in experiments on 14 subjects in concentrations ranging from 5×10^{-6} to $2 \times 10^{-4}M$ and volumes from 0.13 to 0.04 μl, without evidence of local anesthetic effects. In 9 of 14 animals, neuronal firing in the caudate-putamen increased following infusion of amphetamine into the substantia nigra, while in four animals it was unaffected and in one animal activity declined in the caudate-putamen. Infusion of amphetamine into the reticular formation above the substantia nigra, or in pars reticulata of the substantia nigra below pars compacta, usually resulted in increased neuronal activity at the recording electrode in these structures, although the reverse was also seen, and increased neuronal activity typically accompanied these changes at the recording site in the caudate-putamen. It is unclear whether in such instances increased neuronal firing in the caudate-putamen is due to release from dopaminergic inhibition, or increased activity in nondopaminergic elements of the substantia nigra or reticular formation, both of which have access to the caudate-putamen (*51*). Bunney *et al.* (*31*) have reported that the inhibition of dopaminergic neuronal firing produced by intravenously administered amphetamine can be blocked by treatment 15 to 30 minutes prior to amphetamine administration with DL-α-methyl-*p*-tyrosine, a drug that inhibits synthesis of catecholamines [S. Spector, A. Sjoerdsma, S. Udenfriend, *J. Pharmacol. Exp. Ther.* **147**, 86 (1965); M. J. Besson, A. Cheramy, J. Glowinski, *ibid.* **177**, 196 (1971); Weissman *et al.* (*15*)]. We have also been able to reduce or abolish the depression of dopaminergic neuronal firing produced by local infusion of amphetamine with similar pretreatments ($N = 4$). In addition, this compound typically leads to marked increases in spontaneous neuronal activity in both pars compacta of the substantia nigra and the caudate-putamen.

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Reticulocyte Transfer RNA and Hemoglobin Synthesis

Transfer RNA availability may regulate hemoglobin synthesis in developing red blood cells.

David W. E. Smith

In most primates and in some other mammals, such as rabbits, the mature red blood cell of the healthy adult contains approximately 300×10^6 hemoglobin molecules (*1*). In other mammals, which have smaller erythrocytes, and even in lower vertebrates, which generally have larger erythrocytes, the hemoglobin concentration within the cells is about the same as in higher mammals. The amount of hemoglobin is constant from cell to cell, which suggests a program of controlled macromolecular synthesis and degradation during red cell differentiation.

Red cell development takes place in the bone marrow and begins with rapidly dividing precursors that contain no hemoglobin and that synthesize many proteins for mitosis and vegetative life. Differentiation requires several days, during which there are several cell divisions that maintain a population of precursors and produce cells that begin to synthesize hemoglobin. Later in development, when the cells are called polychromatophilic erythroblasts and contain some hemoglobin, DNA and RNA synthesis and cell division cease, and hemoglobin synthesis becomes more rapid. From this stage onward globin synthesis is dependent on preexisting messenger RNA (mRNA), transfer RNA (tRNA), ribosomes, and supernatant factors (*2*).

The nucleus is lost in mammalian erythrocyte differentiation, and the penultimate stage is an anucleate cell called a reticulocyte that still synthesizes protein, over 90 percent of which is hemoglobin (*3*). Although reticulocytes originate in the bone marrow, normally about 1 to 2 percent of circulating red cells are reticulocytes that represent the youngest red cell additions to the peripheral blood. In rabbits under stress, such as bleeding or treatment with phenylhydrazine, a chemical which accelerates red cell destruction, reticulocytes may account for 80 to 90 percent of circulating red cells. These reticulocytes are larger and more active in hemoglobin synthesis than normally circulating reticulocytes and are sometimes called "stress reticulocytes" (*4*). Circulating reticulocytes become mature erythrocytes after 2 to 3 days during which polysomes and ribosomes disappear (*5, 6*) and hemoglobin synthesis ceases. While the reticulocyte is a cell in transition, with its contents and its activity in hemoglobin synthesis changing with age, a certain uniformity of the cells can be achieved by a commonly used schedule of phenylhydrazine injections (*7*), and it is possible to obtain reproducible results in studies on hemoglobin synthesis with cells from different rabbits. The stress reticulocyte preparation is the system in which such important discoveries as the direction of translation (*8*), the mechanism of initiation of protein synthesis in eukaryotes (*9*), and the factors and events involved in peptide chain elongation (*10*) have been made. In this article the experiments discussed have been carried out with this system, except where otherwise indicated.

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The System: Hemoglobin Synthesis in Reticulocytes

Rabbit reticulocytes induced by the injection of phenylhydrazine synthesize hemoglobin at a rate of 20,000 to 30,000 molecules per minute (40,000 to 60,000 molecules per minute each of the alpha and beta subunits) (*11, 12*). The amount of preexisting mRNA in these cells is undoubtedly one determinant of this rate, but several kinds of evidence indicate that there are also determinants at the level of globin mRNA translation. For example, in rabbits there is more mRNA for the alpha subunit than for the beta subunit, with different rates of initiation being a major factor in the synthesis of approximately equal numbers of the subunits (*13*). In sheep there is also a discrepancy between the relative amounts of mRNA and the synthesis of the alpha and beta subunits (*14*). In adult humans, in which there are a major and a minor hemoglobin, there is evidence (*15, 16*), which is still controversial (*17*), that the subunits of the major hemoglobin are translated more rapidly than the subunit found only in the minor hemoglobin. In addition, translation of the minor subunit ceases earlier than that of the major subunits, possibly because of mRNA lability. There is evidence that the alpha subunit of rabbit hemoglobin is translated more rapidly than the beta subunit (*18*), although there is also evidence that the translation time for the two subunits is the same (*19*).

It has been determined experimentally that about 30 seconds are required for the synthesis of a hemoglobin subunit at 37°C (*18–20*). Differences which have been described above in the assembly times for the different subunits would not alter this figure very much. Since a subunit chain is about 150 amino acids long, the average rate of amino acid addition to the nascent peptide chain is about five per second. This rate is slower by a factor of 5 or more than the rate of amino acid addition in bacteria at the same temperature (*21*), and it is slower by a factor of about 2 than the average rate of peptide chain elongation in regenerating liver (*22*).

The determinants of the speed of translation of hemoglobin mRNA in reticulocytes have not yet been identified. It has been suggested that some step early in the process of initiation of globin synthesis is a likely determinant (*13*). The work that we discuss below concerns the possibility that the availability of certain aminoacylated tRNA species may limit hemoglobin synthesis at the level of peptide chain elongation. This possibility was first suggested by Itano (*23*) to explain the apparent deficiency of sickle cell hemoglobin synthesis in sickle cell heterozygotes. Despite equal gene dosage for the two allelic beta chains, only about 35 percent of the hemoglobin in these individuals is hemoglobin S. This phenomenon is the result of the deficient synthesis of hemoglobin S that can be observed in the reticulocytes of sickle cell heterozygotes (*24*). Itano suggested that the mutation to sickle cell hemoglobin might introduce a codon corresponding to a tRNA species that is scarce relative to its participation in hemoglobin synthesis. The requirement for this tRNA species at an additional site might result in its being of limited availability. If the tRNA species were required more frequently for the translation of the mutant subunit than the normal subunits, then the translation of the mutant subunit would be reduced in comparison to that of the normal ones. It is now recognized that deficient mutant subunit synthesis occurs in many of the hemoglobinopathies, with the percentage of the mutant hemoglobin in heterozygote erythrocytes being more or less characteristic for each mutation (*25*). This reduction of mutant hemoglobin synthesis is so frequent that it is not likely that a limiting tRNA species is always the cause.

In an effort to test Itano's hypothesis experimentally, Anderson and Gilbert (*26*) added chromatographic fractions of reticulocyte tRNA to a cell-free system obtained from lysed reticulocytes, and were able to alter the ratio of the amount of alpha hemoglobin subunit synthesized to the amount of beta subunit synthesized. It is not known which tRNA species were included in these fractions and which were missing, nor whether the process that was modified was initiation or elongation, or both, but the inference was made that the rate of translation of hemoglobin mRNA for at least one subunit was altered by changes in the availability of one or more tRNA species.

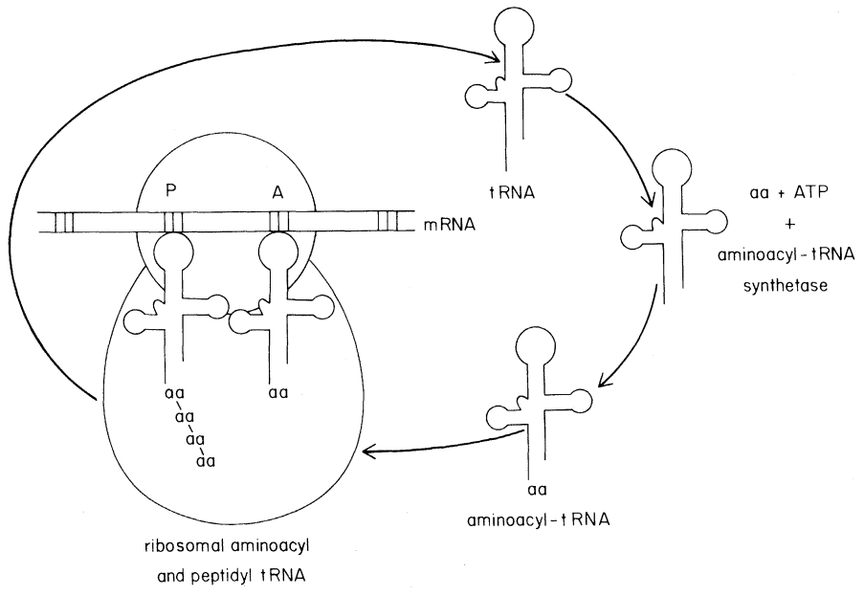

Fig. 1. The tRNA cycle. The various stages through which tRNA is cycled during protein synthesis are shown. The cycling of other components which are also required for protein synthesis such as ribosomes, supernatant factors, and guanosine triphosphate is omitted; however, these have been reviewed (*29*). Although not illustrated, tRNA can be bound to sites on ribosomes that are not involved in protein synthesis; however, such ribosomal attachment is minimal at the Mg^{2+} concentrations of the intact cell.

The tRNA Cycle and the Mechanism of tRNA-Controlled Translation

The concept of tRNA-controlled translation was proposed by Ames and Hartman (*27*) to explain polarity in the synthesis of the enzymes of the histidine operon in *Salmonella*. It was suggested that certain tRNA-codon combinations "modulate" the translation of a polycistronic messenger to provide different amounts of the encoded enzymes. One possible mechanism proposed for modulation was that the limited availability of certain tRNA molecules could delay translation. Translational control is a particularly attractive regulatory mechanism in eukaryotic cells where mRNA is relatively long-lived and control by transcription can function slowly. Its usefulness would seem to be limited to restriction or facilitation of the synthesis of a single protein or a very few proteins in a cell that synthesizes little else. It is too nonspecific a mechanism to be useful in cells in which many kinds of mRNA are being translated simultaneously.

Many experiments have been done to test the tenability of hypotheses of tRNA-controlled translation. In general these investigations have involved the comparison of the tRNA content and chromatographic profiles of tRNA isoaccepting species from cells of different kinds and from cells in different functional states (*28*). Many differences have been found and have generally been interpreted to support tRNA control. In most cases, however, it has been impossible to relate the tRNA changes to the tRNA requirements of protein synthesis because too little is known about the kinds and amounts of protein being synthesized in the cells under study. Hemoglobin synthesis in reticulocytes offers a unique opportunity to assess the relation between tRNA content and protein synthesis for the following reasons: (i) a single protein product that is well characterized is synthesized at a rate that has already been determined; (ii) the system is informationally closed, with protein synthesis dependent on preexisting mRNA and protein synthetic machinery, including tRNA, and these are not altered during study; and (iii) the cells that synthesize hemoglobin can be readily isolated, with virtually no contamination by cells which make other kinds of proteins.

The mechanism of tRNA-controlled protein synthesis is based on the cycling of tRNA during translation. Each time tRNA is involved in amino acid incorporation, it is cycled through several stages (*29*), including free tRNA without an esterified amino acid, tRNA attached to its cognate aminoacyl-tRNA synthetase which sometimes may be attached to a ribosome or be part of a multimolecular complex of synthetases (*30*), free aminoacyl-tRNA, aminoacyl-tRNA attached to its condon on a ribosome, and peptidyl-tRNA in the adjacent position on a ribosome (Fig. 1). After the nascent peptide chain on the tRNA is transferred to the aminoacyl-tRNA on the next codon, the tRNA is released and begins the cycle again. The rapidity of cycling of a tRNA species depends on the frequency with which it participates in protein synthesis, its rate of aminoacylation, its affinity in the aminoacylated form for its codon on a ribosome, and the duration of its ribosomal attachment.

Control of translation by the availability of tRNA may act both positively and restrictively. Limiting amounts of certain tRNA species may delay translation at points where they are required, thus prolonging the synthesis of the polypeptide chain. Conversely, enrichment of certain tRNA species during cellular development and adaptation may facilitate the synthesis of proteins whose translation would have been limited previously. We call this latter process tRNA specialization.

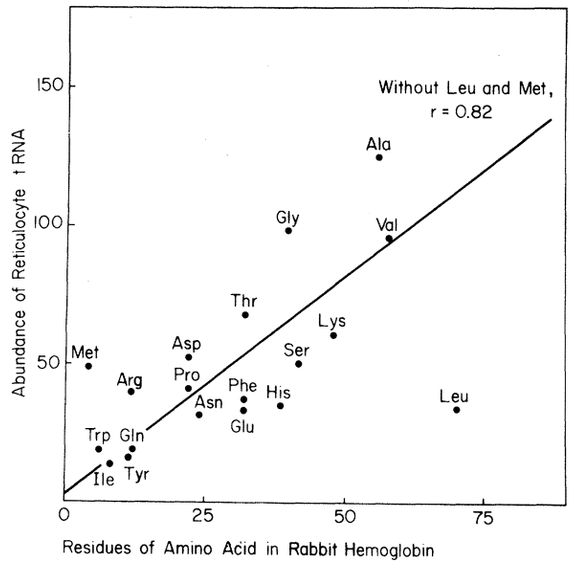

Fig. 2. The relation between rabbit reticulocyte tRNA abundance and amino acid abundance in rabbit hemoglobin. The regression of reticulocyte tRNA content (picomoles of amino acid acceptance per absorbance unit) on amino acid composition of rabbit hemoglobin is plotted. A significant correlation exists. The omission of data concerning leucine and methionine from the calculation of the correlation coefficient, *r* (*32*), is explained in the text. The same correlation coefficient was calculated from these data by Garel (*33*). Abbreviations: Ala, alanine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Glu, glutamic acid; Gln, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Trp, tryptophan; Tyr, tyrosine; and Val, valine.

Recent studies in our laboratory permit a tentative description of the economy of tRNA in reticulocytes during hemoglobin synthesis. There is evidence both that tRNA specialization is involved in red cell differentiation and that tRNA availability is a determinant of the rate of hemoglobin synthesis.

Specialization of Reticulocyte tRNA Content for Hemoglobin Synthesis

Transfer RNA was prepared as quantitatively as possible from reticulocytes and the acceptance activity per absorbance unit for each amino acid was determined (*7*). When the relative abundance of tRNA for the different amino acids was compared with the relative abundance of the amino acids in rabbit hemoglobin (*31*), a strong positive correlation was observed (Fig. 2). There are two clear exceptions to this relationship. Methionine tRNA is very abundant in reticulocytes compared to the amount of methionine in rabbit hemoglobin, even allowing for a labile methionine residue at the amino terminus of each subunit chain (*9*). Leucine tRNA is scarce compared to the abundance of leucine in hemoglobin. These two exceptions are probably important in the determination of the rate of hemoglobin synthesis.

The relative abundance of tRNA for the different amino acids was compared in rabbit reticulocytes and liver (*7*). If reticulocyte tRNA is specialized for hemoglobin synthesis, there are certain respects in which it should be different from the tRNA of liver. These include histidine tRNA, which should be relatively more abundant in reticulocytes, corresponding to the comparative abundance of histidine in rabbit hemoglobin, and isoleucine tRNA, which might be relatively less abundant in reticulocytes than in liver, corresponding to the relative scarcity of isoleucine in rabbit hemoglobin. Each of these predictions was fulfilled in the study, with histidine tRNA three times more abundant in reticulocytes than liver, and isoleucine tRNA two times less abundant (*7*). Since these experiments were performed, evidence has been obtained by another laboratory for tRNA specialization for hemoglobin synthesis in sheep reticulocytes (*34*). Some sheep, if made anemic, synthesize a hemoglobin with a beta chain of abnormal amino acid composition. The tRNA content of the reticulocytes synthesizing this hemoglobin is different from that of the reticulocytes synthesizing the normal adult sheep hemoglobin, with the differences corresponding to the altered amino acid composition of the anemic hemoglobin.

Recently we have obtained evidence that the tRNA content of human reticulocytes is specialized for hemoglobin synthesis (*12*). As with rabbit reticulocytes, tRNA for isoleucine provides an excellent indicator of tRNA specialization, since isoleucine is absent in both subunits of normal, adult human hemoglobin. Transfer RNA for isoleucine is barely detectable (less than 5 pmole of acceptance activity per absorbance unit of tRNA) in human reticulocytes.

Evidence of tRNA specialization for protein synthesis has been obtained in other kinds of cells in which synthesis of a single protein of unusual amino acid composition predominates (*33*). These include

the silk glands of silkworms (*35*), mammary glands at the time of lactation (*36*), the fibroblasts of healing wounds (*37*), and the livers of roosters treated with estrogens that induce the formation of a serine-rich phosphoprotein (*38*). Transfer RNA specialization for protein synthesis can be both an aspect of differentiation, as in reticulocytes, and a functional adaptation of cells that synthesize special proteins for brief periods. The development of a specialized population of tRNA molecules is the likely result of selective transcription of the genes for certain tRNA species, although the processing of tRNA precursors and the postsynthetic modification of tRNA bases may also play a role.

Enumeration and Function of Molecules of Individual tRNA Species

Reticulocytes are unattached cells, and the number of tRNA molecules per reticulocyte was readily determined by extracting tRNA quantitatively from known numbers of cells and assaying it for amino acid acceptance. We have found that there are about 420,000 tRNA molecules in each reticulocyte produced under the stress of phenylhydrazine treatment (*39*). Extracting the erythrocytes of untreated rabbits, which included only 1 to 2 percent reticulocytes, yielded little tRNA. The amount obtained could be attributed to the few reticulocytes present. While results from other laboratories (*6, 40*) indicate that tRNA persists longer than ribosomal RNA, our study shows that it persists only briefly relative to the life span of circulating red cells, which averages 65 to 70 days in the rabbit (*41*).

The tRNA content of each reticulocyte was divided into groups of molecules that accepted each amino acid on the basis of the assays described above. The results show that for most amino acids there are 500 to 1000 tRNA molecules per reticulocyte for each residue of the amino acid in rabbit hemoglobin. Reversed phase column chromatography (*42*) was used to resolve the isoaccepting tRNA species for each amino acid. The distribution of acceptance activity among the isoaccepting species permitted calculation of the number of molecules per cell of each species. There are from 800 to nearly 40,000 molecules of the individual tRNA species per cell. If the volume of a reticulocyte is taken as 100 μm^3 (*2*) and the distribution of tRNA is uniform, the intracellular concentration of a tRNA species represented by 10,000 molecules is about $1.66 \times 10^{-7} M$. This value is of the order of magnitude of the Michaelis constants (K_m's) which have been calculated for tRNA in both aminoacylation (*43*) and polynucleotide-dependent binding to ribosomes (*44*).

In some cases it is possible to relate the number of molecules of a tRNA species to the number of sites in hemoglobin into which the species incorporates its amino acid. The determinations are based on experiments in which labeled amino acids are incorporated into specific sites in hemoglobin by purified aminoacyl-tRNA isoaccepting species.

Two tRNA species for methionine are resolved in our studies. If we assume that these species are homogeneous, there are 19,400 molecules per reticulocyte of methionine $tRNA_1$ ($tRNA_1^{met}$) and 3200 molecules of methionine $tRNA_2$ ($tRNA_2^{met}$). There is evidence (*9*, *45*) that $tRNA_1^{met}$ is the initiating species, $tRNA_f^{met}$. Since there are four initial labile methionine residues (one per subunit chain) per hemoglobin molecule (*9*), it can be calculated that there are 4850 molecules of this species per residue incorporated. There are 800 molecules of the other species ($tRNA_2^{met}$) per residue of methionine incorporated internally in hemoglobin.

The site-specific incorporation of lysine into hemoglobin from the two lysine tRNA species ($tRNA^{lys}$) of reticulocytes was determined (*46*). Assuming that a correlation on the basis of chromatographic position and relative abundance of the two lysine species as isolated in this study and as isolated in our laboratory can be made, we have calculated that there are 512 molecules of $tRNA_1^{lys}$ per reticulocyte per residue it incorporates into hemoglobin and 900 molecules of $tRNA_2^{lys}$ per residue. The $tRNA_1^{lys}$ recognizes the codon AAG, and $tRNA_2^{lys}$ recognizes AAA (A, adenine; G, guanine) (*46, 47*).

We have isolated, by reversed phase column chromatography, two tRNA species each for asparagine and histidine in reticulocytes. These results have been of particular interest because there is only one species of tRNA for each of these amino acids in liver (*48*). An effort has been made to determine the functional significance of the multiple tRNA species for these amino acids in reticulocytes. Coding studies of the species were done by measuring trinucleotide-stimulated binding of the partially purified aminoacylated tRNA's to *Escherichia coli* ribosomes at 10 and 20 m*M* of Mg^{2+} (*49*). Each tRNA species from reticulocytes and the single species from liver responded to both codons for their amino acid, although there were some quantitative differences observed in the binding of asparaginyl-tRNA by the asparagine codons, which may be significant. Studies on the site-specific incorporation of histidine and asparagine into hemoglobin from the tRNA species showed that each species from reticulocytes and liver is able to incorporate its amino acid into all positions in hemoglobin. No quantitative differences were observed which would indicate the preferential use of one of the species of tRNA in either alpha or beta chain translation, but small differences were observed which might indicate preferential, although not exclusive, use of a species to translate certain positions within the subunits (*12, 50*).

There are two likely explanations for the multiplicity of species for these amino acids in reticulocytes. One is that they represent different gene products with different base sequences and possibly different anticodons. In this case the "wobble hypothesis" (*51*) would predict that incorporation differences would not be observed

Table 1. Summary of site-specific incorporation of amino acids into hemoglobin by aminoacyl-tRNA species.

tRNA species	Number of molecules per reticulocyte*	Sites (No.) incorporated per Hb molecule†	tRNA molecules per site‡
$tRNA_1^{met}$ (= $tRNA_f^{met}$)	19,400	4	4,850
$tRNA_2^{met}$ (= $tRNA_m^{met}$)	3,200	4	800
$tRNA_1^{lys}$	20,500	40	512
$tRNA_2^{lys}$	7,200	8	900
$tRNA_1^{his}$ Two species function interchangeably $tRNA_2^{his}$	16,100	38	420
$tRNA_1^{asn}$ Two species function interchangeably $tRNA_2^{asn}$	14,300	24	600
$tRNA^{leu}$ (all isoaccepting species)	15,700	70	224
tRNA for most amino acids			500 to 100

*The values for numbers of tRNA molecules per reticulocyte have been published (*39*). †The numbers of amino acid residues that are incorporated by the tRNA isoaccepting species was determined in several different laboratories. ‡This value was obtained by dividing the number of molecules of a tRNA species by the number of sites in a whole hemoglobin molecule into which it incorporates its amino acid.

because the two codons each for histidine and asparagine differ only by a pyrimidine in the third position. The other is that the two species are products of the same gene with different postsynthetic modifications that give them different chromatographic mobilities. Some of the more complex modifications, such as Q base formation, cause such chromatographic differences and are associated with small changes in coding properties (*52*). On the basis of changes in chromatographic mobility subsequent to treatment with cyanogen bromide and periodate, we believe that the first of the two tRNA isoaccepting species for both histidine and asparagine, and the single species for each of these amino acids in liver, contain Q base (*53*). Whether the differences between the isoacceptors are limited to postsynthetic modifications such as Q base, or also include other base sequence differences, it is probably significant that the small variations in coding at 20 m*M* Mg^{2+} by these species are not amplified at the Mg^{2+} concentrations of intact cells (2 to 3 m*M*) as used in the reticulocyte lysate system to incorporate amino acids from tRNA species. Since no functional differences could be determined between the two tRNA species each for histidine and asparagine in reticulocytes, the use of histidine and asparagine tRNA's can be related to the total number of tRNA molecules for each amino acid and to the total number of residues of each in rabbit hemoglobin. There are, therefore, 600 molecules of asparagine tRNA per asparagine residue and 420 molecules of histidine tRNA per histidine residue. The use of tRNA species in the synthesis of hemoglobin as discussed above is summarized in Table 1.

Frequency of Utilization of Some tRNA Species

If we recall that for most amino acids there are 500 to 1000 tRNA molecules per residue in rabbit hemoglobin, a tRNA species represented by 800 molecules per residue of the amino acid it incorporates may be considered to be of about average abundance. It can be calculated that each molecule of such a species would, on the average, be involved in the incorporation of one amino acid every 2 seconds in a reticulocyte that makes 24,000 hemoglobin molecules per minute. Less abundant tRNA species would, of course, be involved in amino acid incorporation more frequently.

Each time it participates in protein synthesis, a tRNA molecule is attached to a ribosome during the formation of two peptide bonds—one while it is in the aminoacyl-tRNA site, A, and one while it is in the peptidyl-tRNA site, P (*54*). The duration of tRNA attachment to a ribosome is, therefore, in part a function of the availability of the next aminoacyl-tRNA molecule required in the formation of the polypeptide chain, which is also required for release of the tRNA from the ribosome. Transfer RNA can be diverted to ribosomal cellular sites where it is not being actively cycled in protein synthesis. Attachment to these sites is favored by high Mg^{2+} concentrations, and there is evidence that binding to them is minimal at the Mg^{2+} concentration of the intact cell (2 to 3 m*M*).

At an average rate of translation of five peptide bonds per second, 0.2 second is required per bond; therefore, on the average, a tRNA molecule remains attached to a ribosome for 0.4 second each time it incorporates an amino acid. A tRNA species as described, which is involved in protein synthesis once every 2 seconds, would, on the average, be attached to ribosomes 20 percent of the time. Although critical factors such as the affinity of each aminoacyl-tRNA species to its codon on a ribosome remain unknown, it does not seem likely that a tRNA molecule which is attached to ribosomes only 20 percent of the time would be limiting by its lack of availability for protein synthesis.

Leucine tRNA is the least abundant kind of tRNA in reticulocytes relative to the amino acid composition of hemoglobin. While its abundance has not been considered at the level of site-specific incorporation by the several chromatographically separable isoaccepting species, it can be calculated that there are only 224 molecules of leucine tRNA per leucine residue in rabbit hemoglobin. A molecule of this abundance would have to incorporate nearly two amino acid molecules per second in a reticulocyte that synthesizes 24,000 hemoglobin molecules per minute, and would be attached to ribosomes nearly 80 percent of the time, if we assume an average rate of peptide bond formation of five per second. Since aminoacylation and reassociation with a ribosome must occur during the period that the leucine tRNA is unattached, its availability is marginal at best and may limit the rate of hemoglobin synthesis.

Condition of tRNA in Reticulocytes

Determination of the condition of tRNA in reticulocytes provides useful information that can be applied to the problem of tRNA cycling and hemoglobin synthesis and that corroborates some of the conclusions already presented.

Levels of aminoacylation of reticulocyte tRNA were determined from resistance to periodate oxidation of tRNA extracted from fresh reticulocytes under acidic conditions (*55*). It was found that reticulocyte tRNA is highly aminoacylated, varying from 71 percent for glycine tRNA to nearly 100 percent for leucine and phenylalanine tRNA. Periodate-resistant tRNA includes not only free aminoacyl-tRNA but also aminoacyl- and peptidyl-tRNA attached to ribosomes. In view of such high levels of tRNA aminoacylation, it is unlikely that aminoacyl-tRNA synthetase activity or amino acid availability is limiting. Others have shown that deprivation of an essential amino acid decreases tRNA aminoacylation by that amino acid significantly (*56*).

A study was also made of tRNA attachment to reticulocyte ribosomes (*55*). It was determined that there are approximately 10^5 ribosomes (calculated as 80*S* monomers) per reticulocyte. An approximately equal number of tRNA molecules is attached to them when they are isolated in 2.5 m*M* Mg^{2+}. This is about one-quarter of the total reticulocyte tRNA content. This result agrees with the estimate that a tRNA molecule of about average abundance relative to its use in hemoglobin synthesis should be attached to ribosomes about 20 percent of the time. Since ribosomes which are actively synthesizing protein have two tRNA molecules attached per 80*S* monomer (*54*), an upper limit of 50 percent of reticulocyte ribosomes can be engaged in synthesizing hemoglobin. This estimate is substantiated by the independent results of others using different approaches. Several density gradient studies indicate that 30 to 60 percent of reticulocyte ribosomal material is in the polysome fractions, where it can be assumed to be involved in protein synthesis (*54, 57, 58*). Another group has determined that there are approximately 10,000 mRNA molecules for the hemoglobin subunits per reticulocyte in mice (*59*). Since a polysome synthesizing hemoglobin has four to five ribosome monomers attached to each mRNA (*60*), there should be about 50,000 ribosomes participating in hemoglobin synthesis for each reticulocyte.

Ribosomal attachment varies greatly for tRNA's that accept the different amino acids. It ranges from less than 10 percent of the total tRNA for alanine and serine to 65 percent of leucine tRNA. The high level of leucine tRNA attachment to ribosomes confirms the prediction made above that this tRNA, which is the least abundant kind of tRNA relative to its utilization in hemoglobin synthesis, should be attached to ribosomes nearly 80 percent of the time. A high level of ribosomal attachment of

leucine tRNA has been found independently (*61*).

Our results on ribosomal tRNA are applicable to the continuing controversy whether the rate of peptide bond formation is uniform throughout hemoglobin mRNA translation or different bonds are synthesized at different rates. Studies based on rates of labeling of hemoglobin peptides and nascent hemoglobin chains in intact and lysed reticulocytes have provided evidence both for (*8, 62*) and against (*15, 18, 63, 64*) measurable delays or discontinuities during hemoglobin translation. None of the studies was sufficiently sensitive to rule out the possibility of numerous small differences in rates of peptide bond formation throughout translation (*18, 64*). If most ribosomal tRNA molecules are attached to ribosomes which are engaged in hemoglobin synthesis, and if the rate of peptide bond formation is uniform throughout hemoglobin translation, then the abundance of ribosomal tRNA molecules that accept each amino acid should correlate with the abundance of the amino acids in hemoglobin. We found no such relationship. An alternative explanation for levels of ribosomal attachment that can also be discontinued is that most of the ribosomal tRNA is randomly attached to ribosomes that are not actively synthesizing hemoglobin. There is no correlation between ribosomal tRNA for the different amino acids and the abundance of tRNA for these amino acids in the whole reticulocyte. The data on ribosomal tRNA are, in fact, consistent with nonuniform hemoglobin translation, in which the different peptide bonds are formed at different rates. Transfer RNA species that are required immediately ahead of points of slow bond formation would be preferentially attached to ribosomes in this situation. Nonuniform rates of peptide bond formation could be a consequence of the limited availability of some tRNA species.

While nonuniform rates of peptide bond formation may be a major determinant of tRNA attachment to ribosomes, there are at least three situations in which other processes may explain part of the observed ribosomal attachment of tRNA. One of these is methionine tRNA, in which the high level of ribosomal attachment can probably be related to the initiation of protein synthesis as it occurs in eukaryotic cells. Complexes of 40*S* ribosomal subunits with methionyl-$tRNA_f$, which represent an early intermediate in initiation, have been described (*65*). About 10 percent of reticulocyte ribosomal material is in the form of ribosomal subunits (*58, 66*), and 40 to 80 percent of 40*S* subunits in reticulocytes have an attached methionyl-$tRNA_f$ molecule. On the basis of our tRNA enumeration, it can be calculated that there are 4000 to 8000 methionyl-$tRNA_f$ molecules attached to 40*S* subunits per reticulocyte, which accounts for most of the 8440 ribosomal methionine tRNA molecules found in our studies.

It is now recognized that substantial amounts of aminoacyl-tRNA synthetase activity are attached to ribosomes and are included in multimolecular complexes of synthetases (*30, 67*). Transfer RNA that is associated with these synthetases and is not participating directly in hemoglobin synthesis may contribute to the levels of ribosomal tRNA that we have determined, especially in the case of phenylalanine tRNA, because more than 90 percent of the phenylalanyl-tRNA synthetase is attached to ribosomes in reticulocytes.

The high level of ribosomal attachment of arginine tRNA can be explained in large measure by the finding of Protzel and Morris (*68*) that the completed hemoglobin alpha subunit is not released immediately from the ribosome after translation, but remains attached in the form of peptidyl-tRNA for an interval about seven times the average period for peptide bond formation. The carboxy-terminal peptide of this subunit is tyrosylarginine, and arginine tRNA would therefore be attached preferentially to ribosomes pending the release of the new subunit.

Conclusions

Our results and data from other laboratories are consistent with regard to tRNA availability as a controlling factor in hemoglobin synthesis in reticulocytes. Transfer RNA specialization for hemoglobin synthesis is an aspect of red cell differentiation that facilitates the synthesis of this unusual protein. Transfer RNA species that would have been limiting in hemoglobin synthesis in earlier stages of development may be synthesized preferentially so that their relative abundance is increased.

The hypothesis that tRNA availability limits the rate of hemoglobin synthesis is also tenable. An assessment of the utilization of several tRNA species indicates that certain ones may be marginal at best in their availability for hemoglobin synthesis at the rate prevailing in reticulocytes, and that their availability could not support hemoglobin synthesis at a substantially higher rate. The concept of regulation of protein synthesis at the level of translation is especially useful in eukaryotic cells because of their long-lived mRNA that makes regulation at the transcriptional level difficult. A clear picture of tRNA cycling, as has been obtained in reticulocytes, provides a model by which such translational control can be effected in cells where synthesis of a specialized protein predominates.

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NEWS AND COMMENT

Energy Research: A Harsh Critique Says Federal Effort May Backfire

The Energy Research and Development Administration (ERDA) is pursuing "a narrow, hardware-oriented approach" that overemphasizes the importance of increasing energy supplies through complex, new technologies and largely ignores the possibilities of conservation and small-scale technical solutions. As a result, the agency's programs could, ironically, lead to "an increased dependence on foreign energy sources" between now and the year 2000 —the very opposite of the goal enunciated by President Gerald Ford and by ERDA itself.

That surprising conclusion and sharp indictment comes from the congressional Office of Technology Assessment (OTA), which has just completed a comprehensive review of the energy agency's national plan for energy R & D and of the programs launched to achieve the plan's objectives.

The review was requested by the House Committee on Science and Technology, later joined by the Senate Interior and Insular Affairs Committee and the Joint Committee on Atomic Energy. All three have major responsibilities for portions of the ERDA budget.

The analysis was carried out for OTA by six panels of experts drawn from academic, industrial, and nonprofit institutions; environmental and public interest groups; and professional societies.* These panels were backed up by staff members drawn partly from OTA itself, and partly from three universities with active centers for energy policy analysis, namely the Massachusetts Institute of Technology, the University of Oklahoma, and the University of Texas at Austin. In addition, critiques and background papers were solicited from outside groups and individuals. It was unquestionably the most thorough look yet taken at the fledgling energy agency's goals and programs.

In ERDA's defense, it should be pointed out that the agency only became operational on 19 January of this year, and that it was required to submit to Congress by 30 June a national plan for energy research, development, and demonstration. That was barely enough time to find new quarters and hire some key personnel, let alone develop an imaginative, pathfinding plan to solve the much-deplored "energy crisis." Thus it is perhaps not surprising that much of the ERDA effort consists of warmed-over programs inherited from the predecessor agencies that were merged into ERDA, notably the Atomic Energy Commission and the energy portions of the Interior Department. But the OTA evaluators, while sympathizing with the difficulties confronting the new agency, nevertheless pulled few punches because of the immense importance of the agency's task.

The evaluators focused much of their attention on the documents known as ERDA 48, volumes 1 and 2—the "national plan" that was submitted to Congress a few months ago. Volume 1 articulates goals and priorities, while volume 2 sets forth programs to achieve those goals. In general, the evaluators found volume 1 "a significant milestone in the evolution of a long-term national energy policy," though some of the goals were poorly analyzed and appeared to conflict with one another. However, volume 2 was judged markedly inferior and "does not appear adequate to achieve the stated goals," the OTA group concluded.

The evaluators also went beyond the "national plan" and analyzed the President's amended budget, interviewed senior ERDA officials, and talked with key energy staff members from the Environmental Protection Agency, the Federal Energy Administration, and the Office of Management and Budget as well.

They found scores of "deficiencies" which generally fell into two broad categories. One involved an overemphasis on complex, costly technology—the sort of fancy gadgetry that tends to appeal to scientists and engineers, who are often bored by "low technology" approaches to a problem. In OTA's opinion, ERDA has downgraded the less complex technologies that might improve efficiency of energy use, and it has largely ignored such "non-technological" issues as incentives for commercial application, environmental constraints, competition for the use of scarce resources, and public resistance.

The evaluators warn that ERDA might well be successful in developing new technologies, but that these might do little to solve energy problems. As an example, they call it "questionable planning . . . for ERDA to pour large amounts of funds into the development of a commercially fea-

*The overview panel, which prepared a summary of the conclusions to be drawn from the work of the other panels and outside contributors, was chaired by Paul Craig, director of the University of California's Council on Energy and Resources. Other members were Elizabeth Mann Borghese, Center for the Study of Democratic Institutions; John H. Gibbons, University of Tennessee; Jerry Grey, independent consultant; Stanford S. Penner, University of California at San Diego; David J. Rose, Massachusetts Institute of Technology; Robert Socolow, Princeton University; Alvin M. Weinberg, Institute for Energy Analysis; and Wendell H. Wiser, University of Utah. The staff was headed by Jon M. Veigel. Separate panels dealt with fossil programs; nuclear energy; solar, geothermal, and advanced technologies; conservation; and environment and health.