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mRNA-deficient β°-thalassemia results from a single nucleotide deletion

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ABSTRACT

The β-globin gene of a patient with mRNA-deficient β°-thalassemia has been sequenced. We find a single nucleotide deletion in amino acid codon 44 that produces a UGA terminator at codon 60. We have previously shown that the β-globin mRNA of this patient is correctly spliced and polyadenylated, but rapidly turns over with a half-life of less than 30 min. We suggest that the rapid mRNA turnover is influenced by the deletion of this single nucleotide as well as by the nonsense codon.

INTRODUCTION

The β-thalassemias are a heterogeneous group of hereditary anemias that reduce (β^{+}-thalassemia) or eliminate (β°-thalassemia) the synthesis of β-globin polypeptides in erythroid cells. The molecular lesions of some β-thalassemias have been described. These include partial gene deletion (1), inefficient RNA processing (2-7), mRNA instability (8), and translation termination (nonsense) mutations (9-13). Examples of each of these molecular phenotypes have been described in β°-thalassemia. We have characterized the molecular basis of an mRNA-deficient type of β°-thalassemia that is prevalent in a population of Kurdish Jews (8). In these individuals, peripheral blood reticulocytes contain little or no β-globin mRNA (14), and nucleated bone marrow cells contain low levels of β-globin mRNA (1% or less of normal) (8). Bone marrow erythroid cells from these β°-thalassemics synthesize and correctly splice the β-globin mRNA precursor but rapidly degrade the mature β-globin mRNA (8). We have further characterized this mRNA stability mutation by cloning and sequencing a β-globin allele from an affected individual.

MATERIALS AND METHODS

Cloning of the β°-Thalassemic Allele

DNA was prepared from bone marrow cells of a 17-year old male, M.B.,

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with mRNA-deficient β^{o}-thalassemia. Total DNA was digested to completion with Hind III and size-fractionated in a 0.8% agarose gel. Preliminary data showed that the β-globin alleles of M.B. lie within 7.5 kb Hind III fragments. Therefore, 5 kb-12 kb fragments were electro-eluted and ligated to purified arms of the Hind III vector, λ762 (15,16). Lambda phage were packed in vitro (17), and plaques were screened for β-globin gene sequences (18). Of 5 x 10^5 plaques screened, one β-globin recombinant was obtained. The 7.5 kb Hind III β-globin DNA fragment was subcloned into pBR322, from which three additional β-globin gene subclones were constructed to facilitate DNA sequencing (Fig. 1).

DNA Sequencing Strategy

DNA was digested with the appropriate restriction enzyme, treated with calf intestine alkaline phosphatase and RNase A, phenol:chloroform:isoamyl alcohol (1:0.92:0.08) extracted, precipitated with ethanol, and 5' ^{32}P-labeled with polynucleotide kinase. These DNAs were re-cut with a second restriction enzyme, and various DNA fragments were purified from polyacrylamide or agarose gels after electrophoresis. Sequencing was performed by the method of Maxam and Gilbert (19).

RESULTS

Sequence of the mRNA-Deficient β^{o}-Thalassemic DNA

Approximately 1,750 nucleotides of the β-globin gene and flanking regions, including 80 nucleotides 5' and 90 nucleotides 3' to the ends of the mature β-globin mRNA, were sequenced. The DNA fragments used and the extent of sequencing are shown (Fig. 1). Each base-pair was sequenced either on both strands or at least twice on the same strand. The β-globin gene sequence of M.B. differs from that of a non-thalassemic gene by a single base-pair deletion (Fig. 2A). In the nucleotide sequence of amino acid codon 44, a single GC base-pair is deleted (Fig. 2B). This deletion shifts the translational reading frame of the β-globin mRNA, creating a UGA (opal) termination codon at the normal amino acid position 60. The relative contributions of the C deletion and the UGA translational terminator to the β-globin mRNA instability are not known.

DISCUSSION

Possible Mechanisms of β-Globin mRNA Instability

This mRNA-deficient β^{o}-thalassemia results from a previously undescribed single nucleotide deletion. This frameshift generates a downstream

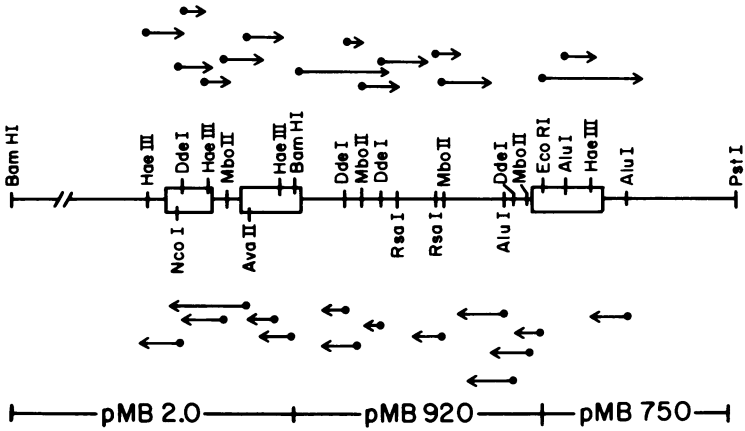

Figure 1: β-Globin gene subclones and sequencing strategy. The initial 7.5 kb Hind III β-globin fragment was subcloned from λ762 into the Hind III site of pBR322. Three β-globin DNA fragments were prepared from this clone by restriction endonuclease digestion, agarose gel electrophoresis, and electro-elution: a 2.0 kb Bam H1 fragment, a 920 bp EcoRI/Bam H1 fragment, and a 750 bp EcoRI/Pst I fragment. These fragments were cloned into appropriately restricted pBR322 and are designated pMB 2.0, pMB 920, pMB 750, respectively. Subclones or purified β-globin DNA fragments from these subclones were used in DNA sequencing studies. DNA fragments were sequenced by the method of Maxam and Gilbert. The site of 5' end labeling (●) and the direction (strand) and extent of sequencing (——>) are indicated.

nonsense mutation. In two other β^{0}-thalassemias, nonsense mutations arise from single nucleotide substitutions at amino acid codon 17 (ref. 9) or 39 (10,11,12). The levels of β-globin mRNA in these patients are reduced to 15% and 5% of normal, respectively. A dinucleotide deletion at codon 8, producing a frameshift nonsense codon at amino acid 22, has previously been described (11), although no mRNA levels have been reported for this patient. The β-globin mRNA content of reticulocytes from the Kurdish thalassemic patient is 1% or less of normal levels, in spite of the fact that transcription of the β-globin genes and processing of the β-globin mRNA precursor to mRNA occur at normal or near normal rates.

The major question raised by these studies concerns the mechanism of the mRNA instability in these syndromes. The simplest interpretation is that the portion of mRNA distal to the nonsense mutation (that which lacks ribosomes) is hyper-susceptible to degradation. However, this hypothesis fails to explain a variety of data: 1. Nonsense mutations in yeast create polar effects; mRNAs with mutations proximal to the initiation codon are less stable than those with mutations distal to the initiation codon (29).

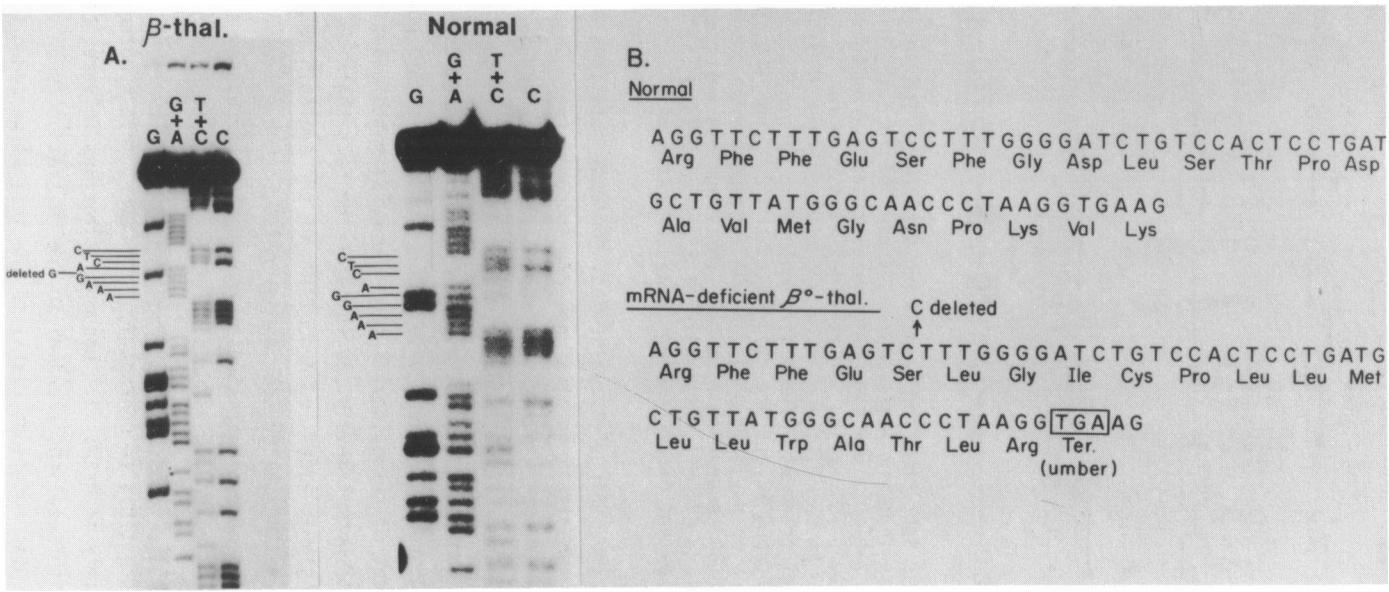

Figure 2: Comparison of normal and β^{o}-thalassemic β-globin gene sequences and coding capacities. A. Sequence of the mRNA-complementary strand of the HaeIII/AvaII 112 bp DNA fragment from an mRNA-deficient β^{o}-thalassemic (M.B.) showing the deletion of a G from codon 44. The normal sequence determined from a clone isolated by Maniatis and co-workers is shown for comparison. B. Comparison of the normal and β^{o}-thalassemic mRNA-homologous DNA strands and amino acid coding capacities.

A pattern of this sort is not observed with three unrelated β^{o}-thalassemias, including the patient described here (8,9,13). On the contrary, the reverse seems to be the case; the instability of β-globin mRNA increases with the distance of the termination codon from the initiation codon. In this context, it is important to note that nonsense polarity in bacteria probably results from premature termination of transcription, not rapid degradation of fully synthesized mRNA (21). Furthermore, in view of the rapid degradation of β-globin mRNA in the Kurdish thalassemic patients, it is possible that little, if any, of the mRNA is transported from the nucleus to the cytoplasm (22). 2. Cells of higher eukaryotes contain relatively stable, untranslated mRNAs (23-26), In S-phase HeLa cells treated with hydroxyurea, histone mRNA is less stable when it is translated than when it is off of polysomes (27). These results are the converse of those expected if mRNAs are significantly more stable on polysomes than off polysomes. 3. Reticulocytes from patients with the hemoglobin variants hemoglobin E ($\alpha_2 \beta_2^{26\ glu \rightarrow lys}$) and hemoglobin Vicksburg ($\alpha_2 \beta_2^{75\ leu \rightarrow 0}$) contain more α- than β-globin mRNA (28,29). Clegg, Weatherall, and co-workers (28) have suggested that the sequence change(s) in hemoglobin E reduces the stability of β^{E}-globin mRNA. Nucleotide sequence changes occur in other hemoglobinopathies at positions (amino acids 17 and 39) where nonsense codons exist in the β^{o}-thalassemias. Homozygotes for these rare variants have not been described, but heterozygotes have slight to moderate anemias (30,31). Although the anemias may result from structural changes in the hemoglobins, other effects, including a reduction in mRNA stability, have not been excluded. We wish to stress that some single nucleotide changes that do not affect translational termination might increase the lability of β-globin mRNA.

In summary, the single nucleotide deletion in the mRNA-deficient β^{o}-thalassemic patient results in an approximately 40-fold reduction in β-globin mRNA stability. Although we have sequenced only one of M.B.'s alleles, it is clear from the mRNA levels of this patient that both alleles produce unstable mRNAs. In the case of the mutation described here, the deletion of a single nucleotide from codon 44 in the β-thalassemic mRNA may directly affect mRNA stability; that is, the instability may not necessarily be linked to translation. It may result from an alteration in the three dimensional structure of the mutant mRNA that increases mRNA susceptibility to cellular nucleases. Further investigation of β-globin alleles that

reduce mRNA stability utilizing cloned genes in a mammalian expression vector may help to define the control of mRNA turnover (32).

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