Proc. Natl. Acad. Sci. USA
Vol. 77, No. 7, pp. 4287–4291, July 1980
Medical Sciences

Processing of human β-globin mRNA precursor to mRNA is defective in three patients with β^+-thalassemia

(nucleated erythroid cells/globin RNA pulse-chase kinetics/RNA splicing)

LYNNE E. MAQUAT*, ALAN J. KINNIBURGH*, LARRY R. BEACH*†, GEORGE R. HONIG‡, JACK LAZERSON§¶, WILLIAM B. ERSHLER‖, AND JEFFREY ROSS*

*McArdle Laboratory for Cancer Research, University of Wisconsin, 450 North Randall Avenue, Madison, Wisconsin 53706; ‡Children's Memorial Hospital, 2300 Children's Plaza, Chicago, Illinois 60614; §Milwaukee Children's Hospital, 1700 West Wisconsin Avenue, Milwaukee, Wisconsin 53233; and; ‖Department of Medicine, Center for the Health Sciences, Madison, Wisconsin 53706

Communicated by Howard M. Temin, March 10, 1980

ABSTRACT **Nucleated bone marrow cells from normal individuals and from three patients with homozygous β^+-thalassemia were pulse-labeled with tritiated nucleosides. The processing of the newly synthesized globin mRNA precursors was monitored by inhibiting additional transcription with actinomycin D for 30 min. Human β-globin mRNA is derived from its precursor via a series of reactions that generate processing intermediates. In nonthalassemic cells the precursor is processed efficiently to mature mRNA during the chase. In contrast, in β^+-thalassemic cells the processing of β-globin RNA is defective. In one patient the β-globin mRNA precursor turns over during the chase, but some of the intermediate RNAs accumulate and are not processed to mRNA. In two other patients a large fraction of the precursor and intermediate RNAs is not processed to mRNA. The α-globin mRNA precursor and intermediates are processed efficiently to mRNA-sized molecules in thalassemic and normal cells. The reduction in the rate of β-globin but not α-globin RNA processing accounts for the α/β globin mRNA imbalance in thalassemic erythroid cells. We discuss the possibility that the genetic lesions in β^+-thalassemia are at splicing signal sites within intervening sequences of the β-globin gene.**

The β-thalassemias are a heterogeneous group of hereditary anemias in man in which β-globin protein synthesis is decreased or absent and α-globin protein synthesis occurs at normal rates (1–6). Erythrocytes from patients with homozygous β^0-thalassemia contain no detectable β-globin protein, and their reticulocytes either lack β-globin mRNA or contain β-globin mRNA that is not translated into functional protein (7–9). The β-globin genes are intact in most, but not all, β^0-thalassemic patients (4, 5, 10). Erythroid cells from patients with β^+-thalassemia contain structurally normal β-globin protein, but there is significantly less β-globin than α-globin protein and the reticulocyte α/β-globin mRNA ratio is increased (11–14). β-Globin mRNAs in β^+-thalassemic and normal reticulocytes are translated with equal efficiencies (15–17), and the β-globin genes in β^+-thalassemia do not contain detectable deletions (4, 5).

Based on these data, the β^+-thalassemia phenotype must result from mutations that specifically reduce the expression of the β-globin structural genes. Such mutations might affect gene transcription, processing of the mRNA precursor, transport of mRNA from the nucleus to the cytoplasm, or stability of mature mRNA in the cytoplasm. The ratios of α-globin to β-globin mRNA sequences in nuclear RNA from normal individuals and from two patients with β^+-thalassemia were approximately equal (13). This result suggests that the transcription rates of the two genes are similar in some patients.

We have investigated the possibility that the β-globin mRNA precursor is processed to mRNA inefficiently in the β^+-thalassemias. Nucleated bone marrow cells were pulse-labeled with ^{3}H-labeled nucleosides and chased with actinomycin D, and globin-specific RNA was analyzed. The results indicate that the processing of β-globin RNA, but not α-globin RNA, occurs inefficiently in three unrelated patients with β^+-thalassemia.

EXPERIMENTAL PROCEDURES

Globin Chain Synthesis, Cell Culture, and RNA Purification. Peripheral blood cells were incubated with [^{14}C]leucine. Globin proteins were fractionated by CM-cellulose chromatography (18).

Nucleated cells were obtained from bone marrow aspirates, and all manipulations prior to cell culturing were performed at 24°C. Each aspirate was passed through a series of progressively larger gauge needles and centrifuged for 10 min at 300 × *g*. The buffy coat was removed and brought to 5 ml with growth medium (RPMI-1640 containing 20% fetal calf serum and 50 units of penicillin and 50 μg of streptomycin per ml). Cells were homogenized by gentle pipetting, transferred to five 1-ml Wintrobe tubes, and centrifuged for 10 min at 300 × *g*. The nucleated cells were pooled, brought to 10 ml with growth medium, and centrifuged for 12 min at 200 × *g*. The cells were resuspended in medium to 2–10 × 10^7 nucleated cells per ml and incubated in a humidified atmosphere of 5% CO_2/95% air at 37°C for 1 hr, at which time [^{3}H]uridine, [^{3}H]cytidine, and [^{3}H]guanosine (ICN) were added to a final concentration of 500 μCi/ml each (1 Ci = 3.7 × 10^{10} becquerels) (19). After 12 min, actinomycin D (Calbiochem) was added to 10 μg/ml, and a portion of the culture (the "pulse" fraction) was harvested. After further incubation (10 or 30 min), an equal volume of culture fluid was harvested (the "chase" fraction). Immediately after the harvest, the cells were chilled, pelleted, and lysed, and total cell RNA was extracted (19).

RNA Fractionation and Hybridization to Filter-Immobilized DNA. Prior to electrophoresis, a portion of each RNA sample was hybridized to [^{3}H] human globin cDNA, and the quantity of steady-state globin mRNA was calculated by C_0t analysis (C_0t is concentration of nucleotide in mol/liter times time in sec). RNA electrophoresis in 4.5% polyacrylamide/98%

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: nt, nucleotides.

† Present address: Department of Biochemistry, University of Washington, Seattle, WA 98195.

¶ Present address: Department of Pediatrics, University of California Davis Medical Center, Sacramento, CA 95817.

Table 1. Hematological data

Patient	Origin	Age, yr	Sex	Hb, g/dl	MCV, μm^3	Reticulocytes, %	HbF, %	HbA, %	HbA_2, %	E:M in BM	Globin chain synthesis in PB α/β	Globin chain synthesis in PB α/non-α
D. S.	Greek	17	M	8.4	73	6.2	32.0	65.0	3.0	6:1	15.6	3.3
D. K.	Italian-American	9	F	9.3	85	1.4	2.0	94.0	4.0	8:1	25.1	5.8
T. T.	Italian	18	F	8.7	82	23.4	55.1	42.3	2.6	2:1	5.6	6.8

D. S. and D. K. receive transfusions monthly and were analyzed just prior to receiving blood. T. T. does not receive transfusions regularly and received no blood during the period of study. Hb, hemoglobin; MCV, mean cell volume; HbF, fetal human hemoglobin ($\alpha_2\gamma_2$); HbA, major adult human hemoglobin ($\alpha_2\beta_2$); HbA_2, minor adult human hemoglobin ($\alpha_2\delta_2$); E:M, erythroid cell-to-myeloid cell ratio; BM, bone marrow; PB, peripheral blood.

formamide tube gels, gel fractionation, and RNA elution were performed as described (20). The recovery of RNA from gel slices did not vary with molecular weight (unpublished observations; see also ref. 20).

pMB9 DNA or recombinant pMB9 DNAs containing human α- (JW101), β- (JW102), or γ-globin cDNA (JW151) (21) were amplified and purified (22) under P2/EK1 containment. Purified plasmid DNA was immobilized on nitrocellulose filters (12.5 μg of DNA per 5-mm-diameter filter) (23). Each filter contained at least 5-fold excess of cDNA sequences relative to the quantity of globin mRNA used in each hybridization reaction. Analytical RNA hybridization to filter-bound DNA was performed as described (24), except that the hybridizing reaction was performed at 65–69°C, the RNase step was omitted, and the hybridized RNA was eluted at 90–95°C. Each reaction tube contained three filters (5 mm diameter, circles): one with pMB9 DNA to serve as a background for nonspecific binding of RNA, one with JW101 (α) DNA, and one with either JW102 (β) or JW151 (γ) DNA. The data in Figs. 1–4 represent the radioactivity (dpm) bound to the cDNA filter minus that bound to the pMB9 filter. For all hybridization reactions the pMB9 background was 0–20 dpm. The dpm were determined by the external standard ratio method, and the counting efficiency was 45–50% for all samples. The extent of hybridization of human RNA to filters was not determined, but approximately 80% of mouse globin mRNAs formed hybrids with filter-bound mouse globin cDNA under similar conditions (table 3 in ref. 24).

RESULTS

Hematological Characteristics of Patients. Hematologic studies on three homozygous patients with β^+-thalassemia are summarized in Table 1. D. S., a man of Greek origin, and D. K., a girl of Italian-American descent, require monthly transfusions to maintain a hemoglobin level of 9 g/dl (normal, 12–14 g/dl). T. T., a woman of Italian descent who was studied previously (25), has mild homozygous β^+-thalassemia and does not receive transfusion regularly. The α-globin/β-globin protein synthesis imbalance is a characteristic feature of β^+-thalassemia.

Processing of Globin mRNA Precursors in Cells of Nonthalassemic Individuals. In murine erythroid cells, the α- and β-globin genes are transcribed into large precursor RNAs that are processed stoichiometrically to globin mRNA within 30 min (19, 20, 26–28). Initial experiments with human cells were performed to determine if human globin mRNA precursors exist and, if so, to measure the efficiency with which they are processed. Nucleated bone marrow cells from a patient with drug-induced hemolytic anemia and from normal individuals were pulse labeled for 12 min and chased for 30 min. RNA was electrophoresed in denaturing gels, and the RNA eluted from gel slices was hybridized to α- and β-globin cDNA filters. Peaks of newly synthesized β-globin RNA—approximately 1900–2000 nucleotides (nt), 1550 nt, 1350 nt, 1150 nt, 950 nt, 900 nt, and mRNA-sized molecules (630–780 nt)—were observed (Fig. 1C). Similar RNA profiles were observed in two normal indi-

Table 2. Summary of pulse-chase experiments

	Hemolytic anemia		Thalassemia					
			D. S.		D. K.		T. T.	
	Pulse	Chase	Pulse	Chase	Pulse	Chase	Pulse	Chase
Radioactivity in α-globin mRNA sequences:								
Distribution (%)								
Precursor	5	0	20	0	10	5	5	5
Intermediate	15	0	25	5	40	25	40	10
mRNA size	80	100	55	95	50	70	55	85
Total (dpm)*	3190	2450	2140	2880	1530	1170	2660	5110
Radioactivity in β-globin mRNA sequences:								
Distribution (%)								
Precursor	5	0	5	0	10	5	5	15
Intermediate	55	15	45	55	55	50	40	65
mRNA size	40	85	50	45	35	45	55	20
Total (dpm)*	1210	910	690	1260	450	315	405	455
Total radioactivity ratio:								
α-globin mRNA/β-globin mRNA	2.6	2.7	3.1	2.3	3.4	3.7	6.6	11.2

Cells were pulse-labeled for 12 min and chased with actinomycin D for 30 min. The percentage (to the nearest 5%) of α- and β-globin mRNA sequences in each RNA class was calculated from Figs. 1–4, as follows: (dpm in RNA size class $\times$ M_r of globin mRNA/M_r of RNA size class).

* The total radioactivity in mRNA sequences is the sum of the mRNA-specific radioactivity in each RNA size class. The values reflect the erythroid-to-myeloid cell ratio, the labeling efficiency of the various cell types in the marrow, and the amount of RNA electrophoresed (see legends to Figs. 1–4).

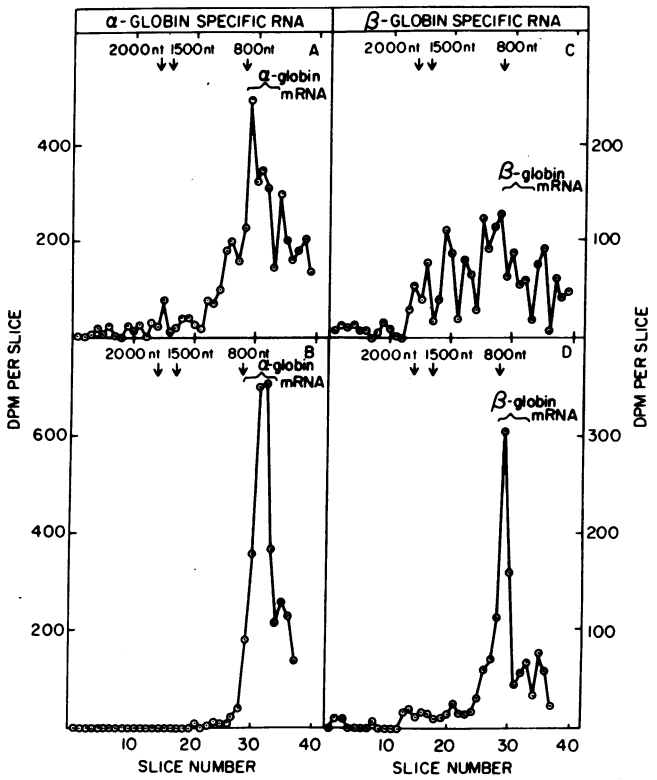

FIG. 1. Pulse-chase analysis of α- and β-globin RNAs from bone marrow cells of a nonthalassemic individual with drug-induced hemolytic anemia. Approximately 6×10^8 nucleated bone marrow cells were cultured at 2×10^7 cells per ml. The cells were pulse labeled and chased, and the RNA was extracted and purified. RNA (175 μg/gel) was coelectrophoresed with 18S mouse rRNA (2000 nt), 16S *Escherichia coli* rRNA (1550 nt), and 12S brome mosaic virus component 4 RNA (800 nt). RNA eluted from each gel slice was hybridized to DNAs (pMB9 and globin cDNA cloned into pMB9) immobilized on filters. The radioactivity bound to the pMB9 filter (0–20 dpm) was subtracted from that hybridized to the cloned cDNA filters. The cDNA-specific dpm are shown. RNA larger than 3000 nt and smaller than 450 nt failed to hybridize to the α or β filters (data not shown). (*Left*) Hybridization of α-globin cDNA to RNA from cells pulse-labeled for 12 min (*A*) and cells chased for 30 min (*B*). (*Right*) Hybridization of β-globin cDNA to RNA from cells pulse-labeled for 12 min (*C*) and cells chased for 30 min (*D*).

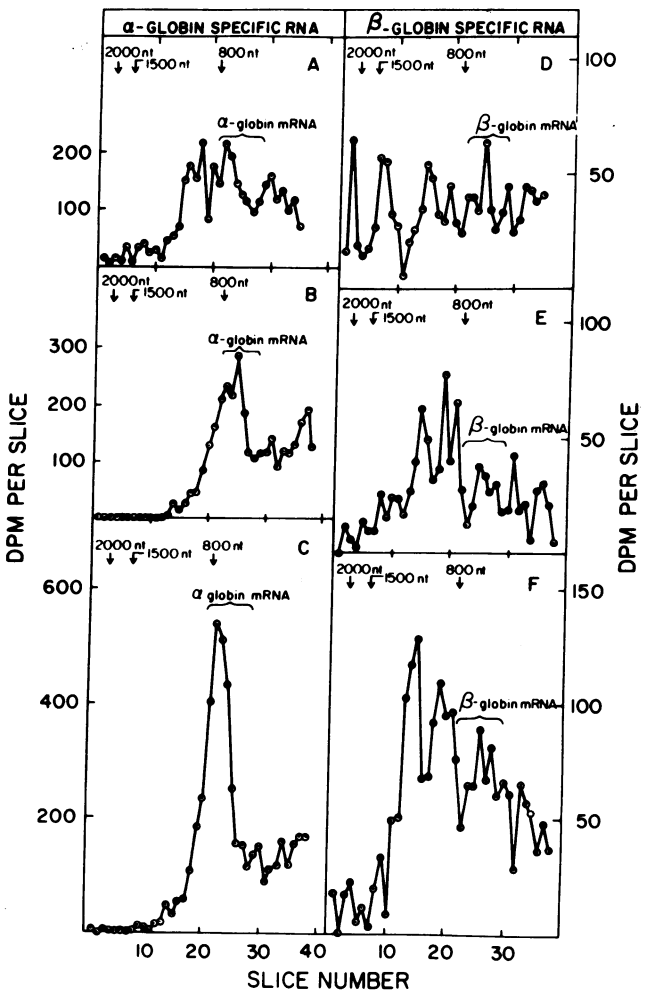

FIG. 2. Pulse-chase analysis of α- and β-globin RNAs from bone marrow cells of D. S. Nucleated bone marrow cells (5.3×10^8) were cultured at 1.8×10^7 cells per ml, pulse-labeled, and chased, and the RNA (120 μg/gel) was electrophoresed, fractionated, and hybridized to α- and β-globin-specific cDNA probes. The cDNA-specific dpm shown represent the radioactivity bound to the cDNA filter minus that bound to the pMB9 filter (0–20 dpm). (*Left*) Hybridization of α-globin cDNA to RNA from cells pulse-labeled for 12 min (*A*), cells chased for 10 min (*B*), and cells chased for 30 min (*C*). (*Right*) Hybridization of β-globin cDNA to RNA from cells pulse-labeled for 12 min (*D*), cells chased for 10 min (*E*), and cells chased for 30 min (*F*).

viduals who did not have hemolytic anemia (data not shown). In some gels, one or more of the intermediate-sized RNAs migrated as shoulders on adjacent RNA peaks. β-Globin RNAs larger than 1900–2000 nt were not detected. Therefore, this RNA is designated as the human β-globin mRNA precursor.

Approximately 60% of the pulse-labeled β-globin mRNA sequences was detected in molecules that were larger than mRNA (Fig. 1; Table 2). After the chase 85% of the β-globin radioactivity was present as mature mRNA (Fig. 1*D*). Little, if any, precursor or intermediate-sized RNAs remained after the chase. We conclude that the precursor and intermediate-sized molecules in these nonthalassemic individuals are substrates in stepwise processing reactions that generate β-globin mRNA with high efficiency. The experiments do not allow us to define precisely the processing pathway or its mechanism.

Peaks of α-globin-specific RNA from pulse-labeled cells were also observed (Fig. 1*A*; similar data from two other normal individuals are not shown). The largest RNA (approximately 1100 nt) is designated the precursor of α-globin mRNA. Intermediate-sized molecules, approximately 900–1000 nt, and mature α-globin mRNA (630–780 nt) were also detected (Fig. 1*A*). The precursor and intermediate-sized RNAs accounted for 20% of the radioactive α-globin RNA, most of which was processed to mRNA-sized molecules during the chase (Fig. 1*B*; Table 2). This result indicates that the RNAs of 900–1100 nt are substrates in processing reactions that generate α-globin mRNA.

Processing of β-Globin mRNA Precursor to mRNA Occurs Inefficiently in Cells from Patients with β^+-Thalassemia. The profile of newly synthesized β-globin RNA from D. S., a Greek patient with severe β^+-thalassemia, was similar to that of the nonthalassemic individuals (compare Figs. 1*C* and 2*D*). The precursor and intermediate-sized RNAs accounted for 50% of the radioactive β-globin RNA (Table 2). The precursor was not observed as a prominent peak after a 10-min chase, indicating that most of the precursor molecules had been processed (Fig. 2*E*). However, a large fraction of the intermediate-sized (900–1200 nt) RNAs was not processed to mRNA after chases of 10 min (Fig. 2*E*) or 30 min (Fig. 2*F*), at which time they accounted for 55% of the radioactive β-globin RNA (Table 2). These results indicate that β-globin mRNA processing intermediates in D. S. are not converted efficiently to mRNA.

The molecular weights of pulse-labeled α-globin RNAs in cells of D. S. were similar to those in normal individuals (compare Figs. 1*A* and 2*A*). The precursor and intermediate-sized RNAs accounted for 45% of the pulse-labeled α-globin mRNA. After 10-min and 30-min chases, 70% and 95%, respectively, of the newly synthesized α-globin RNA was processed to mRNA (Fig. 2 *B* and *C*; Table 2). These results indicate that the α-globin mRNA precursor and intermediates in D. S. are processed to mRNA efficiently and without significant wastage.

In D. K., a patient with severe β^+-thalassemia, pulse-labeled β-globin RNAs were comparable in size to those in nonthalassemic cells (Fig. 3*C*). The precursor and intermediate-sized RNAs accounted for 65% of the newly synthesized β-globin RNA (Table 2). A portion, but not all, of the precursor RNA was processed during the chase, in contrast to D. S. (Figs. 2*F* and 3*D*). The larger of the intermediate-sized RNAs (1300–1600 nt) also turned over, but the smaller intermediates (900–1200 nt) were not processed and accounted for 50% of the total radioactive β-globin RNA. The quantity of newly synthesized β-globin mRNA-sized molecules increased very little (10%) during the chase.

The sizes of pulse-labeled α-globin RNA molecules in D. K. were similar to those of normal individuals (Fig. 3 *A* and *B*). They did not turn over as rapidly in this patient as in the normal control or in D. S. but they were processed more efficiently than the β-globin RNAs (Table 2). Pulse-labeled γ-globin RNAs were also assayed, to investigate γ-globin RNA processing and to monitor the extent of cross-hybridization of γ-specific RNA to β-globin cDNA. Because the quantity of γ-globin RNA (Fig. 3*E*) was 5- to 10-fold lower than that of β-globin RNA (Fig. 3*C*), cross-hybridization of γ-globin RNA to β-globin cDNA could not account for the β-globin RNA peaks.

In the pulse-labeled cells of T. T., 45% of the radioactive β-globin RNA sequences was in precursor and intermediate-sized RNAs (Fig. 4*C*; Table 2), most of which were not processed and accounted for 80% of the β-globin RNA after the chase (Fig. 4*D*). Because the percentage of β-globin mRNA-sized molecules actually decreased to almost one-half during the chase (Table 2), it will be of interest to determine if this mRNA-sized material includes cleavage products generated from precursor or intermediate molecules during the pulse; if so, these products might be ligated during the chase to re-form the larger RNAs (in progress). The α-globin mRNA precursor and intermediate-sized RNAs were processed as efficiently in T. T. as in nonthalassemic individuals (Fig. 4 *A* and *B*). Pulse-labeled γ-globin RNAs were also processed efficiently (Fig. 4 *E* and *F*).

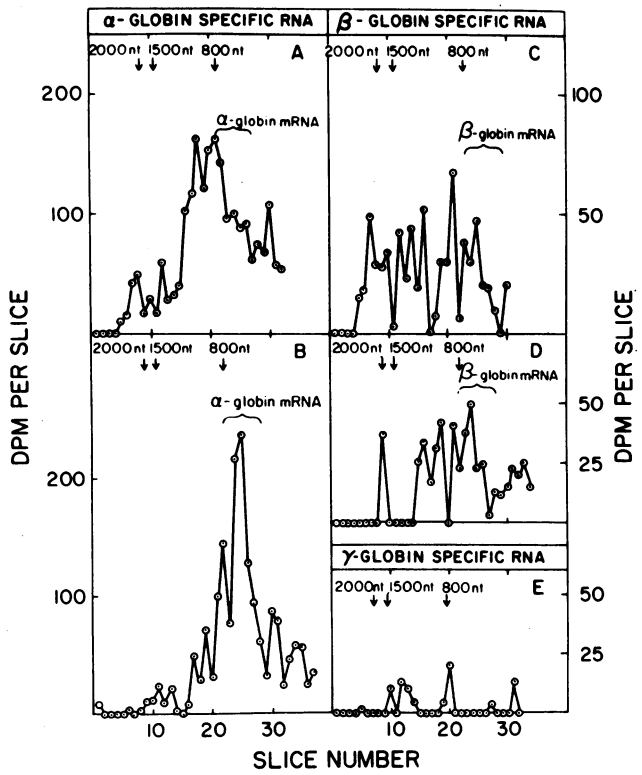

FIG. 3. Pulse-chase analysis of α-, β-, and γ-globin RNAs from bone marrow cells of D. K. Nucleated bone marrow cells (1.1×10^9) were cultured (3.6×10^7 cells per ml) and further processed as described in the legend to Fig. 1. The cDNA-specific dpm are shown and represent the radioactivity bound to the cDNA filter minus that bound to the pMB9 filter (0–20 dpm). RNA larger than 3000 nt or smaller than 450 nt did not hybridize (data not shown). (*Left*) Hybridization of α-globin cDNA to RNA from cells pulse-labeled for 12 min (*A*) and cells chased for 30 min (*B*). (*Right*) Hybridization of β-globin cDNA to RNA from cells pulse-labeled for 12 min (*C*) and cells chased for 30 min (*D*). Hybridization of γ-globin cDNA to RNA from cells pulse-labeled for 12 min (*E*).

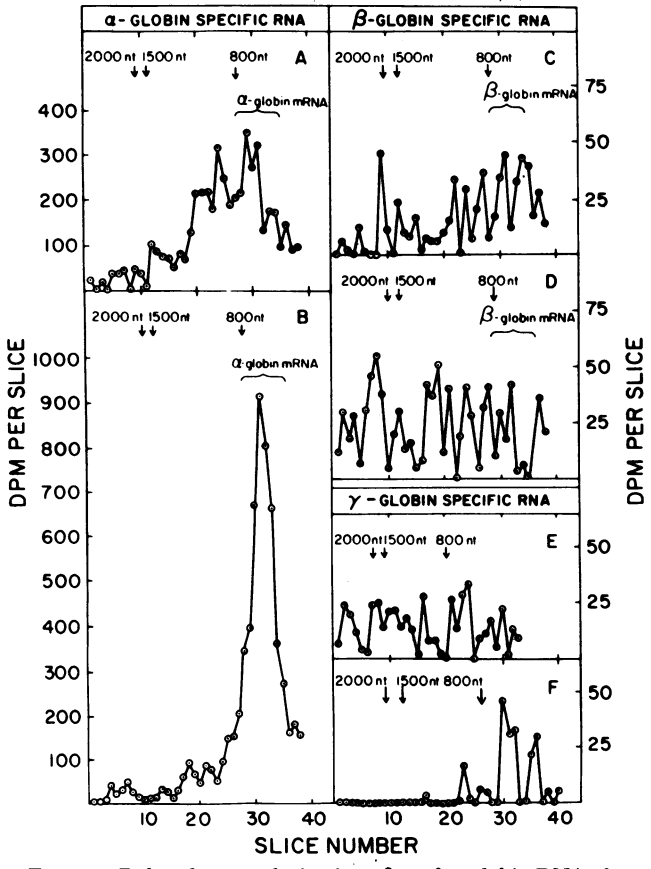

FIG. 4. Pulse-chase analysis of α-, β- and γ-globin RNAs from bone marrow cells of T. T. Nucleated cells (2.7×10^9) were cultured (9×10^7 cells per ml) and analyzed as described in the legend to Fig. 1. The cDNA-specific dpm are shown and represent the radioactivity bound to the cDNA filter minus that bound to the pMB9 filter (0–20 dpm). (*Left*) Hybridization of α-globin cDNA to RNA from cells pulse-labeled for 12 min (*A*) and cells chased for 30 min (*B*). (*Right*) Hybridization of β-globin cDNA to RNA from cells pulse-labeled for 12 min (*C*) and cells chased for 30 min (*D*). Hybridization of γ-globin cDNA to RNA from cells pulse-labeled for 12 min (*E*) and cells chased for 30 min (*F*).

DISCUSSION

These data indicate that imbalanced globin chain synthesis in these three β^+ thalassemic individuals can be accounted for by defective processing of the β-globin mRNA precursor to mRNA. In three nonthalassemic individuals, 75–95% of the radioactive α- and β-globin mRNA sequences was detected in mRNA-sized molecules after the 30-min chase (Fig. 1 and data not shown). In contrast, 55% (at most) of the β-globin RNA was processed to mRNA-sized molecules in thalassemic cells (Figs. 2–4). The processing defect is restricted to β-globin RNAs, because α-globin (and, in T. T., γ-globin) mRNA precursors were processed efficiently to mRNA.

The quantities of newly synthesized β-globin RNA in the pulse and chase fractions were similar in most experiments (Table 2). It is unclear why in some experiments there was almost 2-fold more globin mRNA-specific radioactivity in the chase than in the pulse (Table 2; Figs. 2 *D–F* and 4 *A* and *B*), but similar observations have been noted in experiments with mouse erythroid cells (19). It is also unclear why some β-globin precursor and intermediate molecules turn over during the 12-min pulse but others are not processed during the subsequent 30 min. Perhaps the mutations in the paternal and maternal β-globin alleles of these patients are different, so that the precursors transcribed from each allele are processed differently. It will be of interest to determine the ultimate fate of these unprocessed RNAs.

It is important to note that the precursor and the largest intermediate purified from cells of patient D. S. hybridize at least 10-fold more efficiently to β- than to δ-globin genomic DNA (unpublished observations). Therefore, it is unlikely that we are analyzing δ-globin RNA processing. We propose that the genetic defects in at least some β^+-thalassemias are mutations in one or both of the intervening sequences (introns) of the β-globin gene (29–31). Assuming that the human β-globin mRNA precursor undergoes multiple splicing reactions, as in the mouse (24), the processing defect might vary from patient to patient, accounting for the heterogeneity of the β^+-thalassemia syndromes and for the variety of processing patterns observed in these experiments (Figs. 2–4). In other words, a mutation at one splicing signal site may affect processing to a greater extent than a mutation at another site. Processing defects in some β^+-thalassemias could result also from mutations in enzymes that process β-globin RNA specifically (32, 33) or in structural proteins that bind exclusively to the β-globin mRNA precursor or cleavage intermediates (34). Moreover, we have not excluded the possibility of mutations that reduce both the rates of processing and of transcription.

We thank Milton Johnson for his contributions to the preliminary aspects of this work and Lorri Steffen and Deborah Hass for excellent technical assistance. We are grateful to Drs. Edward Prendergast and Michael Borzy for performing bone marrow aspirations, Dr. John Pellett for providing bone chips, Drs. Bernard Forget and Sherman Weissman for human globin cDNA clones, Dr. Tom Maniatis for human globin genomic clones, and Drs. Gary Altman and Paul Kaesberg for brome mosaic virus. This investigation was supported by Grants CA-07175 and CA-23076 from the National Institutes of Health L.E.M., A.J.K., L.R.B., and G.R.H. were supported by Grants CA-09230, CA-23076, CA-91935, and AM-19046, respectively, from the National Institutes of Health.

1. Weatherall, D. J. & Clegg, J. B. (1979) *Cell* **16**, 467–479.
2. Bank, A. & Ramirez, F. (1978) *CRC Crit. Rev. Biochem.* **5**, 343–368.
3. Forget, B. G. (1978) *Trend Biochem. Sci.* **3**, 86–89.
4. Flavell, R. A., Bernards, R., Kooter, J. M., de Boer, E., Little, P. F. R., Annison, G. & Williamson, R. (1979) *Nucleic Acids Res.* **6**, 2749–2760.
5. Orkin, S., Old, J. M., Weatherall, D. J. & Nathan, D. G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2400–2404.
6. Chang, J. C. & Kan, Y.-W. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2886–2889.
7. Ramirez, F., O'Donnell, J. V., Marks, P. A., Bank, A., Musumeci, S., Schiliró, G., Pizzarelli, G., Russo, G., Luppis, B. & Gambino, R. (1976) *Nature (London)* **263**, 471–475.
8. Old, J. M., Proudfoot, N. J., Wood, W. G., Longley, J. I., Clegg, J. B. & Weatherall, D. J. (1978) *Cell* **14**, 289–298.
9. Benz, E. J., Jr., Forget, B. G., Hillman, D. G., Cohen-Solal, M., Pritchard, J., Cavallesco, C., Prensky, W. & Housman, D. (1978) *Cell* **14**, 299–312.
10. Tolstoshev, P., Mitchell, J., Lanyon, G., Williamson, R., Ottolenghi, S., Comi, P., Giglioni, B., Masera, G., Modell, B., Weatherall, D. J. & Clegg, J. B. (1976) *Nature (London)* **259**, 95–98.
11. Housman, D., Forget, B. G., Skoultchi, A. & Benz, E. J., Jr. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1809–1813.
12. Kacian, D. L., Gambino, R., Dow, L. W., Grossbard, E., Natta, C., Ramirez, F., Spiegelman, S., Marks, P. A. & Bank, A. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1886–1890.
13. Nienhuis, A. W., Turner, P. & Benz, E. J., Jr. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3460–3464.
14. Kazazian, H. H., Ginder, G. D., Snyder, P. G., van Beneden, R. J. & Woodhead, A. P. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 567–571.
15. Nienhuis, A. W. & Anderson, W. F. (1971) *J. Clin. Invest.* **50**, 2458–2460.
16. Benz, E. J. & Forget, B. G. (1971) *J. Clin. Invest.* **50**, 2755–2760.
17. Rieder, R. F. (1972) *J. Clin. Invest.* **51**, 364–372.
18. Honig, G. R., Mason, G. R., Tremaine, L. M. & Vida, L. N. (1978) *Am. J. Hematol.* **5**, 335–340.
19. Ross, J. (1976) *J. Mol. Biol.* **106**, 403–420.
20. Ross, J. & Knecht, D. A. (1978) *J. Mol. Biol.* **119**, 1–20.
21. Wilson, J. T., Wilson, L. B., deRiel, J. K., Villa-Komaroff, L., Forget, B. G. & Weissman, S. M. (1978) *Nucleic Acids Res.* **5**, 536–581.
22. Humphreys, G. O., Willshaw, G. A. & Anderson, E. S. (1975) *Biochem. Biophys. Acta* **383**, 457–463.
23. Melli, M., Ginelli, E., Corneo, G. & Lernia, R. D. (1975) *J. Mol. Biol.* **93**, 23–38.
24. Kinniburgh, A. J. & Ross, J. (1979) *Cell* **17**, 915–921.
25. Heller, P., Yakulis, V. J., Rosenzweig, A. I., Abildgaard, C. F. & Rucknagel, D. L. (1966) *Ann. Intern. Med.* **64**, 52–61.
26. Curtis, P. J. & Weissmann, C. (1976) *J. Mol. Biol.* **106**, 1061–1075.
27. Kwan, S.-P., Wood, T. G. & Lingrel, J. B. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 178–182.
28. Bastos, R. N. & Aviv, H. (1977) *Cell* **11**, 641–650.
29. Lawn, R. M., Fritsch, E. F., Parker, R. C., Blake, G. & Maniatis, T. (1978) *Cell* **15**, 1157–1174.
30. Mears, J. G., Ramirez, F., Leibowitz, D. & Bank, A. (1978) *Cell* **15**, 15–23.
31. Flavell, R. A., Kooter, J. M., deBoer, E., Little, P. F. R. & Williamson, R. (1978) *Cell* **15**, 25–41.
32. Hutchison, H. T., Hartwell, L. H. & McLaughlin, C. S. (1969) *J. Bacteriol.* **99**, 807–814.
33. Hopper, A. K., Banks, F. & Evangelidis, V. (1978) *Cell* **14**, 211–219.
34. Toniolo, D. & Basilico, C. (1974) *Nature (London)* **248**, 411–413.