Intranuclear Defect in β-Globin mRNA Accumulation Due to a Premature Translation Termination Codon

By Kenichi Takeshita, Bernard G. Forget, Alphonse Scarpa, and Edward J. Benz, Jr

We have analyzed a cloned β-thalassemia (β-thal) gene from a patient doubly heterozygous for hemoglobin Lepore and β-thalassemia. Studies of 3H-uridine incorporation into β-globin mRNA in this patient's erythroblasts suggested an intranuclear defect in both β and Lepore (δβ) mRNA synthesis, as did S1 nuclease analysis of nuclear RNA. However, the nucleotide sequence of the β-thal gene revealed only a single base change in codon 39 (CAG → UAG), which created a premature translation termination codon. The 5' flanking sequence, including transcription promoter boxes and the mRNA initiation (CAP) site, were normal. The unexpected effect of this mutation on intranuclear β-mRNA synthesis in vivo was studied by insertion of the cloned gene into a plasmid expression vector and transfection into tissue culture (COS-1) cells. β-Globin mRNA produced by the transfected cells was assessed by S1 nuclease analysis. The β-39 thalassemia gene generated five- to tenfold less β-mRNA than a normal β-gene in both nuclear and cytoplasmic RNA, simulating the results observed in vivo. Moreover, the small amount of β-39 mRNA produced was as stable as normal β-mRNA during an actinomycin D chase, ruling out rapid cytoplasmic turnover as a cause of the reduced accumulation. Cotransfection of the β-39 thalassemia gene with a mutant tyrosine suppressor tRNA gene resulted in restoration of the β-39 mRNA accumulation to near-normal levels. On the basis of these results, we suggest that the low levels of β-mRNA known to exist in the common form of β-thalassemia, β-39 thalassemia, result from a lesion in transcription, or early posttranscriptional processes; the defect appears to be corrected by restoration of proper translational potential to the mutant mRNA, at least in a gene transfer-expression system in tissue-culture cells.

The β-thalassemias are inherited disorders characterized by reduced (β'-thalassemia) or absent (β-thalassemia) synthesis of the β-globin subunit of normal adult hemoglobin, hemoglobin A (HbA:α2β2). Molecular cloning and nucleotide sequencing studies have revealed a variety of mutations that impair β-globin gene function in patients with thalassemia. These mutations include partial gene deletions, impaired function of the transcription promoter region, errors in processing (splicing) of the β-mRNA precursors, due either to alteration of the normal splice sites or to activation of “cryptic” splicing sites, and creation of premature translation termination codons that abolish translation of β-mRNA into β-globin.

A translation termination mutation occurring at codon 39 (CAG → UAG) is known to be a common cause of β-thalassemia among Greek and Italian patients. The β-thalassemia phenotype in homozygotes is readily explained by the inability of the β-39 mRNA to be translated into β-globin; however, the repeated observation that there is also a marked quantitative reduction in the amount of β-globin mRNA in these patients' erythroid cells is not readily explained by this mutation. One frequently invoked hypothesis is that the inability of these mRNAs to be translated leads to accelerated nucleolytic degradation (instability) in the cytoplasm. Other possibilities include the presence of an undetected additional mutation in a transcription control region in the 5' flanking sequences or a second effect of the translation termination mutation on the intranuclear mRNA production, stability, or transport.

To investigate further the cause of reduced β-mRNA accumulation in this form of β-thalassemia, we have analyzed a cloned β-39 thalassemia gene in a surrogate genetics system, permitting comparative analysis of the function of cloned normal β and β-39 thalassemia genes. We have adopted a transient gene expression system that has been shown to be useful for analysis of normal and thalassemic globin gene function in foreign host cells. This system consists of a bacterial plasmid vector containing a viral origin of DNA replication and an enhancer element required for efficient β-globin gene expression into which the genes to be studied can be inserted, and a foreign host cell (COS-1) capable of incorporating and expressing the recombinant vectors. Our results, taken together with studies of β-globin mRNA synthesis by the patient's own erythroblasts, suggest strongly that an intranuclear defect in β-globin mRNA metabolism results from the β-39 mutation. The β-39 thalassemia mRNA was not unstable. Rather, there appears to be a previously unsuspected second effect of this mutation on transcription, nuclear stability, or
nuclear-to-cytoplasmic transport of the mRNA. Our studies also confirm in vivo the suggestion of Humphries et al. that defective production of the Lepore hemoglobin is probably due to, at least in part, or is associated with, impaired transcription of the Lepore (δβ) gene.

MATERIALS AND METHODS

Patients

The patient, R.D., has transfusion-independent thalassemia intermedia, due to double heterozygosity for hemoglobin Lepore and β-thalassemia (β-thal); we previously described this patient with respect to hemoglobin synthesis and β-globin mRNA quantitation in reticulocytes.36 This patient was selected for further study because β-globin mRNA levels are exceedingly low (2% of normal) in both erythroblasts and reticulocytes.36 Analysis of globin genes in spleen DNA obtained at the time of therapeutic splenectomy, using Southern gene blotting techniques,4 revealed that the patient is heterozygous for haplotype I (consistent with hemoglobin Lepore, subtype ββ-39 thalassemia), and haplotype II (consistent with βδ-39 thalassemia), as described by Orkin et al.36 (data not shown).

Studies of Intact Cell Globin Messenger RNA Synthesis

Circulating erythroblasts were obtained from the peripheral blood of this patient (600 erythroblasts/100 WBC) and from a nonthalassemic patient who was studied as a control. The nonthalassemic patient had a leukoerythroblastic peripheral blood picture due to myeloproliferative disease. Purified mononuclear cells were prepared by Ficoll-Hypaque density gradient centrifugation, and incubated with 3H-uridine to measure synthesis of globin mRNA, as described by us previously.31 “Pulse” incubations were conducted for 20 minutes at 37°C in 3H-uridine, and “pulse-chase” incubations were conducted by washing out 3H-uridine after 20-minute incubation and continuing the incubation in the presence of 20 mmol/L nonradioactive uridine for an additional 20 hours. Nuclear and cytoplasmic 3H-RNAs were extracted from these cells by the method of Laevis and Penman,32 with slight modifications described by us previously.31 The 3H-globin mRNAs present in the cells after these incubations were determined by molecular hybridization to cloned, filter-immobilized globin DNA probes, exactly as described by us in an earlier article.31 The ratios of 3H-globin mRNAs were calculated by measuring the number of 3H cpm bound to each globin DNA above a background filter containing only bacterial plasmid DNA sequences (see legend to Table 1).

Molecular Cloning and Nucleotide Sequence Analysis

DNA from a lymphoblastoid cell line prepared from R.D.’s leukocytes was subjected to partial (incomplete) digestion with the restriction enzyme, EcoRI, and DNA fragments 15–20 kilobases (kb) were isolated and ligated to the EcoRI arms of the cloning vector, bacteriophage Charon 4A, as described by Maniatis et al.32 A library of cloned fragments was generated by in vitro packaging and infection of bacterial host cells33 and was screened for β-globin DNA sequences by the 32P recombination screening technique described by Seed.34 Phage containing β-globin DNA inserts was isolated and the DNA fragments containing the β-39 clone were subcloned into bacterial plasmids by standard techniques. The clone used contained the entire β-globin gene in 12 kb of DNA containing the EcoRI fragments C and D, as described by Maniatis et al.,32 as well as the next downstream (3.2 kb) EcoRI fragment 3’ to the β-gene. Nucleotide sequence of the cloned gene was determined by the partial chemical degradation method of Maxam and Gilbert.35 (cf, Fig 2).

Construction of Plasmid Expression Vectors for Transient Expression Analysis of Cloned β-Globin Genes

The parent plasmid expression vector, pTL9, was the gift of Drs Keith Humphries, Timothy Ley, and Arthur Nienhuis.36 This vector has been designated pTLN3.36 This vector is derived from the bacterial plasmid, pBR322, by addition of simian virus 40 (SV 40) sequences, including the SV 40 origin of replication and the 72 basepair (bp) direct repeat DNA sequences that serve as an enhancer element essential for the function of the β-globin gene promoter in this system.36 Two vectors, one containing the β-39 gene and one containing a “corrected” normal β-globin gene derived from the β-39 gene, were constructed. To construct the β-39 expression vector, the 5.0 kb Bgl II fragment of the β-39 thal clone was ligated into the single BamHI site of pTL9. The ligated recombinants were

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**Fig 1.** Nuclear and cytoplasmic β and α mRNA in patient R.D.'s circulating erythroblasts. Nuclear and cytoplasmic RNA were extracted as in Tables 1 and 2 and Materials and Methods, and the steady-state mRNA levels analyzed by S1 nuclease analysis using DNA probe excess as described in the legends to Figs 4 and 5 and Materials and Methods. Note that the mRNA ratio is as low in nuclear RNA as in cytoplasmic RNA, as estimated by intensity of the autoradiography signals. The intensity of each band represents the amount of the radioactive DNA probe protected from S1 nuclease digestion by mRNA present in the RNA samples. β+ -thal, a control reticulocyte RNA sample showing both normal (209 bases) and abnormally spliced (228 bases) β-globin mRNA from a patient with ββ-39 thalassemia.
INTRANUCLEAR DEFECT IN β*-39 THALASSEMIA

Fig 2. Structure of the β*-39 thalassemia gene from patient R.D. The gene was cloned and its nucleotide sequence analyzed as discussed in Materials and Methods. The diagram indicates that the sequence was determined through base -110 in the 5' flanking region. The small open boxes indicate the position of the promoter regions, which were normal. The enlarged area shows the single abnormality found in codon 39 and compares the consequent coding properties of this region to normal.

Fig 3. Plasmid DNA expression vectors used for analysis of normal and thalassemic globin gene function in COS-1 cells. The properties of the vectors are discussed in Materials and Methods. Portions derived from plasmid pBR 322 are shown as wavy lines, and portions containing the SV 40 origins of replication and "enhancer" sequences (pTL9 derivatives) are shown as thin-lined open boxes. Globin gene regions derived from cloned genomic fragments are indicated as heavily lined boxes. The open boxes represent introns and the solid boxes exons. The strategy used to exchange a normal β-globin gene region containing codon 39 is indicated, as described in Materials and Methods.
de novo incorporation of $^3$H-uridine added to the cells after exposure to the drug. RNA was extracted from the cells by proteinase K digestion and phenol extraction, using procedures described by us previously.\textsuperscript{31,32}

$S_1$ Nuclease Analysis of $\beta$ and $\alpha$-Globin mRNA

$S_1$ nuclease analysis of $\beta$-globin mRNA was performed essentially as described by us previously.\textsuperscript{13} The cloned Bam 1.8-kb fragment of the $\beta$-globin gene, which extends from 5' flanking region nearly to the end of exon II, was purified and the 5' ends labeled with polynucleotide kinase and [y$^{32}$P] adenosine triphosphate (ATP). The 5$^{32}$P end label on the strand complementary to mRNA is located at approximately codon 100 (see Fig 4). The DNA probe was denatured by boiling in 80% formamide and hybridized to the RNA samples under conditions\textsuperscript{33},\textsuperscript{39} that permitted DNA–RNA hybridization but no reannealing of the complementary strands of the DNA probe. The hybrid mixtures were then digested with $S_1$ nuclease under standard conditions,\textsuperscript{39} and the $S_1$-resistant radioactive DNA detected by electrophoresis on denaturing polyacrylamide gels, followed by autoradiography.\textsuperscript{13} As shown in Fig 3, normally processed $\beta$-globin mRNA protects a radioactive 209 base fragment, which, in control experiments, was specifically associated with hybridization to authentic $\beta$-globin mRNA. All hybridization reactions were conducted under conditions of at least 20-fold excess of the end-labeled DNA probe, assuring that the intensity of the hybridization signal was proportionate to the amount of $\beta$-globin mRNA in the sample. Equal amounts of total cellular RNA (usually 50–100 $\mu$g) were analyzed for each comparison of normal and $\beta^\alpha$-thal gene expression.

Analysis of $\alpha$-globin mRNA was conducted by analogous procedures, using as probe the bacterial plasmid JW101, containing most of the cDNA sequence complementary to $\alpha$-globin mRNA.\textsuperscript{40} This plasmid was digested with HindIII and 5' end labeled. Normally processed $\alpha$-globin mRNA yields an $S_1$ nuclease-resistant fragment 274 bases long (Fig 5).

**Analysis of $\beta$-Globin mRNA Synthesis in Intact Erythroblasts**

Patient R.D. had been shown by us previously to be doubly heterozygous for hemoglobin Lepore and $\beta$-thalassemia, characterized by very low (2%–5% of normal) $\beta$-mRNA levels. Because previous evidence\textsuperscript{36,44} suggests strongly that the Lepore (fused $\delta\beta$) gene is transcriptionally defective, and because the $\delta\beta$-mRNA can be distinguished from $\beta$-mRNA, patient R.D. afforded an unusual opportunity to observe the function of a single intact $\beta$-thalassemia gene in vivo. By contrast, many “homozygous” patients with $\beta$-thalassemia are, in fact, doubly heterozygous for two different types of thalassemia alleles; attempts to study mRNA metabolism in vivo in such patients is complicated by the varying contributions of the two different alleles. In patient R.D., any structurally normal $\beta$-mRNA detected could be attributed to this single $\beta^\alpha$-thalassemia allele. In addition, patient R.D. possesses 600 circulating erythroblasts per 100 normal WBCs, affording the opportunity for repeated analysis of cells capable of $\beta$-mRNA synthesis without recourse to bone marrow aspiration.
As shown in Table 1, when patient R.D.'s circulating erythroblasts were incubated with $^3$H-uridine for 20 minutes, synthesis of $\beta$-mRNA was abnormally low in the nuclear RNA fraction that contained 90% of the radioactive mRNA. We have previously shown that the techniques used to separate nuclear and cytoplasmic RNA in this experiment and the experiments with COS cells separate unique nuclear and cytoplasmic mRNA fractions, the former being detectable by increased concentration of $\beta$-mRNA precursor sequences (data not shown) (cf, reference 31). Thus, $\beta$-globin mRNA synthesized during a 20-minute exposure to $^3$H-uridine was only 5%-10% of normal. This level of $\beta$-mRNA production was lower than that of the gamma mRNA produced by the patient's gamma genes.

Circulating erythroblasts from a nonthalassemic patient exhibited $\beta/\alpha$ $^3$H-mRNA synthetic ratios of approximately 1.7—a finding consistent with our previous studies of marrow erythroblasts from patients with normal $\beta$-globin gene transcription, in which normal $\beta/\alpha$ $^3$H-mRNA ratios ranged between 0.8 and 1.8. Because the $\beta$-globin DNA probe used to detect $\beta$-mRNA is enriched in 3' end $\beta$-mRNA sequences, which are also represented in the Lepore ($\delta\beta$) mRNA, the results shown actually represent the combined

<table>
<thead>
<tr>
<th>Patient</th>
<th>$^3$H cpm Bound to Filter</th>
<th>$\beta/\alpha$</th>
<th>$\gamma/\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb Lepore-(\beta^-39) thal</td>
<td>205.6, 80.5, 93.0, 49.4</td>
<td>0.20, 1.69</td>
<td>0.28, —</td>
</tr>
<tr>
<td>Nonthalassemic</td>
<td>76.3, 109.2, —</td>
<td>—</td>
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Circulating erythroblasts (400-600) erythroblasts/100 WBCs) were harvested as buffy coat preparations, washed, suspended in medium, and incubated with $^3$H-uridine as described in Materials and Methods and reference 31. Nuclear and cytoplasmic RNA were then isolated, and the amount of $^3$H-RNA binding to filters containing excess amounts (50 ug) of $\alpha$, $\beta$, or $\gamma$-globin complementary DNA prepared from plasmids JW101, JW102, and JW151 was determined. A filter containing only pBR 322 plasmid DNA was included as a control for background binding in each hybridization reaction. Only nuclear RNA results are shown, because >90% of the radioactive RNA is nuclear after a 20-minute pulse labeling incubation. The actual $^3$H cpm bound are shown; $\beta/\alpha$ and $\gamma/\alpha$ $^3$H-mRNA ratios were calculated by dividing the net $\beta$ or $\gamma$ $^3$H cpm (total cpm - cpm bound to pBR322 filter) by the net $\alpha$ cpm. Patients are described in Materials and Methods. The table shows results of each hybridization reaction.
output of both genes from patient R.D. Nonetheless, because the output of δβ-mRNA was also very small, the contribution of Lepore mRNA synthesis does not alter the interpretation of the results.

As shown in Table 2, the small amount of β- and δβ-mRNA sequences produced after a 20-minute pulse labeling were relatively stable during the 20-hour "chase" incubation, during which incorporation of 3H-uridine was blocked by the addition of a vast excess of nonradioactive 3H-uridine. In this experiment, a "chase" incubation, during which incorporation of 3H-mRNA sequences produced after a 20-minute pulse labeling were relatively stable during the 20-hour chase. These results suggest that the defect in β-globin mRNA accumulation due to this patient’s β-39 thalassemia gene occurs first in the nucleus, during or within the first 20 minutes after transcription. A further decline in transported 3H-β-mRNA to cytoplasm also occurs, and this small amount of transported β-mRNA seems to possess sufficient stability to persist at about the same level after the chase.

Table 2. Synthesis and Stability of α and β-globin mRNAs

<table>
<thead>
<tr>
<th>Patient</th>
<th>β/α 3H-RNA Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbLepore-β-thalassemia</td>
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<tr>
<td>Pulse</td>
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<tr>
<td>Chase</td>
<td>0.03</td>
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<td>Nonthalassemic</td>
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<tr>
<td>Chase</td>
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</tr>
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</table>
INTRANUCLEAR DEFECT IN \(\beta^{39}\)-THALASSEMIA

Fig 6. Stability of beta mRNAs encoded by normal and \(\beta^{39}\) thalassemic globin genes. See Results for discussion of the data shown above.

quences has been ruled out by the deletion analysis of Humphries et al (cf, reference 36 and accompanying paper).

As shown in Fig 4, the \(\beta^{39}\) vector promoted the synthesis of five- to tenfold less \(\beta\)-mRNA when transfected into COS-1 cells than the \(\beta\)-normal vector. \(\alpha\)-Globin mRNA, due to cotransfection with pSVOol (cf, Materials and Methods), was equally expressed by COS-1 cells containing \(\beta^{39}\) thal or \(\beta\)-normal genes (Fig 5). This result eliminates differences in transfection efficiency, or RNA recovery, as causes of the observed differences.

To determine whether the low level of \(\beta^{39}\) mRNA present in the COS-1 cell system was due to an intranuclear or cytoplasmic defect, we analyzed purified nuclear and cytoplasmic RNA, as shown in Figs 4 and 5. The level of nuclear \(\beta^{39}\) mRNA was as low, compared to normal \(\beta\)-mRNA and the internal \(\alpha\)-mRNA controls, as the cytoplasmic mRNA. These results imply a nuclear defect in \(\beta\)-mRNA metabolism due to the codon 39 mutation.

Cytoplasmic Stability of \(\beta^{39}\) Thal mRNA in COS-1 Cells

Because the low levels of \(\beta\)-mRNA in patients with translation termination mutations have often been attributed to cytoplasmic instability,\(^{18,42}\) we inhibited transcription in COS-1 cells by addition of actinomycin D (10 \(\mu\)g/mL) to the medium, and measured \(\beta\)-mRNA levels after a 3–4-hour chase incubation period. The chase was initiated 37 hours after transfection to ensure maximal prior accumulation of \(\beta\)-mRNA.

As shown in Fig 6, the amount of cytoplasmic \(\beta^{39}\) thal and \(\beta\)-normal mRNA were both essentially unchanged during the actinomycin D chase. Also unchanged were \(\alpha\)-mRNA levels. These results argue against any role for cytoplasmic instability in the development of the \(\beta^{39}\) thal mRNA deficiency. The 3-hour chase period should have been more than sufficient to detect turnover, as all previous studies have documented reduction in \(\beta\)-thalassemic \(\beta\)-mRNA to the steady-state deficiency levels within 20 minutes of transcription in vivo\(^{13,31}\) (Tables 1 and 2). Moreover, Humphries et al (accompanying paper) have observed the same stability of \(\beta^{39}\) mRNA during longer chase periods.

Effect of an Amber Suppressor tRNA Tyr on \(\beta^{39}\) Thal Gene Expression in COS-1 Cells

Figure 7 shows results obtained when the \(\beta^{39}\) thal gene was cotransfected into COS-1 cells with a normal tyrosine tRNA gene from Xenopus or a gene coding for a mutant suppressor tyrosine tRNA (these plasmids were the gifts of Drs U.L. Rajbanndary and P. Sharp).\(^{37}\) The suppressor tRNA contains a mutated anticodon (3'-AUC,-5') codon complementary to the amber codon at position 39, thus rendering the \(\beta^{39}\) thal mRNA potentially translatable.

The suppressor tRNA gene specifically restored \(\beta^{39}\) thal mRNA levels nearly to normal without effects on normal mRNA; conversely, the normal
tyrosine tRNA had no effects on β-39 thal or β-normal globin mRNA levels (Fig 7). These surprising results suggest strongly that the intranuclear lesion on globin mRNA metabolism arising from the β-39 thal gene is attributable to the 39th codon mutation, as the lesion was corrected with a specific suppressor tRNA. Yet, our data suggest that the fundamental lesion attributable to the 39th codon mutation, as the lesion suggests strongly that the intranuclear lesion on globin mRNA levels (Fig 7). These surprising results tyrosine tRNA had no effects on /3-39 thal or /3-normal mRNA metabolism arising from the f3#{176}-39 thal gene is attributable to the /3#{176}-39 amber codon occurs in the nucleus, prior to cytoplasmic mRNA translation. These apparent conflicts imply, perforce, that the amber codon affects an intranuclear state of the mRNA metabolism and that this stage is restored to normal by the suppressor tRNA independent of the obvious effects of both the mutation and its suppressor tRNA on mRNA translation. The mechanism by which these parameters can affect intranuclear mRNA metabolism are unknown to us, but our results strongly suggest that the effects do exist.

DISCUSSION

Our results and those of the accompanying paper by Humphries et al43 suggest strongly that a mutation causing premature translation termination codon at position 39 of the /3-globin mRNA coding sequence gives rise to a defect in /3-globin mRNA accumulation independent of cytoplasmic mRNA stability. This defect develops rapidly in the nucleus during or within a few minutes after transcription. That the intranuclear lesion in mRNA metabolism is due to the β-39 mutation, rather than to a second abnormality elsewhere in the gene, is apparent from two lines of evidence. (1) Nucleotide sequence analysis of the β-39 thal gene showed no promoter region defects. The expression vector constructs used in these studies (cf, Results and Humphries et al)43 rule out a role for other mutations, as deletion or exchange of normal and β-39 DNA containing these regions had no effect on the results. (2) The defect can be corrected with a specific amber suppressor tRNA (Fig 7). Our data (Tables 1 and 2) also imply strongly that an intranuclear defect in β-mRNA accumulation occurs in vivo.

The low levels of β-39 globin mRNA observed in patients with β-thalassemia (most of whom have mutations resulting directly or indirectly in premature translation termination codons) have previously been attributed to cytoplasmic mRNA instability. The portion of the β-mRNA not protected by polyribosomes was presumed to be exposed to nulease degradation. However, our studies (cf, Fig 6) and those of Humphries et al43 effectively eliminate this explanation, as β-39 thal mRNA and an abnormally spliced β-mRNA43 with a premature terminator were as stable as normal mRNA during actinomycin D chase incubations. These results support, in a more direct fashion, several earlier studies that suggested that β-mRNA deficiency develops in the nucleus in β+ and β-thalassemia patients.15,21,44 In another form of β-thalassemia, in which a premature termination codon is created by a “frameshift” mutation, mRNA instability was so extreme as to be more compatible with intranuclear degradation than cytoplasmic instability.16,42 We have previously shown that a form of β-thalassemia due to an RNA splicing error (causing retention of intron sequences containing an in-phase terminator in processed β-mRNA) results in the same phenomena: development of β-mRNA deficiency in cytoplasm so rapidly that intranuclear or transport defects are likely, and relatively normal stability of the small amount of abnormally spliced mRNA that does enter the cytoplasm.13,31 Indeed, the abnormally spliced species in such patients is readily detected in reticulocytes.13 Finally, studies of a β-thal gene expressed in HeLa cells46 were also consistent with the hypothesis that β-39 thal mRNA is stable in the cytoplasm. These workers did not observe the intranuclear deficiency of β-mRNA that we have observed in COS-1 cells43 (our Fig 4); this difference in behavior of the gene with the same mutation in two different host cells remains unexplained.

On the basis of the above considerations, we concluded that the β-39 thal terminator codon is pleiotropic in its effects on β-globin mRNA metabolism. In addition to blocking translation, the lesion impairs an earlier stage in the metabolism of β-mRNA; this stage reduced nuclear β-mRNA levels in an as yet uncharacterized fashion. As neither we (Fig 4; unpublished data), Moschonas et al,18 nor Humphries et al,43 observed any abnormally spliced β-mRNA species, this mutation probably does not alter mRNA processing. Rather, impaired transcription, accelerated intranuclear degradation, or defective nuclear-cytoplasmic transport with consequent degradation must also result from this mutation, even though one might expect only a lesion at the level of cytoplasmic polyribosomal translation. Precedent exists for coupling of transcription and translation in microorganisms; premature termination codons cause impaired production of transcripts from the same operon, an effect that is corrected by inhibition of the “rho” (transcription termination) protein or presence of a suppressor tRNA.45,46 Although our results (Tables 1 and 2) could be construed as evidence for defective transcription, we are reluctant to draw this conclusion, as the “pulse-labeling” period is sufficient to allow substantial turnover of nuclear mRNA. Precedent for effects of mutations on nuclear-cytoplasmic transport also exist. Zasloff et al47 have shown that a single base change impairs nuclear-to-cytoplasmic transport of a human methionyl tRNA species. It is possible that only fully translated mRNAs, capable of forming polyribosomes
as they cross the nuclear membrane, can achieve efficient egress from the nucleus into the cytoplasm. Further studies will be required to distinguish among these possibilities.

ACKNOWLEDGMENT

We thank Drs Arthur Nienhuis, Timothy Ley, Tom Maniatis, U.L. Rairdbandary, and P. Sharp for the gifts of plasmid DNA expression vectors noted in the text; Dr H. Lazarus, for initiation of the lymphoblastoid cell line from patient R.D.; Dr H.H. Kazazian, for haplotype analysis of DNA from R.D.; and Dr C. Colon, for the gift of COS-1 cells. We thank Ms Gloria Colwell, Ms Helen Grabowski, and Ms Linda Boynton for expert preparation of the manuscript. All recombinant DNA experiments were performed in compliance with the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules. Under current regulations, all experiments in this report are exempt and were performed at the PO level of containment.

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