JCI The Journal of Clinical Investigation

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J Clin Invest. 1974;53(4):955-963. https://doi.org/10.1172/JCI107661.

Research Article

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Translational Control of Hemoglobin Synthesis in Thalassemic Bone Marrow

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ABSTRACT Previous studies of β-thalassemic reticulocytes have implied a decreased amount of functional β-mRNA but unimpaired translation of the β-mRNA present. However, the β/α synthetic ratios in β-thalassemic marrow are higher than those observed in reticulocytes of the same patients. This could imply that marrow cells contain an abnormally functioning β-mRNA no longer active in reticulocytes. To test the function of mRNA found in marrow, intact cells were incubated with [^{35}S]methionine and the relative amounts of nascent α- and β-chains on polysomes of different sizes were measured by tryptic digestion and determination of the specific activities of the respective peptides. Results showed that in normal and β-thalassemic marrow, as well as in reticulocytes, β-chain production, though deficient, occurs predominantly on larger polysomes than the production of α-chains. In one patient with severe thalassemia and very little production of β-chains in marrow or reticulocytes, δ-chain synthesis was found predominantly on larger polysomes than α-chain synthesis. These results indicate that in β-thalassemic as well as in nonthalassemic marrow and reticulocytes, each β- and δ-mRNA initiates protein synthesis at a rate faster than does each α-mRNA, and suggest that the β-mRNA in contact with polyribosomes is normally functioning but quantitatively deficient in β-thalassemic marrow as well as in reticulocytes. No translational defect was detected in a similar study performed in reticulocytes of a patient with hemoglobin H disease, suggesting a normally functioning mRNA in contact with polyribosomes in this condition as well. In both thalassemias, unbalanced synthesis of α- and β-chains was more pronounced on polysomes than in completed chains. This difference possibly reflects a compensatory delay in translation of the nonthalassemic chain, which is present in excess.

This paper was presented in part at the annual meeting of the American Society of Hematology, December, 1972.

Dr. Cividalli is a recipient of an International Postdoctoral Research Fellowship (1 F05 TW-1781-02) from the U. S. Public Health Service, on temporary leave from the Department of Pediatrics, Hadassah University Hospital, Jerusalem, Israel.

Received for publication 29 March 1973 and in revised form 18 July 1973.

INTRODUCTION

The thalassemia syndromes are due to unbalanced globin chain synthesis (1). In reticulocytes of patients with either α- or β-thalassemia, this defect appears to be related to a decrease in the quantity of functional mRNA (2–7). On the basis of previous experiments in rabbit reticulocytes (8), the normal pattern of distribution of nascent α- and β-chains on reticulocyte polysomes of one patient with homozygous β-thalassemia was interpreted to indicate a normal rate of initiation of β-mRNA translation (9). This has recently been confirmed by studies of formyl-methionyl-tRNA$_F$ binding to ribosomes and formation of the initial peptide bond (10). Similarly, direct measurements have not revealed any delay in elongation of the affected chain in thalassemic reticulocytes (11, 12). All these data were, however, collected from the study of peripheral blood reticulocytes. The unbalanced globin chain synthesis in β-thalassemia patients is often more marked in reticulocytes than in erythroid precursors in the bone marrow (13, 14), where the bulk of hemoglobin synthesis takes place. The disparity between the β/α synthetic ratio in marrow cells and reticulocytes suggests that marrow cells might contain an abnormally functioning β-globin mRNA that is no longer active in reticulocytes. It was, therefore, decided to extend studies previously performed in reticulocytes to bone marrow cells of β-thalassemia patients to test the function of mRNA found there. Similar stud-

ies were also performed in reticulocytes of a patient with hemoglobin H disease. In the course of these studies, the degree of chain imbalance on polysomes was compared to that in completed chains. The significance of the difference observed was analyzed in terms of its probable relation to the rates of elongation and release of completed chains from ribosomes.

METHODS

Nascent human globin chains on polysomes were labeled with [^{35}S]methionine[1] and analyzed by minor modifications of methods previously described (9). Studies were performed on bone marrow and/or peripheral blood of patients with β-thalassemia, hemoglobin H disease, and other nonthalassemic hemolytic disorders. Each sample was washed three times in a solution of Krebs-Ringer phosphate buffer adjusted to pH 7.4, to which was added glucose at 200 mg/100 ml, $FeSO_4$ at 100 μg/100 ml, and dialyzed human serum albumin at 1 g/100 ml. The cells were resuspended in the same solution at an approximate hematocrit of 30%, and were incubated for 15 min at 37°C with 100 μCi/ml [^{35}S]methionine and a mixture of essential amino acids minus methionine at individual concentrations of 0.2 mM. 5-μl samples were removed every 2 or 3 min to insure that incorporation of labeled methionine into protein was linear at the time when the polysomes were isolated. To collect polysomes and prevent their degradation, most of the sample was removed after 8 or 9 min and transferred to a tube containing chilled isotonic saline with 50 μg/ml cyclohexemide,[2] added to prevent movement of polysomes across mRNA (15). The cells were washed three times in the same saline solution and hemolysed in 4 vol of chilled solution containing 1 mM Mg acetate, 0.1 mM EDTA, cyclohexemide (50 μg/ml) and polyvinyl sulphate[3] (200 μg/ml), the latter being added to reduce polysome degradation. After gentle agitation for 2 min, tonicity was partially restored with 90 mM KCl. An equal volume of unlabeled rabbit reticulocyte lysate, kept at −70° to preserve polysomes, was added as carrier. The pattern of sedimentation of these polysomes showed that little degradation occurred. Membranes and nonlysed cells were completely removed by collecting only the upper two-thirds of the supernate in two 10-min centrifugations at 17,000*g* at 4°C. To separate the various polysome classes, the membrane-free lysate was layered on top of a 36 ml 20–45% sucrose gradient in HEPES[4] buffer, pH 7.0, (25 mM HEPES, 50 mM KCl, 2 mM Mg acetate, and 50 μg/ml cyclohexemide). Usually not more than 1 ml lysate was used, as larger quantities tended to disrupt the gradient. The gradient was then centrifuged at 26,500 rpm in a Beckman L2-65B ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) in a SW27 rotor for 5½ h at 1°C. After centrifugation, each gradient was pumped from the bottom while OD at 260 nm was recorded in a Gilford recording spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio), and 1-ml fractions were collected. Adjacent tubes were pooled into several groups according to polysome size. To collect total ribosomes, all the fractions were pooled in some experiments (on patients R. R., S. D., and T. R.). Alternatively, the membrane-free lysate was layered on top of 25% sucrose in HEPES buffer and centrifuged at 50,000 rpm in a 60Ti rotor for 2½ h. The supernate hemolysate was collected. The ribosome pellet was washed repeatedly with saline and dissolved by incubation for 1 h in 0.1 NaOH. Globin from a nonthalassemic person with a β/α synthetic ratio of about 1, uniformly labeled with [^{3}H]methionine,[5] was used as internal standard (8, 9). It was added to the ribosome pellet, a sample of the supernate, globin prepared from the supernate, or, in equal amounts, to each polysome fraction. The addition of tritium was arbitrarily determined to provide a mean ^{3}H/^{35}S ratio of about 5, to achieve the best possible accuracy of individual ^{35}S and ^{3}H radiation counts. Bovine serum albumin was also added as carrier, whenever necessary to achieve 5 mg of protein in each sample. Incubation with 0.1 N NaOH to release labeled methionine from tRNA, precipitation with trichloroacetic acid (TCA),[6] trypsin digestion, and paper electrophoresis at pH 6.5 in pyridine acetic acid buffer were performed as previously described (9). Quantitation of the relative synthesis of α- and β-nascent chains was obtained by electrophoretic separation of the respective α- and β-T5 peptides and determination of their radioactivity, since the more commonly used chromatographic separation of whole globin chains in 8 M urea does not permit analysis of incomplete globin chains.

To establish the reliability of the electrophoretic separation technique, α-, β-, γ-, and δ-chains, isolated by urea carboxymethyl (CM) cellulose chromatography from globin uniformly labeled with [^{35}S]methionine (16), and precipitated with 5% TCA, were subjected to trypsin digestion and electrophoretic separation in a similar fashion.

Computations. Globin labeled with [^{3}H]methionine and with a β/α activity rate of about one was added in equal amounts to each polysome fraction. Therefore, the relative amounts of α- and β-nascent chains in each polysome fraction would be proportional to the ratio of ^{35}S/^{3}H activity in the methionine containing T5 peptides. Determination of the β/α synthetic ratio in each fraction was obtained by dividing the ratio of ^{35}S/^{3}H counts in the βT5 peak by the ^{35}S/^{3}H counts in the αT5 peak. This method does not require equal recovery of the two peptides, since tritiated globin is used as an internal standard.

When calculated in this fashion, the βT5/αT5 ratio in the supernate represents the synthetic ratio of completed β-chains (plus contaminating δ-chains, see Results) over completed α-chains. In calculating the β/α ratio of nascent chains, one important correction was necessary because the carboxyl-terminal residue of αT5 is at position 40 (of 141 residues), whilst that of βT5 is at position 59 (out of 146). Hence, if ribosomes are distributed uniformly over the mRNA and the time necessary for release of completed chains is negligible in comparison to that necessary for elongation of nascent chains (8), then 72% [(141–40)/141] of the α-ribosomes would contain a nascent αT5 peptide, but only 60% [(146–59)/146] of the β-ribosomes would contain a nascent βT5 peptide. To correct for this

[1] Supplied by Amersham/Searle Corp., Arlington Heights, Ill., at a sp act of 23–41 Ci/mmol.

[2] Supplied by Sigma Chemical Co., St. Louis, Mo.

[3] Distributed by General Biochemicals Div., Mogul Corp., Chagrin Falls, Ohio.

[4] *N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid, supplied by Calbiochem, San Diego, Calif.

[5] Supplied by Amersham/Searle Corp., at a sp act of 8 Ci/mmol.

[6] *Abbreviations used in this paper:* CM, carboxymethyl; TCA. trichloroacetic acid.

difference, the βT5/αT5 synthetic ratio on ribosomes must be multiplied by 1.20 (72/60) so as to make the results comparable to those found in the released supernate proteins.

RESULTS

Fig. 1 illustrates the results of paper iontophoresis of trypsin digests of whole globin labeled with [^{3}H]methionine and of α, β, γ, and δ-chains labeled with [^{35}S]methionine and isolated from globin of a nonthalassemic patient (R. R.). Similar results were obtained when chains isolated from globin of a thalassemic patient (S. D.) were analysed. Adequate separation of αT5 and βT5 labeled with [^{35}S]methionine has already been documented (9), and the reproducibility of repeated measurements of the ratio βT5/αT5 is shown in Table I. The use of cyclophosphamide and carrier rabbit polysomes together with an internal standard of tritiated methionine-labeled globin permitted the development of normal-appearing polysome patterns and accurate assessment of the synthesis of methionine-containing peptides in nascent and completed globin chains. Certain technical problems do arise, however, with the use of this method to determine β/α synthetic ratios in thalassemic patients. For example, δT5 cannot be separated from βT5, which it closely resembles. γT13 may contaminate αT5, but even in the hemoglobin of the patients with thalassemia, the γ/α ratios estimated by urea CM-cellulose chromatography (16) were smaller than 0.09 and the recovery of γT13 after trypsin digestion was less than 20% of the recovery of αT5. Therefore, the contribution of γT13 to αT5 was ignored.

Results of nascent chain distribution on bone marrow polysomes of a patient with immune hemolytic anemia (R. R.) and of another patient with hemolytic anemia due to unstable hemoglobin (T. L.), are represented in Figs. 2 and 3. As has already been reported in human (9, 17) and rabbit (8, 18, 19) reticulocytes, whereas the β/α synthetic ratio in completed chains was close to one, β-nascent chains predominated on heavier polysomes than α-nascent chains. Therefore, the ratio βT5/αT5 decreased from heavy to light polysomes.

The distributions of nascent α- and β-chains were also determined on the polysomes of the marrow cells of three patients with homozygous β-thalassemia. Patient S. D., whose bone marrow study is represented in detail in Fig. 4, had a δ/β and a γ/α synthetic ratio in completed chains of 0.05. Hence the contributions of δT5 to βT5 radioactivity and of γT13 to αT5 radioactivity were negligible. In this patient there was a marked deficiency of completed β-chains and of nascent β-chains on polysomes, but their distribution relative to nascent α-chains remained unaltered. As in the nonthalassemic cells shown in Figs. 3 and 4, the peak of β-chain synthesis occurred on polysomes corresponding in size to the

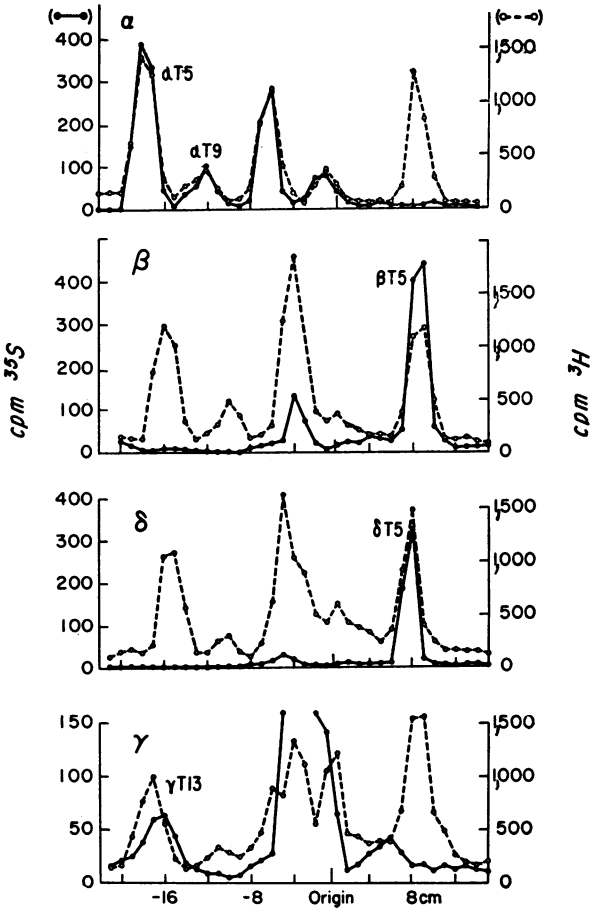

FIGURE 1 Electrophoresis of trypsin digests of isolated human α-, β-, γ-, and δ-chains labeled with [^{35}S]methionine (solid lines). Each electrophoresis of isolated chains was carried out in the presence of a trypsin digest of [^{3}H]-methionine-labeled intact $\alpha_2\beta_2$ globin (broken lines). The type of isolated chain is shown in the upper left hand corner of each panel. The radioactivity associated with the [^{35}S]methionine containing peptides of each isolated chain is shown above or beside the appropriate peak. The notation, such as αT5, means that the peptide is the fifth tryptic peptide from the N-terminus of the α-chain. The isolated chains contained equal amounts of ^{35}S radioactivity.

pentasomes of the rabbit, while α-chains were made on lighter polysomes. Again the ratio βT5/αT5 decreased from heavy to light polysomes.

A similar pattern was observed in patient J. B. (Fig. 5) with more severe β-thalassemia, but the measurements were less accurate because of the very low β/α synthetic ratio. In fact, her marrow cells were nearly devoid of β-chain synthesis (as estimated by urea CM-cellulose chromatography), and β-chain synthesis was exceeded by δ-chain synthesis. Fig. 5 illustrates that in this patient the sum of nascent δ-chains and the few residual β-chains were also found on heavier polysomes

TABLE I

β/α Synthetic Ratios of Nascent Chains Attached to Ribosomes and of Released Chains in the Supernate

Patient	Diagnosis	Source of cells	$(\beta T5/\alpha T5)_{rib}$	$(\beta/\alpha)_{rib}$*	$(\beta/\alpha)_{sup}$
R. R.	Immune hemolytic anemia	Bone marrow	0.88‡	1.05	0.98(0.96)‖
J. G.	Pyruvate kinase deficiency	Peripheral blood	0.83§	1.00	1.08
S. D.	β-thalassemia	Bone marrow	0.36‡	0.44	0.54(0.48)‖
M. G.	β-thalassemia	Bone marrow	0.15§	0.18	0.47
A. S.	β-thalassemia	Bone marrow	0.26§	0.31	0.50
A. S.	β-thalassemia	Peripheral blood	0.25§	0.30	0.47
T. R. (1)	Hemoglobin H disease	Peripheral blood	10.4‡	12.5	5.4(4.5,4.8)‖
T. R. (2)	Hemoglobin H disease	Peripheral blood	17.2§	20.4	3.9

* $(\beta T5/\alpha T5)_{rib} \cdot 1.20$ (See Methods).
‡ Value obtained in pooled polysome and monosome fractions separated on sucrose gradient.
§ Value obtained in pelleted ribosomes.
‖ Repeated measurements on same supernate sample performed to check reproducibility.
rib, ribosomal; sup, supernate.

than were the nascent α-chains. The same results were found in a third patient, J. S. (data not shown).

Results obtained in peripheral blood reticulocytes of T. R., with hemoglobin H disease, are illustrated in Fig. 6. This patient was studied twice. On each occasion nascent β-chains predominated on heavy polysomes while the small amount of α-chains synthesized appeared on lighter polysomes.

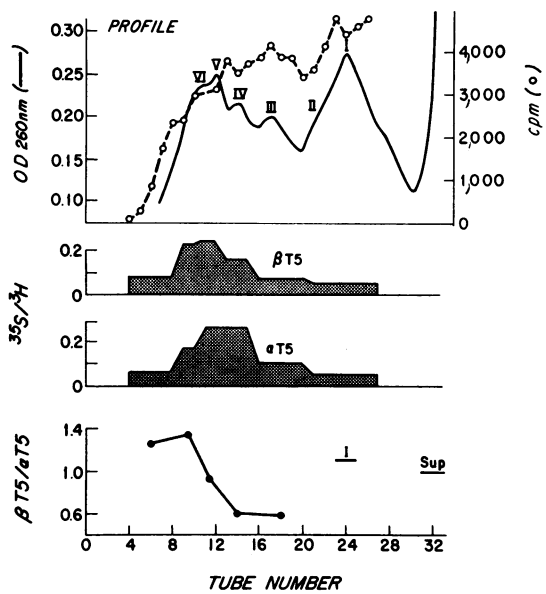

FIGURE 2 Distribution of nascent α- and β-chains on marrow polysomes of R. R. with immune hemolytic anemia. In the upper panel are represented the optical density of carrier rabbit polysomes (solid lines) and incorporated radioactivity into human nascent chains (broken lines) along the polysome gradient; rabbit polysome classes are marked with roman numerals. In the middle panels are represented the distributions of nascent α- and β-chains and in the lower panel, their ratios in the various polysome classes. Sup, supernate. I, single ribosomes.

Results of β/α ratios in nascent chains attached to ribosomes and in completed and released chains in the supernate are compared in Table I. To obtain the β/α synthetic ratio on ribosomes, the βT5/αT5 ratio was corrected for the difference in position of the two peptides (see Methods). This small correction is strictly valid only for the nonthalassemic samples, where it is known that the number of completed chains attached to ribosomes is negligible relative to that of elongating chains (8). In the case of α- or β-thalassemia, it is possible that an appreciable fraction of the ribosome-bound βT5 or αT5 peptides, respectively, are in completed chains (see Discussion). If so, the correction factor would be less than the ratio of 1.20 used for the nonthalassemics (but always more than 1). However, whether or not the

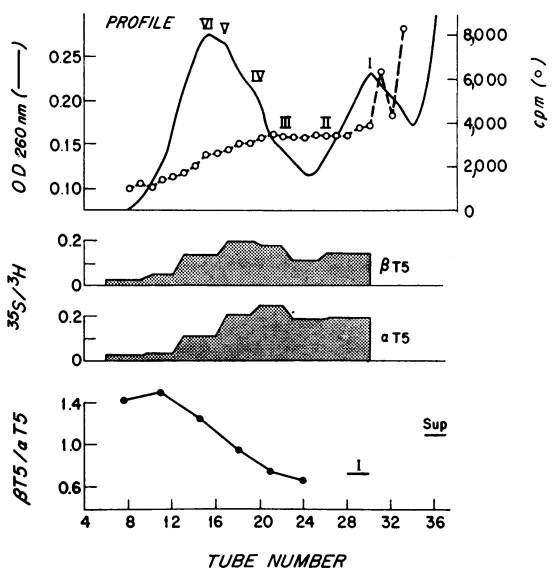

FIGURE 3 Distribution of nascent chains on marrow polysomes of T. L. with hemolytic anemia due to unstable hemoglobin. Symbols are the same as in Fig. 2.

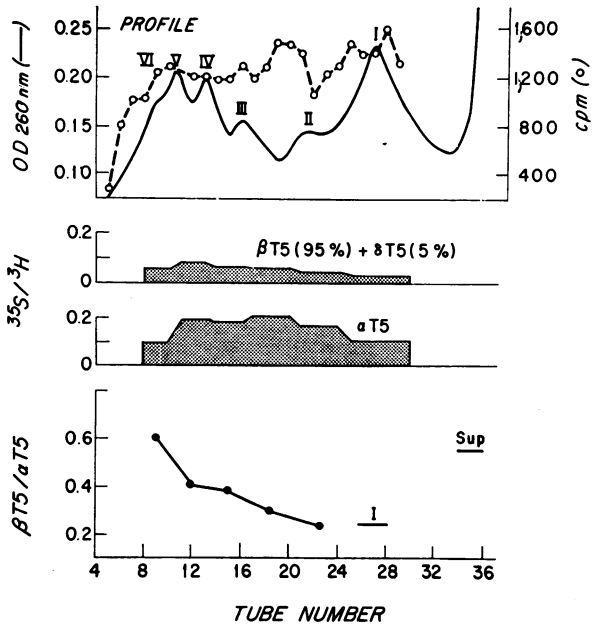

FIGURE 4 Distribution of nascent chains on marrow polysomes of S. D. with a mild form of homozygous β-thalassemia. Symbols are the same as in Fig. 2.

correction was applied, chain imbalance in thalassemia was always more marked in ribosomes than in the supernate, and the difference was particularly striking in hemoglobin H disease. The experiment in Fig. 7 demonstrates that this difference is real and does not result from technical problems involved in the electrophoretic separation technique, even in the presence of very marked α- and β-chain imbalance where determination of the actual β/α synthetic ratio may not be very accurate. Since this result was not expected and suggested the possibility of translational influences on the production of normal chains in thalassemia, additional experiments were performed to rule out obvious potential artifacts.

To determine the stability of newly synthesized completed chains, the following experiments were performed. First the β/α synthetic ratio in the supernate was determined after 9 and 45 min of labeling at 37°C. In every case in which this experiment was performed (J. G., M. G., A. S., T. R.), the 9 and 45 min ratios were similar. Second, when reticulocytes of the patient with hemoglobin H disease (T. R.) were pulse-labeled in the usual manner, washed, and incubated at 37°C for an additional period of up to 1 h, there was no measurable change in the β/α synthetic ratio of completed chains.

To define whether completed chains produced in excess, such as the β-chains in hemoglobin H disease, might bind to polysomes during the sucrose gradient separation procedure, the following experiment was performed. The reticulocyte-rich red cells of patient

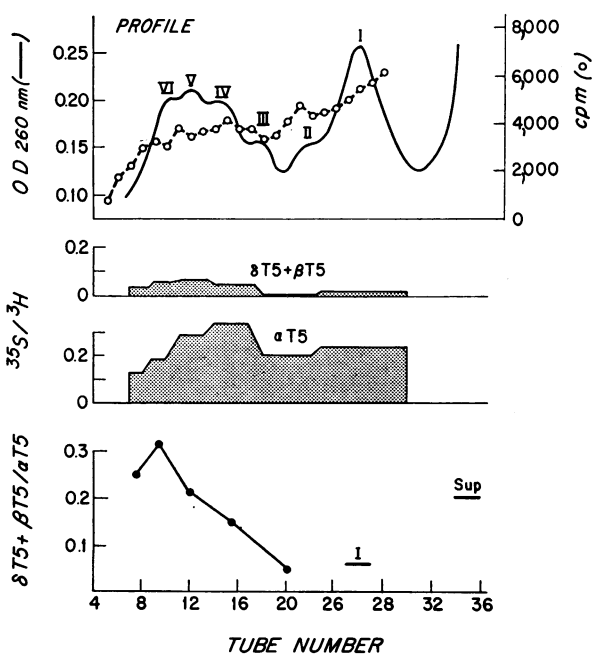

FIGURE 5 Distribution of nascent chains on marrow polysomes of J. B. with a severe form of homozygous β-thalassemia. Symbols are the same as in Fig. 2.

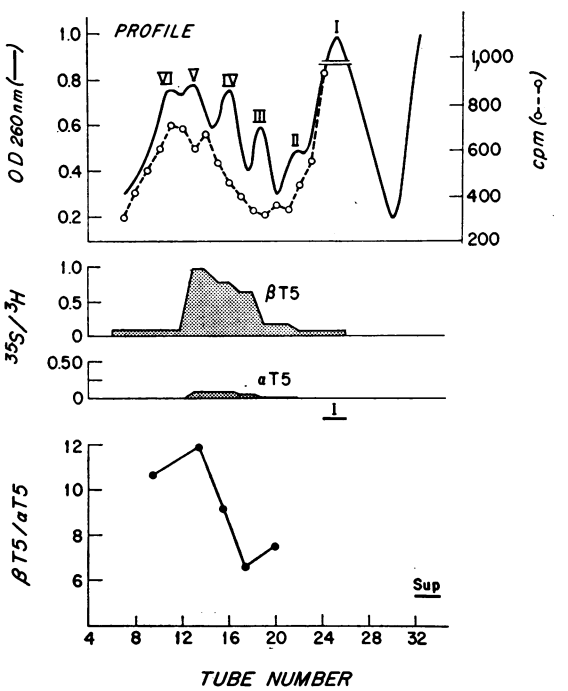

FIGURE 6 Distribution of nascent chains on polysomes in reticulocytes of T. R. with hemoglobin H disease. Symbols are the same as in Fig. 2.

T. R. with hemoglobin H disease were labeled with [^{35}S]methionine in the usual manner. The cells were lysed and the lysate divided into two portions. One was centrifuged at 40,000 rpm for 1 h to remove membranes and ribosomes and the other was centrifuged for 10 min at 10,000 rpm to remove only membranes. 0.75 ml of each were mixed separately in duplicate with 0.25 ml of a rabbit reticulocyte lysate containing polysomes and the mixtures were centrifuged through a sucrose gradient as described in Methods. The radioactivity on the polysomes was then determined and found to be present only when 10,000-rpm hemoglobin H lysate was utilized. No radioactivity was found in the polysome zone of the gradient when labeled but ribosome-free hemoglobin H lysate was mixed with rabbit lysate containing polysomes.

DISCUSSION

The functional analysis of mRNA, utilized here, examines the size of polysomes on which each chain is synthesized as an index of the rate of initiation and elongation. It is based on the assumption that the number of ribosomes on a message, or the size of polysomes on which a given protein is synthesized, is directly proportional to the rate of initiation and inversely proportional to the rate of elongation of the nascent chains. Experiments in rabbit reticulocytes have demonstrated that polysomes associated with nascent β-chains are larger than those associated with nascent α-chains (8, 18). In contrast to earlier work (19), recent measurements have shown (8) that there is no difference in the rate of elongation of α- and β-globin chains in rabbit reticulocytes. The different distribution of α- and β-nascent chains on polysomes has therefore been attributed to a faster rate of initiation of β-mRNA than α-mRNA (8, 15). In bone marrow cells from nonthalassemic patients, nascent β-chains are also associated with heavier polysomes than are α-chains (Figs. 2 and 3). The same β/α ratio in nascent and completed chains (Table I) probably indicates a similar rate of elongation for the two chains (see below). It would therefore appear that β-globin chains are initiated at a faster rate per mRNA molecule than are α-chains in human bone marrow cells as well as in reticulocytes.

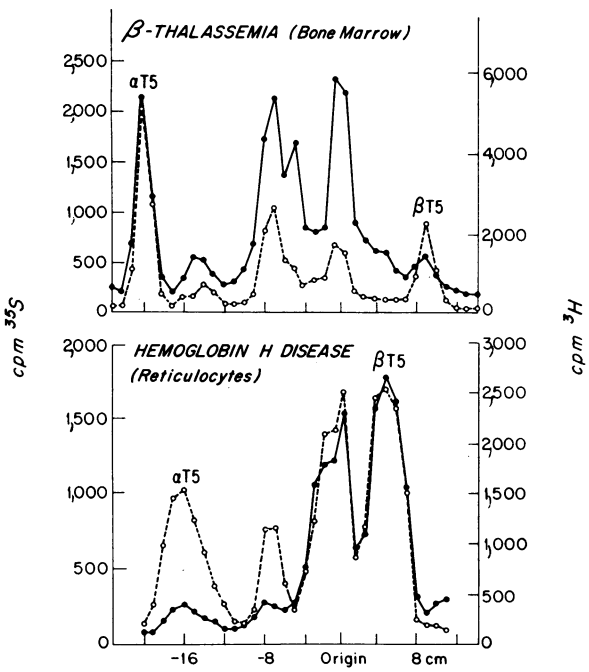

FIGURE 7 Electrophoresis of trypsin digests of nascent and released chains in bone marrow cells of M. G. with β-thalassemia (top) and in reticulocytes of T. R. with hemoglobin H disease (bottom). Solid lines represent [^{35}S]-methionine activity in digested ribosomes; broken lines represent [^{3}H]methionine activity in digested supernate of the same patient. Normal globin was not added in this case as internal standard, and, therefore, β/α synthetic ratios could not be measured. It is, however, evident that chain imbalance is more marked on ribosomes than in released chains.

The same normal distribution of nascent β-chains was observed in bone marrow cells of three patients with homozygous β-thalassemia (Figs. 4 and 5). Among them was patient S. D., with mild homozygous β-thalassemia, in whom contamination of βT5 with δT5 was minimal. The elongation time of β-chains is not prolonged in β-thalassemic reticulocytes (11) and may be even faster than normal (12). It could not be prolonged in this particular patient since otherwise we would have found less, rather than more, chain imbalance in his nascent compared with completed, chains (see below). Hence we conclude that the normal pattern of distribution of nascent chains on his marrow polysomes indicates that initiation of translation of a β-mRNA molecule in contact with ribosomes must occur at a more rapid rate than initiation of translation of an α-mRNA molecule, like the occurrence in nonthalassemic cells. If there were a delay in initiation of his β-chains, they would be attached to lighter polysomes, and the ratio βT5/αT5 would not decrease from large to small polysomes. Recent studies of formyl-methionyl-tRNA$_F$ binding to ribosomes have confirmed the validity of this approach in demonstrating a normal initiation of mRNA translation in thalassemic reticulocytes (10). In the bone marrow cells of the two other patients with severe homozygous β-thalassemia, the sum of nascent β- and α-chains also predominated on heavier polysomes than did the α-chains, suggesting that translation of δ-mRNA as well as of β-mRNA is also initiated at a faster rate than that of α-mRNA.

A normal distribution of nascent α- and β-chains was found on polysomes of reticulocytes from a patient with hemoglobin H disease, suggesting normal rates of initia-

tion of the two globin chains in this condition as well. However, this approach to estimation of mRNA function is not particularly well suited to the study of α-thalassemia syndromes. A decrease in the rate of initiation of translation of α-mRNA and resultant synthesis of α-chains on smaller polysomes would not result in a reversal of the normal tendency of βT5 to predominate on larger polysomes than αT5, as would occur if there were a decrease in the rate of initiation of β-chains in β-thalassemia. A shift of α-chain synthesis to even smaller polysomes (which might occur if α-mRNA were initiated more slowly than usual) would be particularly difficult to observe when the overall rate of production is markedly reduced.

It must be carefully emphasized that this approach to estimation of mRNA function in thalassemia does not provide direct quantitative information about the amount of β-mRNA in the cells. Only the function of β-mRNA that is normal enough to make contact with polysomes is estimated here. For example, an abnormally unstable mRNA that failed to be initiated at all would not be measured. The fact that β-chain synthesis is reduced in β-thalassemia while the β-mRNA in contact with ribosomes functions normally provides strong circumstantial evidence that the total amount of β-mRNA is reduced in β-thalassemia marrow cells, but recent direct measurements of β-mRNA content by hybridization assays (6, 7) have provided much firmer evidence for this important conclusion.

In nonthalassemic individuals the β/α synthetic ratio of nascent chains attached to ribosomes was similar to that of completed chains in the supernate (Table I). Since very few completed chains, labeled near the C-terminus with [^{3}H]tyrosine, were found attached to ribosomes of rabbit reticulocytes (8) and nonthalassemic human erythroid cells (unpublished observation), a similar β/α synthetic ratio in nascent and completed chains suggests that α- and β-nascent chains remain attached to ribosomes for a similar length of time, viz., the time necessary for the translation of the two chains is approximately equal.

This is not the case in thalassemia, where chain imbalance in nascent chains was always more marked than in the supernate (Table I). Various explanations for this unexpected finding are possible:

Very rapid preferential breakdown of uncoupled chains. This possibility has been suggested to explain the difference in β/α synthetic ratio of reticulocytes and bone marrow cells in thalassemia trait (20). However, if this were the case, one would expect to find a greater difference between polysomes and supernatant in β-thalassemia than in hemoglobin H disease, since uncoupled α-chains are probably more unstable than uncoupled β-chains (21). Moreover, if a large proportion of uncoupled chains were precipitated or degraded very quickly, chain imbalance would be expected to decrease upon prolonged incubation. This did not occur in our studies (see Results) or in studies by others (22). The different β/α synthetic ratio on ribosomes and in the supernate could still be explained if a fraction of the newly released uncoupled chains were destroyed immediately upon their release. However, it would be extremely unlikely that a large proportion of the newly formed nonthalassemic chains were precipitated or degraded so rapidly, since the remaining excess uncoupled chains were not differentially destroyed upon further incubation (see Results).

Precipitation of uncoupled chains with ribosomes. This possibility could explain the results in pelleted ribosomes, but not those in individual or pooled polysome and monosome fractions separated on a sucrose gradient (Fig. 6 and Table I). Adhesion of previously released completed chains to ribosomes does not seem to occur in nonthalassemic individuals. If it did occur, it would be unlikely that the normal pattern of distribution of nascent chains on polysomes of different sizes would be maintained. Finally, direct measurement of the effect of completed chains on polysome radioactivity in hemoglobin H disease revealed no adhesion of completed chains to polysome fractions.

Different translation time for the two chains. If there were a delay in translation or release of the affected chain in thalassemia, it would remain attached to ribosomes for a longer period, and there would be *less* imbalance on the ribosomes than in the supernate. However, since the opposite is true, we postulate that *more* time is required to translate the chain produced in excess, and that this "normal" chain remains attached to ribosomes for a longer period. Our results at present cannot define the stage at which the postulated delay in translation would occur. They are compatible with one or more of the following possibilities: first, more rapid elongation of the thalassemic chain; second, delayed elongation of the normal chain; and third, delayed release from ribosomes of the completed normal chain. (A fourth possibility, enhanced release of the thalassemic chain, would not have a significant effect on the translation time, since release is a very rapid process under normal circumstances). Among these three possibilities we tend to prefer the third, since it is the only one that would not necessarily alter the pattern of distribution of nascent chains on polysomes, which was found to be normal in thalassemia. This normal pattern could, however, also result from the combined effect of increased rates of initiation and elongation. But preliminary experiments with nascent chains labeled near the C-terminus with [^{3}H]tyrosine suggest the presence of more completed chains attached to ribosomes in thalassemia than in nonthalassemic hemolytic disorders.

If indeed there is a longer translation time of the so-called normal chain in thalassemia, this could represent a compensatory mechanism striving to achieve balanced chain synthesis. Existence of this mechanism would explain why less imbalance in synthetic ratios was observed in intact cells of patients with hemoglobin H disease than in cell-free systems to which mRNA extracted from these cells was added (7). This difference was not consistently observed in β-thalassemic reticulocytes (23), but there the degree of compensation may indeed be low. This postulated mechanism could also be the basis for the specific inhibition of α- or β-chain synthesis, which is reportedly induced in rabbit cell-free systems by addition of homologous chains (24–26).

It has been disturbing to the above interpretation of our results that a similar β/α synthetic ratio in ribosomes and in supernate was found in a previous study on bone marrow cells of a patient with sickle-thalassemia (27), Further studies on bone marrow cells of patients heterozygous for β-thalassemia are necessary to determine whether the postulated compensatory mechanism is responsible for the different β/α synthetic ratio in bone marrow and in reticulocytes. The necessity for very marked erythroid hyperplasia to obtain sufficient label attached to ribosomes constitutes the main difficulty in performance of these studies.

Our studies thus confirm that translation of functioning β-mRNA proceeds normally in β-thalassemia marrow cells as well as in peripheral reticulocytes. Therefore, the reduction in β-chain production does not seem to be due to defective mRNA translation, but is more likely due to decreased availability of normal functioning β-mRNA. No abnormalities of mRNA translation have been detected in hemoglobin H disease reticulocytes. Finally, we have presented data that suggest a compensatory delay in the translation of the complementary, nonthalassemic chain. The exact mechanism of this delay and its effectiveness in various thalassemia syndromes remains to be evaluated.

ACKNOWLEDGMENTS

The authors are grateful for the excellent technical assistance of Miss Grace Lam.

These studies were supported by United States Public Health Service Grants AM-15322, AM-05581, and 1-K4-6M50, 175-01. Some of these studies were carried out on a Clinical Research Center USPHS grant, FR-00128.

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