tion of 1 μc/ml. Incubation was carried out at the site from which the algae were collected, with the vials floating naturally in the water. Several incubation times were used, but because of the slow division rates the longer period of incubation (60 minutes) yielded the best autoradiograms for suitable analysis. The distribution of radioactive and nonradioactive cells along a number of filaments can be seen (Table 1). As in the two-membered cultures, there is no evidence for preferential growth at either base or tip of a filament, although radioactive cells occurred in clusters of four to eight or more cells interspaced with clusters of nonradioactive cells. This nonrandom distribution was verified by statistical analysis (*8*) and is seen only in natural situations. From the data, according to the assumptions discussed earlier and using the average percentage of radioactive cells throughout all of the filaments studied, one can estimate the average growth rate of *L. mucor* in nature. Since 43.8 percent of the cells are radioactive, the estimated generation time is 685 minutes. In a similar situation for Long Island Sound, the estimated generation time is 660 minutes. Similar generation times were found in the other habitats studied. These generation times are considerably longer than those determined for two-membered or pure cultures. This difference in generation times is not surprising in view of differences between a natural environment and laboratory cultures with respect to macro- and micro-environmental factors.

My technique should be adaptable to the estimation of the growth rate of any microorganism that can incorporate tritiated thymidine, for which pure cultures are available, and that can be recognized in nature microscopically. Because a wide variety of microorganisms meet these requirements, this technique may have wide application in microbial ecology.

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Tryptophan Deficiency in Rabbit Reticulocytes: Polyribosomes during Interrupted Growth of Hemoglobin Chains

Abstract. *Of several amino acids essential for optimum hemoglobin synthesis by the rabbit reticulocyte, only omission of tryptophan results in polyribosome disaggregation. This disaggregation is prevented by the omission of both tryptophan and an amino acid that is relatively more essential than tryptophan for hemoglobin synthesis. Since tryptophan is located only near the amino-terminal ends of both chains of rabbit globin, the results indicate that single ribosomes and those in polyribosomes are in a dynamic state in the intact cell.*

The synthesis of proteins in a variety of cell types occurs on polyribosomes (*1*). It has been suggested that single ribosomes (or their subunits) become associated with the end of messenger RNA (mRNA) coding for the amino-terminal end of a peptide chain, and then, in concert with aminoacyl transfer RNA (tRNA), enzymes, and cofactors, travel along the mRNA to translate the nucleotide code into an amino acid sequence (*2*). After completion of one round of protein synthesis, the single ribosomes become detached from the mRNA and remain as single ribosomes until they initiate the synthesis of a new protein molecule. Experimental evidence in support of this model has come principally from studies with cell-free preparations (*3*), and it is therefore of interest to ascertain whether recycling of free ribosomes with those in polyribosomes takes place during protein synthesis in the intact cell (*4*, *5*).

The α- and β-chains of rabbit globin contain the common amino acids nearly all of which are distributed at a number of different sites throughout the protein molecule (*6*). A unique characteristic of tryptophan is its location near the amino-terminal end of rabbit hemoglobin, at position 14 of the α-chain and in positions 15 and 37 of the β-chain (*6*). Growth of the peptide chains proceeds from the amino-terminal end (*7*), and therefore during a relative deficiency of tryptophan the rate of

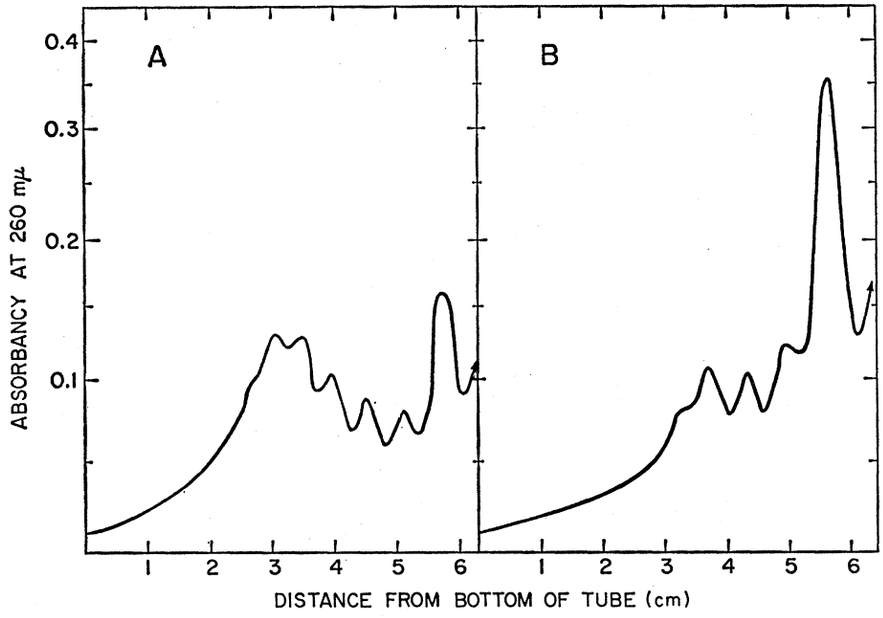

Fig. 1. Reticulocyte polyribosomes during amino acid deficiencies. Conditions corresponding to (A) and (B) are described in Tables 1 and 2.

Table 1. The effect of single amino acid deficiencies upon hemoglobin synthesis and the ribosome-polyribosome profile of reticulocytes. Cells were prepared and incubated (*5*). Amino acids determined essential by Borsook *et al.* (*10*) were present at their recommended concentrations. An additional supplementation, found to enhance hemoglobin synthesis by approximately 20 percent, was also included: L-alanine, L-arginine, L-asparagine, glycine, L-isoleucine, L-proline and L-threonine at 0.2m*M* and L-cystine at 0.05 m*M* concentrations. L-Leucine and L-lysine were omitted from the medium and were added after a 10-minute temperature equilibration as the 1-C^{14}- or 6-C^{14}-labeled compounds, respectively, at 0.5 m*M* final concentration. The incubation was continued for 20 minutes, at which time the cells were lysed, and the lysate was prepared for analysis of the ribosome-polyribosome profile (*5*). The cellular support of hemoglobin synthesis was determined by estimation of radioactivity incorporated into the protein of the ribosome-free supernatant (*5*). Hemoglobin synthesis is expressed as percentage of the completely supplemented control. Cells supplemented with all amino acids had a ribosome-polyribosome profile as indicated in Fig. 1A and incorporated 2 μmole of leucine per gram of protein during the 20-minute incubation period. In another experiment the fully supplemented control incorporated 1.3 μmole of lysine per gram of protein during the same period.

Amino acid omitted	Hemoglobin synthesis (percent of control)	Ribosome-polyribosome profile (Fig. 1)
Histidine	14	A
Valine	24	A
Leucine	26	A
Phenylalanine	26	A
Tryptophan	43	B
Lysine	55	A
Serine	60	A
Tyrosine	74	A

Table 2. Effect of omission of another essential amino acid with tryptophan on hemoglobin synthesis and the ribosome-polyribosome profile. Incubation conditions were as in Fig. 1 and Table 1. Hemoglobin synthesis is expressed as percentage of the completely supplemented control. Additional controls in which tryptophan and each amino acid listed were individually omitted were carried out alongside the doubly deficient sample. Omission of tryptophan did not decrease the rate of hemoglobin synthesis below that found when histidine, valine, leucine, and phenylalanine were omitted singly. Omission of either lysine, serine, or tyrosine did not decrease the rate of hemoglobin synthesis below that found when tryptophan alone was omitted.

Amino acid omitted with tryptophan	Hemoglobin synthesis (percent of control)	Ribosome-polyribosome profile (Fig. 1)
Histidine	18	A
Valine	24	A
Leucine	26	A
Phenylalanine	25	A
Lysine	42	B
Serine	40	B
Tyrosine	42	B

translation of mRNA would be retarded at the sites of tryptophan residues. If there is a normal rate of translation beyond these sites, the inhibition would lead to polyribosome disaggregation because of a failure in the system to maintain the steady-state number of ribosomes on mRNA. A similar study has been made by Baglioni and Colombo (*8*), who used a high concentration of tryptamine to interfere with tryptophan activation (*9*). The isolated rabbit reticulocyte, however, is deficient in tryptophan and cannot support hemoglobin synthesis at an optimum rate unless tryptophan and several other amino acids are included in the incubation medium (*10*). We now report results obtained by omission of individual amino acids in an otherwise complete incubation medium.

A deficiency of tryptophan, but not that of other amino acids required for optimum hemoglobin synthesis (essential amino acid), results in polyribosome disaggregation (Fig. 1, Table 1). Although valine occupies the amino-terminal position of both α- and β-chains of rabbit globin (*11*), a deficiency of this amino acid does not result in polyribosome disaggregation. This may be because valine occupies many other positions throughout the α- and β-chains (*6*) so that a deficiency of this amino acid results both in a diminution of peptide-chain initiation and in interruptions of chain growth at valine sites throughout the protein molecule.

Thus, a deficiency of an amino acid occupying a position near the amino-terminal end of a protein does not result in polyribosome disaggregation; for disaggregation to occur, this amino acid must be located only near the amino-terminal end. This point is further illustrated by the effect of removing another essential amino acid along with tryptophan (Table 2). If an amino acid is more essential than tryptophan (that is, if its omission from the medium lowers the rate of hemoglobin synthesis below that observed when tryptophan is omitted) then an omission of that amino acid along with tryptophan does not result in polyribosome disaggregation. Disaggregation is observed if a less essential amino acid is omitted along with tryptophan, for in such cases tryptophan remains the limiting amino acid for hemoglobin synthesis.

Prolonged incubation of rabbit reticulocytes in tryptophan-deficient medium does not result in the complete breakdown of polyribosomes, apparently because a limited supply of this amino acid is made available by the breakdown of protein associated with cell maturation (*10, 12*). The effects of tryptophan deficiency on both polyribosome structure and protein synthesis are completely reversible upon the addition of tryptophan. A normal polyribosome profile can be established in 3 to 4 minutes after addition of this amino acid. Our results support the view that single ribosomes and those in polyribosomes are in a steady state during protein synthesis in the intact reticulocyte.

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