Translation of β -Globin m-RNA in β -Thalassemia and the S and C Hemoglobinopathies

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ABSTRACT Genetic and biochemical evidence indicates that in β -thalassemia there is impaired synthesis of the β -globin chains of hemoglobin A. In patients heterozygous for the hemoglobinopathies, hemoglobin S and hemoglobin C, the mutant β -chain is produced in smaller amounts than normal β^A . Defective m-RNA translation has been suggested as a possible cause of decreased β -globin polypeptide synthesis in thalassemia and the hemoglobinopathies. In the present study, the ribosomal assembly of β -globin chains was examined in the peripheral, nucleated red blood cells and reticulocytes of patients with Cooley's anemia, thalassemia intermedia, sickle thalassemia, sickle cell anemia, hemoglobin C disease, and in hemolytic anemias not associated with a hemoglobinopathy. The translation times of β^A , β^S , and β^C did not differ significantly (average times: β^A = 75 sec, range 43–114, β^S = 69 sec, β^C = 92 sec). In thalassemia, no evidence was found for a delay in translation as the cause of the marked impairment of β -globin synthesis. In several specimens of peripheral blood from thalassemic patients, the translation time of the β -chain was even shorter than in nonthalassemic specimens (average time = 45 sec, range 35–59). The results suggest that the defect in β -globin synthesis in β -thalassemia is due to impaired initiation of β -globin chain assembly or a quantitative deficiency in m-RNA.

INTRODUCTION

In β -thalassemia there is deficient synthesis of hemoglobin A due to a reduced rate of production of β -globin chains (1–3). Most structural mutants of normal human hemoglobin also appear to have reduced synthetic rates;

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the abnormal species generally occur in smaller amounts than hemoglobin A in the blood of heterozygotes (4).

The process of m-RNA translation, in which amino acids are sequentially joined on the ribosomes to form polypeptides, has been suggested as a possible point of delay in globin production in thalassemia and the hemoglobinopathies (5, 6). However, Clegg, Weatherall, Na-Nakorn, and Wasi found no difference in the rate of β -globin m-RNA translation in reticulocytes from nonthalassemic and thalassemic persons from Thailand (7).

The present paper reports studies of β -globin m-RNA translation in several patients of Italian descent with classical Cooley's anemia and in an American Negro with "thalassemia intermedia." Translation times for the β -polypeptide chain were measured in the nucleated red blood cells and reticulocytes of the peripheral blood of thalassemic patients and persons with hemolytic anemia unassociated with a hemoglobinopathy. In addition, β -chain translation rates were studied in the reticulocytes of patients with sickle cell anemia, hemoglobin C disease, and sickle thalassemia.

METHODS

Handling of blood samples. Venous blood was collected in heparinized tubes, packed in ice, and immediately processed.

Hemoglobin synthesis in vitro. Incubation of reticulocyte-rich blood with radioactive amino acid was performed as previously described (8). The plasma was removed by low speed centrifugation and the reticulocytes were concentrated by centrifuging the red blood cells in 1 ml conical Pyrex tubes at 15,000 rpm for 1 hr. The top third of the packed cells was removed and washed once with 0.13 M NaCl, 0.005 M KCl, 0.0074 M $MgCl_2$ (NKM), and once with the incubation mixture of Lingrel and Borsook (9) lacking valine.

The cells were resuspended in incubation medium and preincubated for 10 min at 37°C. 250 μ Ci of valine- 3H was then added to 1–2 ml of cell suspension. After 3, 6, and 9 min of incubation, portions of the cells were removed and

pipetted into 10 vol of ice-cold distilled water containing 0.01 N NaCN, 0.01 N NaF. In order to obtain hemoglobin uniformly labeled with ^{14}C , cells were incubated with 10 μCi of valine- ^{14}C for 5 hr or 22 hr.

Preparation of β -globin and measurement of m-RNA translation. The method was based on that of Dintzis (10) using the technique detailed by Clegg et al. (7) and the present author (8). Membranes were removed from the radioactive hemolysates by centrifugation at 15,000 rpm for 1 hr. Ribosomes were sedimented at 30,000 rpm for 3 hr. The clear hemolysates were dialyzed extensively against water to remove unincorporated radioactive amino acid and finally recentrifuged to remove any precipitate. In some experiments, at this point, the 3, 6, and 9 min ^3H -hemolysates were mixed with enough uniformly labeled ^{14}C -hemolysate to give an approximately 1:1, ^3H cpm: ^{14}C cpm, ratio for the 6 min fraction and identical ^3H -hemoglobin: ^{14}C -hemoglobin concentration ratios for all three time samples.

Heme was removed by the acid-acetone method (11) and the globin lyophilized. In one experiment, hemoglobin F was separated from hemoglobin A, before the acid-acetone step, by column chromatography on Amberlite CG-50 using developer II (13). The polypeptide chains of globin were separated by column chromatography on carboxymethyl-cellulose in sodium phosphate buffers containing 8 M urea according to Clegg, Naughton, and Weatherall (12). In samples from patients with thalassemia or sickle thalassemia, the γ - and β -chains, or β^A - and β^S -chains were separated by a modified procedure using an initial 0.008 M-0.02 M sodium phosphate gradient. At this point, in experiments in which the ^3H - and ^{14}C -hemoglobins had not previously been mixed, β -chain uniformly labeled with ^{14}C

was added to 3, 6, and 9 min ^3H β -chain fractions to give a 2:1 ratio of ^3H cpm to ^{14}C cpm in each sample. The samples were then amino-ethylated, digested with trypsin, and fingerprinted. The ^3H and ^{14}C radioactivity in the tryptic peptides was measured as previously described (8) except that dual label counting was performed using Aquasol (New England Nuclear Corp., Boston, Mass.) in place of Bray's solution.

Materials. L-Valine-(2,3- ^3H), 6 Ci/mmol and L-valine- ^{14}C , 260 mCi/mmol were purchased from Schwarz/Mann (Orangeburg, N. Y.)

RESULTS

Clinical. Table I lists the patients whose blood was used for the study of β -globin m-RNA translation. Five patients of Italian descent, ages 11-25 yr, with Cooley's anemia or β -thalassemia major were examined. All had severe anemia and, except for one patient, required blood transfusions at 1-2 month intervals. However no patient was transfused within a 6 wk period before study. All patients had undergone splenectomy. Reticulocyte counts ranged from 0.5 to 6.5% and nucleated red blood cell counts varied from 30,000 to 200,000 per mm^3 . A 33 yr old Negro patient with a milder disorder, "thalassemia intermedia," was also studied. He had hepatosplenomegaly, no growth defects, and was able to maintain an hematocrit value of 34% with no transfusions. The peripheral blood morphology of this pa-

TABLE I
Diagnoses, Hematologic Values, and Translation Data of Patients Studied

Patient	Diagnosis	Reticulo- cytes	Nucleated RBC	^3H N-term/ ^3H C-term			Calculated translation time
				3 min	6 min	9 min	
		%	per mm^3		%		sec
R. R.	Thal. major		137,000	61	77		
			180,000	58	73		
		5.3	220,000	64	84	83	55
A. M.	Thal. major		99,000	40	58		
		0.5	204,000	47	65	81	
L. G.	Thal. major	2.6	175,600	73	90	96	34
R. G.	Thal. major	6.5	25,700	69	77	88	
			34,500	69	90	93	
			28,000	70	81	93	
S. I.	Thal. major	4.0	92,000	77	81	97	40
J. D.	Thal. intermed.	4.0	300	56	74		
				67	85	94	38
P. H.	Sickle thal.	3.2	0	62; 60*	84; 81*	88; 88*	59; 59*
D. R.	S. S.	13.0	Few	42	69	82	69
S. P.	S. S.	5.2	Few	50	76	83	
T. M.	Hb C	5.0	0	35	54	67	
G. L.	Hb C	4.0	0	36	65	78	92
M. F.	Hemolysis	18.0	0	38	59	72	114
L. U.	Hemolysis	30.0	0	72	88	88	43
S. T.	Hemolysis	3.5	0	50	69	78	
D. L.	P-K. Def.	75.0	Few	38	70	81	77

* The first number is the value for β^A ; the second is for β^S .

FIGURE 1 Incorporation of valine- ^3H into globin polypeptide chains in vitro. The left panel shows the radioactivity in the α - and β -chains after 9 min of incubation of reticulocytes from a patient with pyruvate kinase deficiency. The polypeptide chains of hemoglobin were separated on a carboxymethylcellulose column in 8 M urea. Synthesis of α - and β -chains was equal. The difference in size of the optical density peaks is due to the greater extinction coefficient of the β -chains. The difference in size of the radioactivity peaks is due to the fewer valine residues in the α -chain. The pre- α -peak of radioactivity is frequently seen in short time incubation experiments. The significance is not known. The right panel shows the radioactivity in the α -, β -, and γ -chains after 9 min of incubation of cells from a patient with thalassemia intermedia. The combined synthesis of β - and γ -chains was half that of α -chains.

FIGURE 2 Gradients of relative radioactivity in β -globin polypeptide chains after 3, 6, and 9 min. The radioactivity in the valine residues along the chain is expressed as a percentage of the radioactivity of the C-terminal amino acid. ^3H : ^{14}C ratios were measured in the tryptic peptides and plotted against position in the chain. A straight line was determined by the least squares method and extrapolated to position 146. The values for the N-terminal and interior valines were then normalized to the calculated value for position 146. The left panel shows the results of an experiment using reticulocytes from a patient with pyruvate kinase deficiency. The right panel shows the results in an experiment with peripheral blood from a patient with Cooley's anemia. X—X, 3 min; ●—●, 6 min; ○—○, 9 min.

tient, except for the presence of few nucleated red blood cells, was identical with the severely affected patients. One 16 yr old patient with sickle cell thalassemia was investigated. He had a hematocrit value of 33%, no transfusion requirements, a reticulocyte count of 3.2%, and no nucleated red blood cells in the peripheral blood.

Except for the patient with sickle thalassemia, all thalassemic patients studied had 40–60% fetal hemoglobin in the peripheral blood. All showed a marked deficit in β -chain synthesis with excess α -chain production. Fig. 1 compares the incorporation of valine- ^3H into the polypeptide chains of globin in a patient with pyruvate kinase deficiency and a thalassemic individual. In the nonthalassemic patient there was equal synthesis of α - and β -chains, cognizance being taken of the fewer valine residues in the α -chains. In the patient with thalassemia, total α -chain production was 1.9 times total β -plus γ -chain synthesis.

Assembly of β^A -polypeptide chains in patients without thalassemia or hemoglobinopathy. The technique and interpretation of the study depend upon the fact that the growth of polypeptide chains proceeds by stepwise addition of amino acids from the NH_2 -terminus to the COOH -terminus (10, 14). When radioactive amino acid is added to cells actively synthesizing hemoglobin, the tracer is first incorporated into the growing ends of the

partially completed chains already on the polyribosomes. As the polypeptides are completed and released from the ribosomes, radioactivity appears first near the C-terminal end of the hemoglobin in solution. Later, with the completion of chains, a large part of whose synthesis has taken place in the presence of the radioactive amino acid, a gradient of decreasing radioactivity appears in the finished hemoglobin and spreads from the COOH -terminus to NH_2 -terminus. Upon continued incubation, all newly formed chains are then uniformly labeled. Although the specific activity of the NH_2 -terminus of the hemoglobin in solution never equals that of the COOH -terminus, the percentage difference in radioactivity decreases until uniform labeling is approached. The rate at which the radioactivity spreads from the C-terminal to the N-terminal end and the rate at which the slope of the gradient of radioactivity, relative to the C-terminal value, decreases are dependent on the rate of translation. Both rates are independent of chain initiation and the quantity of m-RNA present. A nonuniform rate of translation along the polypeptide chain will affect the shape of the gradient of radioactivity (15).

The left panel of Fig. 2 shows the results of an experiment using reticulocytes obtained from a patient with pyruvate kinase deficiency. β -Globin uniformly labeled with valine- ^{14}C was added to β -globin from each

FIGURE 3 Derivation of translation time of β -chains. The left panel shows the specific activities of the valine residues, expressed as $^3\text{H}:^{14}\text{C}$, along the length of the β -polypeptide chains after 3, 6, and 9 min in an experiment on pyruvate kinase-deficient reticulocytes. The right panel shows the specific activities of the NH_2 -terminus and the COOH -terminus plotted against time of incubation. These specific activities were derived from the data in the left panel. The translation time is the horizontal distance between the lines and the calculated value is shown in Table I.

time sample of the valine- ^3H incubation. The specific activity of each tryptic peptide containing valine was measured as the $^3\text{H}:^{14}\text{C}$ ratio. The ratios were plotted against the position of the valine residues in the β -chain, and the curve was extrapolated to the C-terminus. The $^3\text{H}:^{14}\text{C}$ ratios were then normalized to the calculated C-terminal value and the data replotted. In this experiment the N-terminal amino acid was 37% labeled at 3 min. By 9 min the ratio had increased to 81% (Fig. 2). The curves obtained were straight lines indicating that the rate of translation was uniform along the polypeptide chain (15). Table I lists the relative percentage of ^3H found in the N-terminal valine in experiments with three other patients with hemolytic anemia. The graphs of radioactivity were linear in all experiments. In two instances the percentage labeling of the NH_2 -terminus compared with the COOH -terminus at 3 min was similar to the first experiment. In one experiment a value of 72% labeling of the NH_2 -terminus at 3 min suggested a more rapid translation rate. However, in this experiment, the relative labeling of the NH_2 -terminus was the same at 6 and 9 min suggesting nonuniform synthesis of protein during the time of incubation or some experimental error.

In the experiment with pyruvate kinase-deficient reticulocytes, ^{14}C -globin was added to the ^3H -globin samples to give identical ^3H -globin: ^{14}C -globin concentration ratios for all three time points. This procedure allowed comparison of the specific activities of the valine residues not only within one time sample but among all three samples. The left panel of Fig. 3 shows the $^3\text{H}/^{14}\text{C}$ ratios of the valine residues of the 3, 6, and 9 min samples plotted against position in the β -chain. The lines are approximately parallel and evenly spaced, demonstrating the maintenance of a constant absolute difference in radioactivity between the COOH -terminus

and NH_2 -terminus and an almost steady rate of synthesis during the 3–9 min portion of the incubation. By placing time scales on the vertical axis, and ignoring the time required for the radioactive amino acid to enter the synthetic cycle, the translation time, the time required to synthesize one complete β -chain, can be estimated by measuring the vertical distance between the N-terminal amino acid and the C-terminal amino acid for each sample. In this experiment the 3, 6, and 9 min lines indicated translation times of 113, 108, and 99 sec respectively. The right panel of Fig. 3 shows the specific activities of the N- and C-terminal amino acids graphed against time of incubation. The translation time can also be estimated from the average horizontal distance between the two lines (16, 17). The average translation time was calculated from the following equation (17):

Translation time (sec)

$$= \frac{(\text{Mean sp. act. C-term.}) - (\text{mean sp. act. N-term.})}{(\text{slope C-term. (sec}^{-1}\text{)} + (\text{slope N-term. (sec}^{-1}\text{)})}$$

$$77 = \frac{3.57 - 2.6}{1.32 \times 10^{-2} + 1.20 \times 10^{-2}}$$

In two other experiments with nonthalassemic cells without a hemoglobinopathy, the calculated average translation times were 43 and 114 sec (Table I).

Assembly of β^s and β^c . Experiments were performed in two patients with sickle cell anemia. After 3 min of incubation the relative per cent of tritium in the N-terminal amino acid was 42 and 50% in the two experiments (Table I). The left panel of Fig. 4 shows the radioactivity gradients in the β^s -chains in one experi-

FIGURE 4 Gradients of relative radioactivity in mutant β -globin polypeptide chains after 3, 6, and 9 min of incubation with valine- ^3H . The left panel shows the results in sickle cell anemia. The right panel is from an experiment on cells from a patient with hemoglobin C disease. $\times$ — $\times$, 3 min; $\bullet$ — $\bullet$, 6 min; $\circ$ — $\circ$, 9 min.

FIGURE 5 Derivation of translation time of β -chains in β -thalassemia. The left panel shows the specific activities of the valine residues along the β -chain. The right panel shows the specific activities of the C-terminal and N-terminal amino acids at 3, 6, and 9 min.

ment. There is no evidence for nonuniform translation along the length of the chain. The three specific activity lines in this experiment indicated the translation time to be 79, 95, and 94 sec. The translation time of β^s calculated from the equation was 69 sec.

In two patients with hemoglobin C disease the N-terminal amino acid was 35 and 36% labeled after 3 min. The right panel of Fig. 4 illustrates the radioactivity gradients from one patient. The translation time of β^c -globin was calculated in the second patient and was 92 sec (Table I). Thus β^A , β^s , and β^c appear to be translated at grossly similar rates.

Translation of β -globin m-RNA in patients with β -thalassemia. 13 experiments were performed on peripheral blood from 6 patients with homozygous β -thalassemia (Table I). In four patients, repeated studies were performed at intervals from 1 month to 1 yr and the subsequent experiments produced results similar to the original studies (Table I). In four patients with thalassemia major, the relative percentage of tritium in the N-terminal amino acid at 3 min ranged from 58 to 77% (mean, 68). The blood of one patient, A. M., was studied twice and showed 40 and 47% labeling of the NH₂-terminus at 3 min. The cells of one patient with thalassemia intermedia, J. D., gave results of 56 and 67% labeling on two occasions (Table I).

The right panel of Fig. 2 shows the 3, 6, and 9 min gradients, expressed as per cent of the ³H radioactivity in the C-terminus in one patient with thalassemia. The

rate of translation is uniform along the length of the β -chain. The left panel of Fig. 5 shows the specific activity along the β -chain at the three time intervals. The estimate of translation time, including the equilibration time, from the 3, 6, and 9 min samples was 65, 62, and 68 sec respectively. The right panel of Fig. 5 shows the lines of specific activity of the N- and C-terminal ends at 3, 6, and 9 min. The translation time calculated from the equation was 54 sec. Translation times were calculated in three other thalassemic patients and ranged from 34 to 40 sec (Table I).

A comparison of the results obtained with thalassemic and nonthalassemic patients indicates that although there was overlap between the groups, the translation time of the β -chain was reproducibly shorter in most specimens of thalassemic cells (Table I).

Translation of β^A and β^s in sickle thalassemia. A patient with sickle thalassemia afforded the opportunity of studying simultaneously the translation of a thalassemic β -chain and a nonthalassemic β -chain in the same cells. The patient's peripheral blood contained approximately 18% hemoglobin A and 80% hemoglobin S. Upon incubation of reticulocytes, incorporation of valine-³H into the β^A -, β^s -, and α -chains was in a ratio of 1:2.2:4.6 (Fig. 6). This result indicates the deficit of β^A -synthesis compared with β^s -production and the over-all excess of α -chain synthesis.

Fig. 7 shows the results of the translation study. The left panel shows the ³H/¹⁴C ratios in the valine residues

FIGURE 6 Incorporation of valine- ^3H into β^A -, β^S -, and α -polypeptide chains by reticulocytes from a patient with sickle thalassemia. The cells were incubated for 9 min. The globin chains were separated by column chromatography on carboxymethylcellulose in 8 M urea.

of the β^A -chain at 3, 6, and 9 min. The right panel shows the values for the β^S -chain. At each corresponding time point the percentage labeling of the N-terminal amino acid, was almost identical for both chains (Table I). The 3, 6, and 9 min lines of specific activity indicated translation times of 68, 58, and 63 sec for β^A and 71, 69, and 60 sec for β^S . The translation time calculated from the equation was 59 sec for both β^A and β^S . Thus the time required to assemble a thalassemic β -chain and a nonthalassemic β -chain is identical in the same cells.

DISCUSSION

The finding of linear gradients of radioactivity along the peptide chains indicates that the translation of normal human β^A -globin m-RNA proceeds at a uniform rate from the N-terminal amino acid to at least position 135. Past that point a deviation from a uniform rate of translation would not have been detected by the method used in the present study. No evidence has been found for a control point, near the site of the attachment of heme, separating regions with different rates of peptide chain growth, as postulated by Winslow and Ingram (18). The present results are therefore in accord with prior studies on normal human hemoglobin by Clegg and associates (7) and the present author (8). Similar results were obtained in studies of rabbit hemoglobin synthesis by Hunt, Hunter, and Munro (19) and Luppis, Bargellesi, and Conconi (20).

A uniform rate of addition of amino acids to the polypeptide chain was also found in the present experiments on the mutant chains β^S and β^O . The presence of two valine residues in the first tryptic peptide of the β^S -chain interferes with the interpretation of the effect of the site of the mutation on the uniformity of translation. However, within the error of the method, the results of the studies on β^O indicate that chain assembly proceeds with no marked change in rate through and past the position of the mutation. The lack of an effect of a gross structural mutation on the uniformity of translation was previously described in studies of the synthesis of hemo-

FIGURE 7 Specific activity gradients in β^A - and β^S -polypeptide chains after incubation of cells from a patient with sickle thalassemia.

globin Gun Hill (8) in which there is a deletion of a sequence of five amino acids.

The average rate of translation of the β -chain, as indicated by the relative percentage of tritium found in the N-terminal amino acid, and by actual measurement of translation time, did not differ significantly in reticulocytes from patients with sickle cell anemia, hemoglobin C disease, or hemolysis without hemoglobinopathy. Thus the lesser amount of hemoglobin S and hemoglobin C compared with hemoglobin A in heterozygotes cannot be ascribed to slow translation of the mutant β -chains.

The experiments performed on cells from patients with β -thalassemia indicate that the translation of β -globin m-RNA is also uniform in this disease. No evidence for a slower average rate of β -chain translation was found to explain the severalfold deficit in β -chain synthesis in β -thalassemia. Measurement of the rate of translation of β^A and β^S in sickle thalassemia provided a nonthalassemic chain as an internal control and confirmed the findings in homozygous β -thalassemia. These results agree with the findings of Clegg and associates in their study of Thai patients with β -thalassemia (7). Thus a defect in translation appears to have been ruled out in β -thalassemia in three distinct ethnic groups.

Although in the present study there was a considerable spread in the results among individual patients within the two groups of thalassemic and nonthalassemic patients, repeated experiments on the same patients indicated that the method was capable of good reproducibility. An increase in the number of time samples taken would have increased the reliability of the calculated translation time determinations. However the large amount of blood required for such experiments would have made them less feasible especially for the thalassemic patients. In addition, translation time measurements might have been more reliable if samples were taken after longer periods of exposure of the cells to the radioactive isotope. However such longer incubations would have rendered less reliable the determination of uniformity of translation which is best seen in the early time samples with steep radioactivity gradients. Taking account of these conditions, the available data suggest that in most of the patients with β -thalassemia, the rate of β -chain translation (mean = 45 sec) was somewhat faster than in the cells of most of the control subjects (mean = 79 sec). The difference is statistically significant at the 5% level. The more rapid translation of β -globin m-RNA in β -thalassemia is a surprising finding and is contrary to the results obtained by Clegg et al. (7), who found no difference in translation rates between their thalassemic patients and controls. Those investigators estimated the translation time of β -chains in cells from patients with

thalassemia to be approximately 90 sec (7). This interval is similar to the average value of 79 sec for nonthalassemic cells in the present experiments but longer than the 45 sec for cells from patients with thalassemia. This latter fast translation time is closer to the 20 sec time for rabbit β -chains reported by Knopf and Lamfrom (16) and Hunt, Hunter, and Munro (17). The more rapid rate of translation does not appear to be a genetic characteristic of thalassemic β^A -chains since some acceleration of translation of β^S was also suggested in the experiment on cells from the patient with sickle thalassemia (Table I).

Two artifactual causes of apparent accelerated translation seem to be unlikely. Nonuniform radioactivity in the ^{14}C - β -globin synthesized in thalassemic cells could result in an apparent increased uniformity of labeling of the ^3H -chains. However, in some experiments on thalassemic cells the ^{14}C -globin had been made in nonthalassemic cells and was known to give the usual gradients of radioactivity when mixed with pulse-labeled nonthalassemic ^3H -globin. Failure to stop completely protein synthesis at the chosen time points could result in continued release of uniformly labeled β -chains resulting in the calculation of spuriously accelerated translation rates. However it seems unlikely that even nucleated red blood cells would continue to synthesize hemoglobin for many minutes after immersion in a large volume of ice water containing fluoride and cyanide. Knopf and Lamfrom observed no protein synthesis in rabbit reticulocytes incubated for 4 hr at 0°C (16). In addition, the results obtained with sickle thalassemic blood containing no nucleated red blood cells suggest that a resistance of nucleated cells to the method used to stop synthesis cannot completely explain the increased radioactivity of the N-terminal valine in thalassemic cells at 3 min.

The presence of nucleated cells might provide an explanation of the findings if translation tended to slow as cells aged. Again, the absence of nucleated red cells from the peripheral blood of the patient with sickle thalassemia argues against this explanation. A comparison of the translation time for β -chains in bone marrow cells and peripheral blood reticulocytes from the same individual would be revealing. A decrease in the supply of cofactors required for the initiation of chain synthesis has been shown to occur as cells age (21).

Another possible explanation of more rapid translation of β -globin chains in thalassemic cells is the presence of excess α -chains. It has been suggested that α -chains assist in the removal of completed β -chains from polyribosomes (22, 23). If the rate of removal of completed β -chains from the ribosomes limits the rate of translation of the m-RNA, then the large excess of α -chains found in thalassemic cells could accelerate translation. This acceleration would also be expected to

affect nonthalassemic β -chains in thalassemic cells and would be compatible with the findings on translation of β^s in sickle thalassemia. Experiments in a cell-free system with added α -chains might provide information in this regard.

Finally, if defective translation is not the cause of the deficient β -globin synthesis in β -thalassemia, then it would seem that either a reduced amount of m-RNA or a defect in the initiation of chain synthesis is a likely explanation.

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