

Nucleotide Pools and Regulation of Ribonucleic Acid Synthesis in Yeast

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Nucleotide pools were measured in growing and amino acid-starved *Saccharomyces cerevisiae*. During amino acid starvation there are neither significant changes in the endogenous nucleoside triphosphate pool levels nor measurable synthesis of guanosine 5'-diphosphate, 3'-diphosphate. Stable ribonucleic acid synthesis does not appear to be regulated by changes in the triphosphate pools or by the unusual nucleotide guanosine 5'-diphosphate, 3'-diphosphate.

Amino acid starvation of *Escherichia coli* produces two major effects: (i) blockage of net protein synthesis and (ii) blockage of net ribonucleic acid (RNA) synthesis in strains carrying the RC stringent allele (*rel*⁺) (18). Mutant strains of *E. coli* carrying the RC relaxed allele (*rel*⁻) also stop protein synthesis upon amino acid starvation but continue to accumulate RNA (18). Many other differences between stringent and relaxed strains of *E. coli* have been noted (8). Of particular interest are the differences in uptake of purine and pyrimidine precursors by amino acid-starved bacteria (9) and the production of the usual nucleotide guanosine 5'-diphosphate, 3'-diphosphate (5'ppG3'pp) during starvation of stringent bacteria (4, 5, 14, 19).

Amino acid starvation of auxotrophic mutants of yeasts also results in stopping the net synthesis of RNA (13, 15, 20). Although the effect of amino acid withdrawal on RNA synthesis in yeasts is outwardly similar to the effect observed in bacteria, differences between the two organisms have been noted. A major difference involves the response to antibiotics. Cycloheximide, an inhibitor of protein synthesis in eukaryotic organisms such as yeast, and chloramphenicol, an inhibitor of protein synthesis in bacteria, both inhibit polypeptide elongation by similar mechanisms, and both bind to ribosomes (16, 17, 21). Addition of chloramphenicol to either growing or amino acid-starved bacteria results in the stimulation of RNA synthesis (1, 8, 12), whereas addition of cycloheximide to either growing or amino acid-starved yeasts retards the synthesis of stable RNA (15). The different effect of antibiotics on RNA synthesis suggests that the mechanism of regulation of stable RNA synthesis in yeasts is different from

the regulation in bacteria as has been suggested previously (15).

To clarify further the regulation of RNA synthesis in yeasts, we examined the effects of amino acid starvation on the nucleoside triphosphate pools and also looked for the appearance of any unusual nucleotides.

For these experiments *Saccharomyces cerevisiae* S2072A (obtained by R. Mortimer), requiring arginine, leucine, and tryptophan, was used. Amino acid starvation was initiated by rapid filtration of the culture and suspension in the same medium lacking the required amino acids. The growth of the starved and growing culture was monitored by incorporation of [¹⁴C]methionine (protein synthesis) and [¹⁴C]uracil (RNA synthesis). The results showed an immediate cessation of [¹⁴C]methionine incorporation after removal of the amino acids and no net incorporation of [¹⁴C]uracil after about 30 min, as has been previously reported (reference 15, Fig. 4 and 5). Measurements of the nucleoside triphosphate pool levels were made on separate yeast cultures that had been labeled either with [³²P]- or [¹⁴C]uracil for several generations to assure uniform labeling. Table 1 gives the levels of both the ribonucleoside and deoxyribonucleoside pools in exponentially growing yeasts. The triphosphate pool concentrations measured in samples taken one generation apart yielded similar values, indicating that steady-state labeling was achieved. Moreover, the values obtained by either [³²P]- or [¹⁴C]uracil labeling are in close agreement. Once the values for the concentration of the nucleoside triphosphates in exponentially growing cells were established, we measured the pool levels during amino acid starvation. Figure 1 shows the triphosphate pool levels during amino

TABLE 1. Nucleoside triphosphate pool levels in exponentially grown yeasts^a

Nucleotide	Nucleoside triphosphate ^a (mol/mg [dry wt] × 10 ⁻⁹)	Values for [¹⁴ C]uracil (mol/mg [dry wt] × 10 ⁻⁹)
ATP	4.8 (±0.5)	
dATP	0.15 (±0.02)	
GTP	1.5 (±0.05)	
dGTP	0.05 (±0.005)	
CTP	1.1 (±0.1)	0.72 (±0.1)
dCTP	0.10 (±0.02)	
UTP	1.5 (±0.05)	1.4 (±0.1)
dTTP	0.15 (±0.05)	

^a Yeasts were grown in Wicherham medium (22) with low phosphate (0.756 mM) and either [³²P]- (130 μCi/μmol) or [¹⁴C]uracil (0.5 mCi/ml; specific activity 52 mCi/mmol). After radioactivity had been incorporated for one to two generations, samples were collected and analyzed for nucleotide pools by thin-layer chromatography (2, 6, 7). Nucleoside triphosphates were localized by ultraviolet absorption and autoradiography. Radioactive areas were cut from the chromatogram and counted. The counts were converted to nanomoles using the specific activity of the medium and dry weight of a predetermined cell number; 4.1×10^7 cells equals 1 mg (dry weight). Abbreviations are as follows. ATP, dATP, GTP, dGTP, CTP, dCTP, UTP, and dTTP: adenosine, deoxyadenosine, guanosine, deoxyguanosine, cytidine, deoxycytidine, uridine, and deoxythymidine 5'-triphosphate, respectively.

^b Average of two samples.

acid starvation. In particular, note that the ribonucleoside triphosphate levels during amino acid starvation are equal to or greater than the levels measured during growth. Thus, even though net RNA synthesis stops after 30 min of starvation, there is no reduction in the level of the RNA precursors for at least 2.5 h. This would appear to eliminate a mechanism in which RNA synthesis was regulated by substrate availability, a proposal that has been suggested as a possible mechanism for the regulation of stable RNA synthesis in bacteria (10).

It has been reported that the uptake and phosphorylation of nucleosides, especially pyrimidines, is inhibited in amino acid-starved bacteria (9). This inhibition seems to be another consequence of stopping net RNA synthesis in bacteria, and we wished to know if a similar inhibition occurred in amino acid-starved yeasts. Thus, we measured the uptake of [³H]uridine and its conversion to uridine 5'-triphosphate in growing and amino acid-starved yeast cultures. Two hours after removal of the amino acids both the growing and starved

cultures were pulsed for 15 min with [³H]uridine. At the end of the pulse, samples were analyzed for radioactivity in uridine 5'-triphosphate. The amino acid-starved culture had 85% of the activity found in the growing culture (growing, 900 counts/min; starved, 760 counts/min). The total incorporation into trichloroacetic acid-precipitable material in the starved culture was only 9% that of the growing control (growing 7,100 counts/min; starved 615 counts/min). These data showed that yeast cells, in contrast to *E. coli*, are able to convert exogenous uridine to uridine 5'-triphosphate even when net RNA synthesis is stopped.

Finally, a search was made for the unusual nucleotide 5'ppG3'pp in either growing or amino acid-starved yeasts. A large body of evidence suggests that this nucleotide is directly or indirectly involved in the regulation of RNA synthesis in *E. coli*. Yeast cultures were labeled with [³²P]- or [¹⁴C]guanine, and the acid-solu-

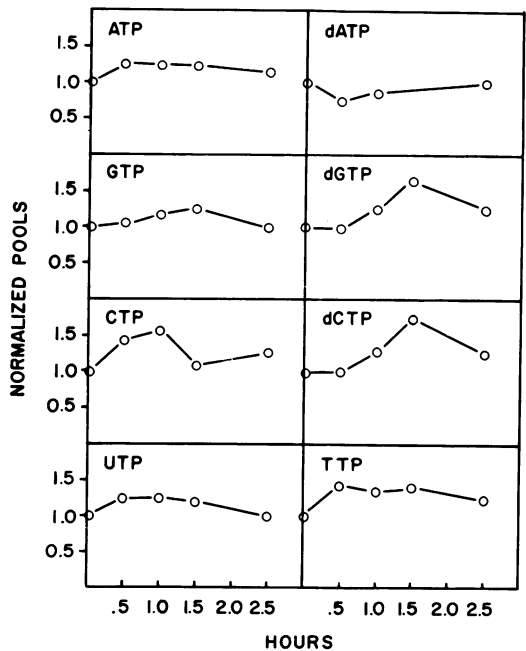


FIG. 1. Nucleoside triphosphate pool levels during amino acid starvation of yeast strain S2072A. Yeast were grown in ³²P-labeled medium and samples were taken as described Table 1, footnote a. Starvation was effected by collecting the growing yeasts on a membrane filter (Millipore Corp.) and resuspending them in identical ³²P-labeled medium with and without the required amino acids. Pool size was determined as described in Table 1 (footnote a), and the values were normalized by dividing by the average of two pool samples taken from an exponentially growing culture.

ble extracts were analyzed for the presence of 5'ppG3'pp. The sensitivity of the chromatographic assay would have permitted detection of 0.05 nmol/mg. of protein (dry weight). None of the labeling procedures in either growing or amino acid-starved yeast cultures gave any evidence for the presence of 5'ppG3'pp. Extracts of ³²P-labeled yeasts, growing or starved, do show the presence of several ³²P-labeled compounds that migrate more slowly than guanosine 5'-triphosphate by thin-layer chromatography. However, none of these compounds migrate with marker 5'ppG3'pp (generously supplied by M. Cashel) nor do they appear if the yeasts are labeled with [¹⁴C]adenine or [¹⁴C]guanine. These compounds are presumed to be polyphosphate, which has been reported to occur in yeasts (11).

The foregoing results suggest that the amino acid regulation of RNA synthesis in yeasts is not caused by the synthesis of 5'ppG3'pp or by changes in the RNA precursor pool levels. Yeast are able to take up and phosphorylate uridine from the medium in the absence of net RNA synthesis, which *E. coli* is unable to do. All of these observations support the idea that the mechanism that regulates RNA synthesis is different in the two organisms.

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