully
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protein concentration was assayed by the procedure of Lowry et al. contained 50 mM glycine-KOH (pH 9.2); 7.5 mM MgCl₂; 5 pg of 0.5 mM [³H]UTP or [³H]ATP at 0.2 Ci/mmol; radioactivity. soluble fraction were analyzed for radioactivity after the addition of lysate was transferred to a scintillation vial prior to analysis. Zero-time background was subtracted removed by centrifugation at 6000 × g. Incorporation of radioactive glucose and nicotinic acid, the whole cell was boiled for 15 min in the case of protein labeling), and aliquots of the soluble fraction were analyzed as described above. Zero-time background was subtracted (29). Reaction mixtures (100 µl) contained 20 mM Tris-HCl (pH 7.9); 50 mM MgCl₂; 0.6 mM each of ATP, CTP, GTP; 10 µM UTP; 5 µCi of [³H]UTP; 2 mg/ml of bovine serum albumin; 1 mM dithiothreitol; 5 mM NaF; 20 µg of native calf thymus DNA (in the case of solubilized polymerase); and 15 µl (2 to 3 µg of protein) of the nuclei preparations or 5 to 25 µl (2 to 14 µg of protein) of the soluble polymerase preparations. Following incubation at 25°C for the indicated times, the reaction was stopped by the addition of 1 ml of ice-cold 10% CCl₄:COOH-2% sodium pyrophosphate, and the acid-insoluble material was collected and analyzed as described above. Zero-time background was subtracted from all data points and represents 2 to 12% of the total radioactivity.

Nucleic Acid RNA Polymerase Activity—Nuclei and solubilized RNA polymerase were prepared as previously described (25). For analysis of intact nuclear activity, nuclei were suspended in buffer containing 10 mM Tris-HCl (pH 7.9); 0.3 M sucrose, 4 mM MgCl₂, and 1 mM dithiothreitol. The soluble RNA polymerase preparation corresponded to the fraction described as "supernatant of sonicate" by Higashiyama et al. (25).

The RNA polymerase assay was based on that of Goldberg et al. (26). Enzyme activity was determined in 100 µl reaction mixtures containing the following: 0.1 mM Tris-HCl (pH 7.9); 5 mM MgCl₂; 0.6 mM each of ATP, CTP, GTP, 10 µM UTP; 5 µCi of [³H]UTP; 2 mg/ml of bovine serum albumin; 1 mM dithiothreitol; 5 mM NaF; 20 µg of native calf thymus DNA (in the case of solubilized polymerase); and 15 µl (2 to 3 µg of protein) of the nuclei preparations or 5 to 25 µl (2 to 14 µg of protein) of the soluble polymerase preparations. Following incubation at 25°C for the indicated times, the reaction was stopped by the addition of 1 ml of ice-cold 10% CCl₄:COOH-2% sodium pyrophosphate, and the acid-insoluble material was collected and analyzed as described above. Zero-time background was subtracted from all data points and represents 2 to 12% of the total radioactivity.

Nuclear DNA was determined by the method of Burton (27), and protein concentration was assayed by the procedure of Lowry et al. (28).

Analysis of Nucleoside Triphosphate Pools—For the preparation of Tetrahymena extracts, 5 × 10⁹ cells were collected at 500 × g for 10 min at 0°C and resuspended in 0.5 ml of cold 10% CCl₄:COOH. Samples were sonicated for 10 s with a Bronwill Biosonik III at 30% maximum output using the small probe. The insoluble material was removed by centrifugation at 6000 × g for 10 min. The supernatants were extracted three times with 2 ml of anhydrous ether, the aqueous phases were neutralized by the addition of 20 µl of 2 M Tris-HCl, pH 7.9, and the residual ether was evaporated under a stream of cool air.

The activities of ATPase, CTPase, GTPase, and UTPase were determined by a slightly modification of the method of Jamieson and Webb (29). Reaction mixtures (100 µl) contained 20 mM Tris-HCl (pH 7.9); 50 mM KCl; 0.1 mM dithiothreitol; 5 mM MgCl₂; 10 µg of poly(dA-dT); 2.5 µM [³H]ATP or [³H]GTP at 0.2 Ci/mm; 4 units of Escherichia coli RNA polymerase (Enzo-Biochem); and 2 to 4 µl (ATP determination) or 20 to 35 µl (UTP determination) of cell extract. Standard curves were constructed using 100 to 1000 pmol of the limiting nucleotide to be measured. Levels of dTTP were measured essentially as described by Lindberg and Skoog (30). Reaction mixtures (100 µl) contained 50 mM glycine-KOH (pH 9.2); 7.5 mM MgCl₂; 5 µg of poly(dA-dT); 2.5 µM [³H]ATP at 5 Ci/mm; 0.13 unit of E. coli DNA polymerase I (Miles Laboratories, Inc.); and 10 to 20 µl of cell extract. Standard curves were constructed using 4 to 24 pmol of dTTP. The above reactions were incubated at 37°C for 30 min and stopped by the addition of 1 ml of 10% CCl₄:COOH-2% sodium pyrophosphate containing 10 µg/ml of yeast tRNA as carrier. Acid-insoluble material was analyzed as described above.

RESULTS

Effect of Amino Acid Starvation on Uridine Uptake and RNA Synthesis—To initially examine the effect of amino acid starvation on RNA synthesis and uridine uptake in Tetrahymena, cells were incubated in a defined medium containing 19 amino acids plus or minus leucine, and the incorporation of [³H]uridine into RNA (CCl₄:COOH insoluble) and the acid-soluble pool was followed. As shown in Fig. 1, the incorporation of [³H]uridine into RNA was greatly inhibited in the starved cells as compared to control cells (upper panel), but starved cells also exhibited a large decrease in the amount of [³H]uridine present in the acid-soluble pool (lower panel). The effects shown in Fig. 1 occur within the first half-hour of incubation and remain unchanged for at least 6 h. In other studies not shown, we found that these effects could be reversed in less than 1 h by addition of the missing amino acid. During the course of the experiments both starved and unstarved cells continue to divide, with a doubling time of approximately 6 to 7 h for starved cells and 3 h for control cells.

In an attempt to overcome the inhibition of [³H]uridine uptake, the concentration of uridine in the medium was increased to as much as 1.5 mM, 10-fold over the normal concentration used. The results of these experiments are shown in Fig. 2. Uridine uptake into the acid-soluble pool of starved cells increased from 35% to 66% of control levels, but it was apparent that even at very high concentrations of uridine in the medium, the uptake effect could not be completely reversed. Incorporation of [³H]uridine into RNA increased from 15% to 28% of control. It should be noted that in these experiments there was always a greater inhibition of RNA labeling than precursor uptake. Also, the increase in RNA synthesis reached a maximum at a point (0.4 mM uridine) where the uptake of uridine was still increasing. These results indicated that the inhibitory effect of amino acid starvation on the incorporation of [³H]uridine into RNA could not be explained solely by inhibition of uridine uptake.

To determine whether the inhibition of uridine uptake and RNA synthesis was specific for leucine starvation several amino acids essential for Tetrahymena (31) were individually deleted from the medium. The results are summarized in Table 1. Starvation for leucine, phenylalanine, and arginine

![Fig. 1. Inhibition of uridine incorporation into acid-insoluble and soluble fractions following leucine starvation.](image-url)
Amino Acid Starvation in Tetrahymena

resulted in large decreases in the incorporation of \[^{3}H\]uridine into RNA and the acid-soluble pool. The effect of methionine starvation was less dramatic which may indicate that this amino acid is not an absolute requirement for the organism under the conditions used. Table I also shows that deletion of each of the amino acids resulted in less than a 30% decrease in the incorporation of \[^{14}C\]leucine or valine into protein (hot acid-insoluble material). The time course of \[^{14}C\]valine incorporation into protein in the absence of leucine is shown in more detail in Fig. 4. These data confirm that there is a relatively small effect of amino acid starvation on protein synthesis in the first 3 h, presumably due to significant levels of amino acids still remaining in the cells.

These results indicated that in the absence of an amino acid, the inhibition in the uptake of \[^{3}H\]uridine and resulting incorporation of label into RNA was more pronounced than the effect on protein synthesis. Therefore, it appeared that the cell was not responding to a generalized inhibition of protein synthesis during the course of the experiments. To obtain more information on this point the effects of two protein synthesis inhibitors, cycloheximide and emetine, were also examined. As shown in Fig. 4, both inhibitors also caused a dramatic decrease in \[^{3}H\]uridine uptake and incorporation into CCl₄COOH-insoluble material similar to the results seen after amino acid starvation. In these experiments, however, protein synthesis was inhibited by greater than 95% (data not shown). In the presence of either cycloheximide or emetine the cells visibly deteriorated somewhat after 1 h, but the decrease in \[^{3}H\]uridine uptake and RNA synthesis could be seen as early as the first half-hour. Thus, starvation for an amino acid or treatment of cells with protein synthesis inhibitors yielded the same overall effect on uptake and incorporation of uridine into RNA, but the degree of inhibition of protein synthesis was quite different in the two cases.

Effect of Amino Acid Starvation on the Uptake of Other Nutrients—It appeared from the results above that one of the primary effects of amino acid starvation on \[^{3}H\]uridine incorporation into RNA was on the transport of \[^{3}H\]uridine into the cell. To examine the specificity of the transport effect, the incorporation of various compounds into the acid-soluble pool was examined in response to leucine starvation. As seen in Table II, the uptake of \[^{3}H\]guanosine and \[^{3}H\]deoxyguanosine

![URIDINE CONCENTRATION (mM)](image)

**Fig. 2.** Effect of increasing exogenous uridine concentration on the incorporation of \[^{3}H\]uridine into acid-soluble (A) and insoluble (C) fractions of leucine-starved cells. Cells were incubated for 3 h at 30°C in complete medium (control) or medium minus leucine in the presence of varying concentrations of uridine as indicated and 25 μCi/ml of \[^{3}H\]uridine. In these experiments the concentration of guanosine in the medium was increased to 1.6 mM (10-fold over the normal concentration). Samples were prepared as described under "Experimental Procedures." Incorporation of \[^{3}H\]uridine into control cells ranged from 2.1 to 2.8 nmol/10^5 cells for acid-soluble and 1.1 to 1.8 nmol/10^5 cells for acid-insoluble fractions.

<table>
<thead>
<tr>
<th>Amino acid composition of medium</th>
<th>[^{3}H]Uridine</th>
<th>[^{14}C]Amino acid (acid insoluble)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid insoluble</td>
<td>Acid soluble</td>
</tr>
<tr>
<td>Complete</td>
<td>0.45</td>
<td>3.23</td>
</tr>
<tr>
<td>Minus leucine</td>
<td>0.10</td>
<td>1.90</td>
</tr>
<tr>
<td>Minus phenylalanine</td>
<td>0.36</td>
<td>3.10</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>1.02</td>
</tr>
<tr>
<td>Complete</td>
<td>3.22</td>
<td>2.46</td>
</tr>
<tr>
<td>Minus arginine</td>
<td>0.38</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>0.34</td>
<td>2.88</td>
</tr>
<tr>
<td>Minus methionine</td>
<td>0.20</td>
<td>2.18</td>
</tr>
</tbody>
</table>

![HOURS](image)

**Fig. 3.** Time course of incorporation of \[^{14}C\]valine into protein. Cells were incubated at 30°C in complete medium (O) or medium minus leucine (C) in the presence of 1.9 mM \[^{14}C\]valine (8 μCi/ml). At the indicated times 0.5-ml aliquots were withdrawn, and the cells were lysed in 0.6% sodium dodecyl sulfate. Acid-insoluble material was collected and analyzed as described under "Experimental Procedures" following precipitation in 10% CCl₄COOH.
Amino Acid Starvation in Tetrahymena

FIG. 4. Inhibition of uridine incorporation into acid-insoluble and soluble fractions in the presence of protein synthesis inhibitors. Cells were incubated at 30°C in complete medium alone (○) or complete medium with 10 μg/ml of cycloheximide (□) or 20 μg/ml of emetine-hydrochloride (△). [3H]Uridine was present at 0.15 mM (10 μCi/ml). At the indicated times 1-ml aliquots were removed and prepared as described under “Experimental Procedures.”

TABLE II
Effect of leucine starvation on the incorporation of various compounds

<table>
<thead>
<tr>
<th>Radioisotope</th>
<th>Complete medium</th>
<th>Medium minus leucine</th>
<th>10^3 radiolabel/10^5 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>[3H]Guanosine</td>
<td>Acid insoluble</td>
<td>1.56</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>Acid soluble</td>
<td>2.52</td>
<td>0.87</td>
</tr>
<tr>
<td>[3H]Deoxyguanosine</td>
<td>Acid insoluble</td>
<td>0.008</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Acid soluble</td>
<td>0.013</td>
<td>0.004</td>
</tr>
<tr>
<td>[3C]Valine</td>
<td>Acid soluble</td>
<td>3.13</td>
<td>4.32</td>
</tr>
<tr>
<td>[3C]Glucose</td>
<td>Total</td>
<td>998</td>
<td>114.6</td>
</tr>
<tr>
<td>[3C]Nicotinic acid</td>
<td>Total</td>
<td>0.41</td>
<td>0.32</td>
</tr>
</tbody>
</table>

was inhibited similarly to uridine suggesting that all purine and pyrimidine nucleosides are affected. As expected, incorporation of [3H]guanosine and [3H]deoxyguanosine into acid-insoluble material (RNA and DNA, respectively) was also inhibited. However, the uptake of radioactive valine and glucose into the acid-soluble pool was unaffected, although the uptake of nicotinic acid was decreased by about 20%. In studies not shown, the uptake of [3C]leucine following starvation for phenylalanine, arginine, or methionine was also unaffected. The results indicate that amino acid starvation does not result in a generalized inhibition of the uptake of nutrients and may be a unique effect on purine and pyrimidine ribo- and deoxyribonucleosides.

The effect of leucine starvation on the incorporation of [3H]thymidine into DNA and the acid-soluble pool was examined in more detail. As shown in Fig. 5, the kinetics of [3H]thymidine incorporation into DNA resembles the results seen for [3H]uridine incorporation into RNA. Also, in the absence of leucine the uptake of [3H]thymidine was markedly depressed within one-half hour.

Effect of Amino Acid Starvation on the Internal Nucleotide Pool Size—In view of recent reports that purine nucleotide pools are depressed in certain mammalian cells starved for amino acids (32, 33), pool sizes were also examined in Tetrahymena cells starved for leucine. As shown in Table III, the levels of ATP and UTP remained essentially unchanged following leucine starvation. However, the dTTP pool size was lowered by about 40%, a value which must be taken into account in analyzing DNA synthesis under starvation conditions.

Effect of Amino Acid Starvation on Nuclear RNA Polymerase Activity—Although the decreased in vivo incorporation of [3H]uridine into RNA upon amino acid starvation could be
lacking the ability of uptake of [3H]-uridine into the cell, there are many reports of decreases in RNA polymerase activity after amino acid starvation (9-15). In addition, the results described above in Fig. 2 suggest that the decrease in uridine incorporation into RNA could not be due solely to an effect on precursor uptake. In the present experiments, RNA polymerase activity (using endogenous template) was assayed in nuclei isolated from control and amino acid-starved cells. Greater than 80% of the endogenous activity in these cells corresponded to eukaryotic RNA polymerase I, i.e. α-amanitin insensitive (data not shown). Fig. 6 shows that after amino acid starvation there was a large decrease in nuclear polymerase activity. In various experiments, this decrease ranged from 40 to 75%. In other studies not shown, we found that the level of RNA polymerase activity in nuclei isolated from leucine-starved cells could be restored to control (unstarved) levels following a 3-h refeeding.

To determine whether the starvation-induced decrease in polymerase activity was due to a loss of activity on endogenous template or to a decrease in total enzyme activity, the amount of polymerase activity in solubilized nuclear extracts was determined using native calf thymus DNA as exogenous template. As seen in Fig. 7, the amount of total soluble polymerase activity was similar in control and amino acid-starved cells. The results were the same in the presence or absence of α-amanitin.

**DISCUSSION**

In bacterial cells the availability of amino acids regulates the "stringent response" which affects several metabolic processes including protein and RNA synthesis and the uptake of nucleic acid precursors (8, 34). A similar response also appears to be operative in eukaryotic cells (35). However, there are a number of conflicting reports in the literature regarding the effect of amino acid starvation on RNA synthesis in eukaryotes. In particular, the role of trinucleotide pool sizes (32, 33, 36) and protein synthesis inhibition (9, 14, 18, 19) in the regulation of RNA synthesis remains unclear. In this report we have investigated all of the above parameters in *Tetrahymena* in response to starvation for a single essential amino acid.

Recently, Adair and Wolfe (37) showed that the uptake of [3H]uridine into *Tetrahymena* cells was dramatically inhibited when cells were transferred to non-nutrient buffer, and the authors concluded that the regulation of transport was under the control of nutrients other than the nucleosides themselves. Our present findings show that nucleoside transport can be controlled by the availability of amino acids in the medium. In studies not shown, deletion of glucose from the medium had no effect on either uptake of [3H]uridine or RNA synthesis. The marked inhibition of [3H]uridine uptake caused by amino acid starvation could not be overcome completely by increasing the concentration of uridine in the medium to 10-fold over the normal concentration, although addition of the missing amino acid caused a rapid recovery of uptake to control levels. Also, of the nutrients tested, the transport effect appeared to be specific for ribo- and deoxyribonucleosides.

The extent and rapidity of the decrease in the uptake of nucleic acid precursors suggest that it may be a primary physiological response to amino acid starvation. This is supported by the fact that similar observations have been made in yeast (10), mouse fibroblasts (20), and even in *E. coli* (38, 39). In eukaryotes, nucleosides are believed to be transported by facilitated diffusion (40, 41). Adair and Wolfe (37) have suggested that the inhibition of [3H]uridine uptake in *Tetrahymena* following shiftdown in non-nutrient buffer involves an alteration of the plasma membrane which results in a
decrease in the number of functional transport sites. It would be of interest to determine whether this or another mechanism is operative under conditions of starvation for a single amino acid.

In early studies with *Tetrahymena*, Byfield and Scherbaum (42) showed that the addition of amino acids to inorganic medium greatly stimulated RNA synthesis *in vivo*. By using the opposite approach, i.e. amino acid starvation, several investigators have demonstrated amino acid control of RNA synthesis in other eukaryotes including yeast (9, 10), HeLa cells (11, 12), Ehrlich ascites cells (13, 14), and mouse fibroblasts (15). Our data clearly demonstrate the inhibitory effect of amino acid starvation on RNA polymerase activity in isolated *Tetrahymena* macronuclei. This effect accounts for our observation that the apparent decrease in RNA synthesis *in vivo* was always greater than the decrease in uptake of the radioactive precursor. The inhibition of nuclear RNA synthesis was probably not due to an alteration of the polymerase molecules themselves since the activity of soluble RNA polymerase preparations, using calf thymus DNA as template, was the same for control and amino acid-starved cells. This finding also indicates that the lower polymerase activity in starved cells is not related to greater damage to the DNA template or RNA product due to higher nuclease activity since the unfraccionated nuclear extracts which contain the polymerase activity should also contain all other soluble enzyme activities. Although the identity of the nuclear RNA polymerase(s) has not been established, greater than 80% of the activity that we measure is α-amamin insensitive, a property of eukaryotic RNA polymerase I, the enzyme responsible for the synthesis of rRNA (43, 44).

It has been suggested that RNA polymerase I can exist in two separate pools, one pool of “free” polymerase and one of polymerase “engaged” in a transcription complex (45, 46). Much data support the concept that amino acid starvation or treatment of cells with protein synthesis inhibitors results in a shift in the proportions of the “free” and “engaged” forms without altering the total polymerase activity (11, 13, 46–49). “Free” polymerase can be detected by measuring RNA synthesis intact nuclei in the presence of exogenous template such as poly(dA-dT). In studies not shown, we have found that macronuclei from amino acid-starved cells contain the same or only slightly higher amounts of “free” polymerase than control nuclei. Thus, the levels of “free” polymerase cannot account for the fact that total soluble polymerase activity remains the same whereas endogenous polymerase activity is greatly decreased in nuclei from starved cells. One possibility is that a fraction of polymerase molecules is “engaged” on endogenous template but become transcriptionally inactive (or less active) under starvation conditions.

It has also been proposed that rRNA synthesis may be regulated by intracellular levels of ATP and GTP since these pools were found to decrease following amino acid starvation in Ehrlich ascites cells (22, 35). In Friend leukemia cells and rat kidney cells, however, histidine starvation did not lower ATP and GTP pools even though RNA synthesis was inhibited (36). In addition, Gross and Pogo (50) found that amino acid starvation produced an expansion of UTP and CTP pools in yeast. We have found no change in ATP or UTP levels in starved *Tetrahymena* cells, and, therefore, changes in nucleotide pools cannot explain the observed effects on RNA synthesis.

Our studies with [3H]thymidine and [3H]deoxyguanosine are suggestive that DNA synthesis is also inhibited by amino acid starvation, and there is ample evidence in the literature that inhibition of protein synthesis results in decreased DNA synthesis (51–53). Since the uptake of these precursors was also inhibited, however, one must be cautious in interpreting *in vivo* labeling experiments. The finding that the dTTP pool size was decreased under starvation conditions further complicated the issue. This line of investigation was not pursued in the present studies.

The relationship between protein synthesis and RNA synthesis in eukaryotes is not clear. The concept that inhibition of protein synthesis itself triggers the reduction of RNA synthesis is supported by many studies employing protein synthesis inhibitors (10, 11, 46). It has been proposed that a short-lived protein exists which regulates RNA polymerase I activity (9, 14, 18, 19). On the other hand, conditions have been found under which RNA synthesis in isolated nuclei was inhibited even though protein synthesis was not affected or even stimulated (14, 50, 54). Our data do not reveal a close correlation between inhibition of amino acid incorporation into protein and reduced RNA synthesis or nucleoside uptake.

The data are suggestive that the cell can, in some way, sense the drop in amino acid levels even before there is a large effect on protein synthesis. It was previously suggested that the effects of amino acid starvation and those of protein synthesis inhibitors may occur by different mechanisms (52, 46).

In bacteria at least some of the effects of the “stringent response” appear to be mediated by the unusual nucleotide ppGpp which accumulates during amino acid starvation (8). Hershko et al. (35) postulated the existence of a common intracellular mediator involved in the similar set of reactions observed in eukaryotic cells. None has yet been identified. However, it has been suggested that the increased levels of uncharged tRNA which result from amino acid starvation could trigger a number of metabolic changes that have been observed (12, 20). Experiments are in progress to test this hypothesis in *Tetrahymena*.

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REFERENCES

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