Proc. Natl. Acad. Sci. USA
Vol. 76, No. 5, pp. 2400–2404, May 1979
Genetics

Partial deletion of β-globin gene DNA in certain patients with β^0-thalassemia

(restriction enzyme mapping/molecular hybridization/3′ sequences/genetic heterogeneity)

STUART H. ORKIN*†, JOHN M. OLD‡, DAVID J. WEATHERALL‡, AND DAVID G. NATHAN*

*Division of Hematology and Oncology, Children's Hospital Medical Center, The Sidney Farber Cancer Institute, and the Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115; and ‡Nuffield Department of Clinical Medicine, University of Oxford, Radcliffe Infirmary, Oxford, England

Communicated by Bernard D. Davis, March 8, 1979

ABSTRACT **We have used restriction endonuclease mapping of cell DNA to investigate the structure of the β-globin gene in β-thalassemias. Among 17 individuals with β^+- and β^0-thalassemia, we observed three patients of Indian origin with β^0-thalassemia whose DNA revealed a consistent mapping abnormality. In one β allele in each diploid cell, 0.6 kilobase of DNA was deleted from β-specific *Pst* I and *Bgl* II restriction fragments. This deletion involved 3′ β-globin gene sequences and eliminated the *Eco*RI site normally present at codons 121/122, but it did not extend to the *Bam*HI site at codons 98–100 on the 5′ side of the 0.90-kilobase intervening sequence normally present in β-globin genes. Partial β-globin gene deletion appears, therefore, to be a primary molecular defect seen in certain patients with β^0-thalassemia.**

β-Thalassemias comprise a class of inherited disorders of humans characterized by deficiency of the β-globin chains of hemoglobin (1). In β^+-thalassemia, both β-globin synthesis and mRNA are reduced in reticulocytes and in their precursors. In β^0-thalassemia, no β-globin chains are present and β-globin mRNA sequences may be either entirely absent, or present but nonfunctional (2, 3). Hybridization kinetic analysis with radioactive β-globin cDNA probes has previously indicated that the β-globin locus is present in cells from patients with β^0-thalassemia in which no β-globin mRNA sequences can be detected (4–8). However, the resolution of these studies was probably not sufficient to exclude partial gene deletions or other structural rearrangements in β-globin loci.

The precise molecular lesions responsible for β^+- and β^0-thalassemias are thus unknown. The technique of restriction endonuclease mapping of cellular DNA now permits closer examination of the structure of globin genes in these syndromes (9–14). Studies of Mears *et al.* (12), Flavell *et al.* (13), and Lawn *et al.* (15) in normal DNAs have identified cleavage sites for several restriction enzymes neighboring the closely linked β- and δ-globin loci. With these data as a framework for restriction mapping, we have now identified a distinctive group of β^0-thalassemias in which the β-globin locus contains an extensive deletion within its 3′ region.

MATERIAL AND METHODS

Patient Material. Diagnoses of homozygous β-thalassemia had been previously established in all patients by hemotologic and family studies, including routine blood counts, hemoglobin electrophoresis, and globin chain synthesis. Three patients with β^0-thalassemia had the molecular defect characterized in this work. Patient 1, our index patient, is a 6-year-old Indian girl with classical, severe β-thalassemia. Both parents are typical carriers of β-thalassemia with elevated levels of Hb A_2. DNA was isolated from spleen tissue removed at Children's Hospital Medical Center in December 1977. Patients 2 and 3 are β^0-thalassemics of Indian background, whose families had settled in East Africa and finally moved to the United Kingdom. High molecular weight cellular DNAs were prepared by proteinase K digestion and phenol extraction from spleen tissue or peripheral white blood cells (9).

Molecular Procedures. *Eco*RI and *Pst* I were purchased from Boehringer Mannheim and *Bgl* II and *Bam*HI from Bethesda Research (Rockville, MD). Cellular DNA samples were digested to completion with restriction enzymes and electrophoresed in 1.0% agarose as described (9, 10). After electrophoresis, β-globin-specific cellular DNA fragments were detected by molecular hybridization with β-globin [^{32}P]cDNA (200–400 cpm/pg) (9) after the blotting procedure of Southern (16). β-Globin cDNA, approximately 90% free of α-globin DNA, was synthesized from Hb H mRNA by avian myeloblastosis virus DNA polymerase with oligo(dT) as primer (9). After hybridization, the filters were rinsed under stringent conditions (13) to reduce potential crosshybridization of β and γ sequences. Fragments bearing globin sequences were detected by autoradiography with Kodak X-Omat film and calcium tungstate intensifying screens.

Short cDNA, enriched for sequences complementary to the 3′ region of the β-globin gene sequence (12), was prepared from β-globin cDNA by preparative electrophoresis in 3.0% SeaPlaque agarose (Marine Colloids, Rockland, ME) (15). The region of gel containing cDNA less than about 200 nucleotides in length was excised and cDNA was recovered from melted agarose by hydroxyapatite chromatography. To corroborate results with short cDNA, we performed competition experiments in which nonradioactive DNA specific for the gene sequence 5′ to the *Eco*RI site from the human β-globin plasmid JW-102 (17) was added to radioactive total β-globin cDNA. Plasmid DNA (kindly provided by David Housman) was treated with *Hha* I and *Eco*RI, and the β-globin 5′ fragment was isolated by gel electrophoresis (17). Heat-denatured plasmid DNA was added to normal-length β-globin [^{32}P]cDNA at the start of filter hybridizations in excess empirically determined to compete with hybridization of the cDNA to *Eco*RI-digested cellular DNA fragments that contain 5′ gene sequences. The appropriate ratio of 5′ β-globin plasmid DNA to β-globin [^{32}P]cDNA was determined in a series of pilot experiments in which filter strips containing *Eco*RI-digested DNA were hybridized with a fixed concentration of labeled probe and an increasing amount of unlabeled competitor. The plasmid DNA was handled under P1 conditions.

Separation of 4.4- and 3.8-kilobase (kb) *Pst* 1 fragments of

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: kb, kilobase.

† To whom reprint requests should be addressed at: Children's Hospital Medical Center, 300 Longwood Avenue, Boston, MA 02115.

the DNA of patient 1 (see below) was achieved by preparative electrophoresis in 0.7% SeaPlaque agarose in a horizontal gel unit. After the bromphenol blue marker had migrated 170 mm, 5-mm sections of gel were excised. DNA was recovered from melted agarose, purified by hydroxyapatite chromatography, extensively dialyzed, and concentrated for further analysis. DNA prepared in this manner is undegraded and fully susceptible to restriction enzyme digestion (unpublished observations). The size of globin-specific fragments was estimated by comparison with λ phage DNA treated with *Eco*RI and *Hin*dIII. Size estimates of DNA fragments electrophoresed in neutral agarose gels (9, 14, 15, 18), as in this work, tend to be somewhat smaller than those estimated by others who have used alkaline gels and the size determination of single-stranded DNA (12, 13).

RESULTS

Alteration of Specific DNA Fragments in Some β^0-Thalassemia DNAs. Cellular DNA samples from normal and β-thalassemic individuals were digested to completion with restriction enzymes, electrophoresed in agarose, and subjected to the filter hybridization procedure described by Southern (16), with β-globin [^{32}P]cDNA as the probe. Selected restriction enzyme cleavage sites within and surrounding the β-globin locus in normal DNA are depicted in Fig. 1 (12, 13). Pertinent to the arguments to be presented here are the following: (*i*) the cellular β-globin gene, including its intervening sequences, is approximately 1.6 kb long (15); (*ii*) *Pst* I and *Bgl* II each produces single cellular DNA fragments containing β-globin gene sequences; (*iii*) *Eco*RI cleaves in both 5′ and 3′ flanking regions close to the β-globin gene and also within the coding sequence at codons 121/122 (19, 20); (*iv*) *Bam*HI cleaves close to the β-globin gene on the 5′ side as well as intragenically at the sequence for amino acids 98–100 (19, 20); and (*v*) a large intervening DNA sequence of about 900 base pairs is situated between codons 104 and 105 of the coding sequence (12, 13, 15). [The 5′-3′ orientation of the β-globin gene is defined by convention as that represented in mRNA (13).] Furthermore, β-globin cDNA probe will hybridize with both β and δ loci owing to the close homology of these gene sequences (12, 13, 15).

Normal DNAs digested with *Pst* I contain two fragments that hybridize with β-globin cDNA (12, 13), 4.4 and 2.2 kb long (Fig. 2*A*, lane 1). The larger fragment contains the β-globin gene, whereas the smaller contains the δ (13). Three patients with β^0-thalassemia, all of Indian background, had an additional

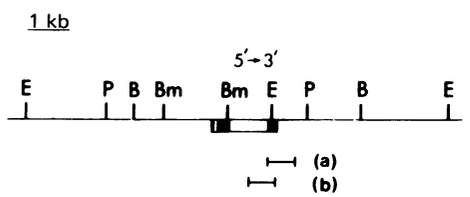

FIG. 1. Restriction map of β-globin gene region of normal human DNA. The order of restriction sites was established by Flavell *et al.* (13) and Mears *et al.* (12). Distances between sites represented here were derived from our own digests of normal DNA and are slightly smaller than those reported. Enzymes: E, *Eco*RI; P, *Pst* I; B, *Bgl* II; Bm, *Bam*HI. Blackened boxes indicate sequences represented in mRNA. Open boxes depict the two intervening sequence regions in normal β-globin genes (15). The smaller intervening sequence is located between codons 26 and 44 (exact position not yet determined) (15), and the larger, about 0.9 kb, between codons 104 and 105 (15). The bracketed lines marked (a) and (b) indicate the extreme locations of the 0.6-kb deletion seen in some β^0-thalassemia DNAs (see text and *Discussion*).

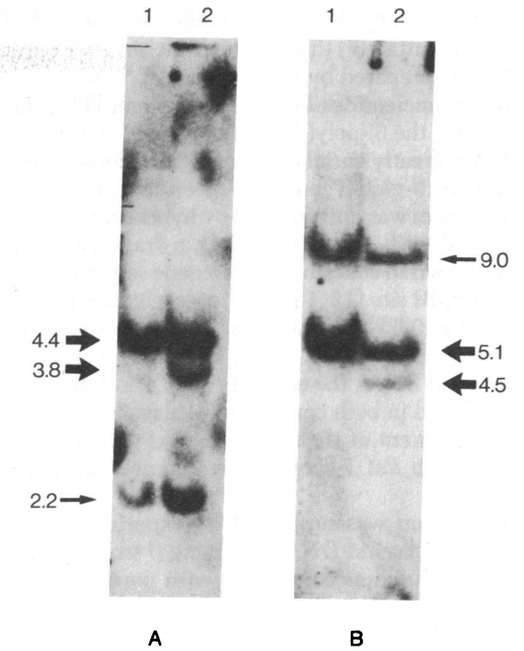

FIG. 2. β- and δ-globin-specific DNA fragments in *Pst* I and *Bgl* II digests. Normal DNA (lane 1) and β^0-thalassemia DNA of patient 1 (lane 2) were digested to completion with either *Pst* I (*A*) or *Bgl* II (*B*) and electrophoresed in 1.0% agarose. Fragments were identified by hybridization with β-globin cDNA after blotting (16). Approximately 15 μg of DNA was applied per lane. Heavy arrows indicate β fragments and lighter arrows δ fragments.

Pst I fragment labeled with β probe, approximately 3.8 kb in size (Fig. 2*A*, lane 2). In the family of patient 1, the abnormal *Pst* I fragment was inherited from the mother, who had β-thalassemia trait and the same restriction pattern with *Pst* I as her affected child. These results initially suggested that one β allele in each of our β-thalassemics might be structurally abnormal and the other might be apparently normal by this technique.

Similar experiments were performed with a different enzyme, *Bgl* II, whose recognition site, A-G-A-T-C-T, differs from that of *Pst* I, C-T-G-C-A-G (21). Normal DNAs digested with *Bgl* II contain two fragments that hybridize with β-globin cDNA (13) (Fig. 2*B*, lane 1). The entire β-globin gene is present in the smaller fragment (5.1 kb), and the δ gene in the larger one (9.0 kb) (13). DNA from our three unusual β-thalassemics yielded an additional labeled band, approximately 4.5 kb long (Fig. 2*B*, lane 2). Thus, in digests with either *Pst* I or *Bgl* II these unusual DNAs gave a new cellular DNA fragment 0.6 kb shorter than the normal β-specific fragment. Moreover, double digestion with *Pst* I and *Bgl* II also produced a new fragment, 0.6 kb shorter than the normal β-specific double-digest fragment of 3.5 kb (not shown). We conclude, therefore, that 0.6 kb of DNA are deleted from the β-globin gene region on one chromsome in these patients with β^0-thalassemia. The presence of a normal-appearing 4.4-kb *Pst* I fragment in addition indicates that these individuals are not homozygous at the molecular level but contain two different types of β^0-thalassemia genes.

Among other patients, mostly of Mediterranean origin, whose DNA was analyzed after *Pst* I digestion, six with β^+-thalassemia and eight with β^0-thalassemia had normal-appearing hybridization patterns with β probe (not shown).

Deletion of a 3′ β-Globin Region. The 0.6-kb deletion in the Indian thalassemic DNAs might lie either entirely 5′ or 3′ to the β-globin gene, or within the gene (and/or its intervening sequences), or it might extend from the gene into a flanking re-

gion. To localize the position of the deletion, we used cDNA specific for the distal (3′) third of the β-globin gene sequence. This probe was prepared by isolating partial cDNA transcripts (less than 200 nucleotides long). Use of oligo(dT) as primer, complementing the 3′-poly(A) on the mRNA, ensures that this short cDNA is greatly enriched in DNA complementary to the 3′ portion of the β-globin gene. The specificity of this probe for 3′ gene sequences was documented by hybridization to normal DNA digested with *Eco*RI (Fig. 3*A*); a fragment of 3.7 kb, previously shown to contain β-globin gene sequences 3′ to the intragenic *Eco*RI site (12, 13), was intensely labeled, whereas the fragment of 5.6 kb containing sequences 5′ to that site (12, 13) was only faintly visible. When this probe was hybridized to *Pst* I-digested DNAs, the normally sized 4.4-kb fragment was distinctly labeled in both control and thalassemic samples, but the 3.8-kb fragment of thalassemic DNAs was not (Fig. 3*A*). Thus, the 3.8-kb *Pst* I fragment is deficient in 3′ gene sequences.

This conclusion was supported by experiments in which nonradioactive plasmid DNA containing β sequences 5′ to the *Eco*RI site was used as competitor with normal-length, ^{32}P-labeled β-globin cDNA. The 3.8-kb *Pst* I fragment of the thalassemic DNAs was not appreciably labeled, whereas the 4.4-kb fragment was clearly visible (Fig. 3*B*).

Deletion of Intragenic *Eco*RI Site. One landmark within the 3′ region of normal β genes is the *Eco*RI site at codons 121/122 (19, 20). To define further the deletion in β^0-thalassemic DNA, we investigated whether this site was present or absent. Because the deletion appeared to involve extensively the 3′ gene region, we anticipated that this site might be missing in the partially deleted genes. In that case, the 3.8-kb *Pst* I fragment would not be cleaved by *Eco*RI. If the patients had been homozygous for the partially deleted locus, examination of DNA cleaved with both enzymes would have provided a direct test for the presence or absence of an intragenic *Eco*RI site. However, the *Pst* I site within the normal 5′ β *Eco*RI fragment produces a normal double-digest fragment of similar size (3.6 kb) (see Figs. 1 and 4). Hence it has proved exceedingly difficult to be certain whether the fainter 3.8-kb fragment has been destroyed or remains in *Pst* I plus *Eco*RI digests of our β^0-thalassemia DNAs.

To circumvent this difficulty, we separated the 4.4- and 3.8-kb fragments of *Pst* I-digested DNA of patient 1 by preparative electrophoresis and individually treated them with *Eco*RI (50 units/μg of DNA for 1 hr) in the presence of λ phage DNA. The latter served as an internal control for digestion during the incubation and was visualized under ultraviolet light after being stained with ethidium bromide. The 4.4-kb *Pst* I DNA yielded two fragments containing β-globin gene sequences, 3.6 and 0.8 kb (Fig. 4). The normal-sized *Pst* I fragment thus contains an intragenic *Eco*RI site, as present in normal loci (12, 13). Treatment of the deletion-containing, 3.8-kb *Pst* I DNA with *Eco*RI, on the other hand, did not result in cleavage of the β-specific band (Fig. 4). Thus, the deletion eliminated the intragenic *Eco*RI site normally present at codons 121/122. Double digestion of DNAs with *Bgl* II and *Eco*RI confirmed this conclusion: the 4.5-kb *Bgl* II fragment was not cleaved by *Eco*RI (Fig. 5*A*).

Because the deletion within the 3′ region is only 0.6 kb in size and involves the intragenic *Eco*RI site, and because this site is 1 kb from the *Bam*HI site in the gene (12, 13, 15), we would not expect the deletion to include this *Bam*HI site. Such is indeed the case. When *Pst* I-digested DNA was treated with *Bam*HI,

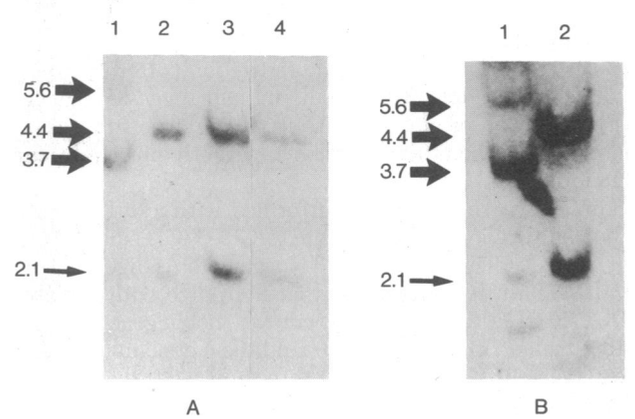

FIG. 3. Deletion of β-globin 3′ gene sequences in 3.8-kb *Pst* I fragment. (*A*) Hybridization with short β-globin [^{32}P]cDNA. Lane 1, normal DNA digested with *Eco*RI; lanes 2, 3, and 4, normal, patient 1, and patient 2 DNAs, respectively, digested with *Pst* I. (*B*) Hybridization with total β-globin [^{32}P]cDNA in the presence of competitor plasmid DNA containing β-globin gene sequences 5′ to the intragenic *Eco*RI site. Lane 1, normal DNA digested with *Eco*RI; lane 2, β^0-thalassemia DNA from patient 1 cleaved with *Pst* I. β-Globin gene specific DNA fragments are identified with heavy arrows and δ fragments with lighter arrows. The prominent fragment labeled in lane 1 (3.7 kb) contains β-globin gene sequences 3′ to the intragenic *Eco*RI site (12, 13). Faint bands at 5.6 and 2.1 kb in lane 1 contain β- and δ-globin 5′ gene sequences, respectively (12, 13). Indistinct labeling below the 4.4-kb band in *Pst* I-digested thalassemic DNAs near 3.8 kb, more evident in *B*, is attributable to detection of β-globin 5′ gene sequences by small amounts of contaminating 5′ β probe in the short cDNA (*A*) and incomplete competition of 5′ sequences by plasmid DNA (*B*). In our experience, the 3.8-kb *Pst* I fragment is visible only to the extent to which 5′ β and 5′ δ *Eco*RI fragments are seen in parallel lanes on the same filter sheet. Compare hybridization of *Pst* I-digested thalassemia DNAs shown here with Figs. 2*A* and 5*B* in which, in contrast, normal-length β-globin [^{32}P]cDNA prominently labels the 3.8-kb band.

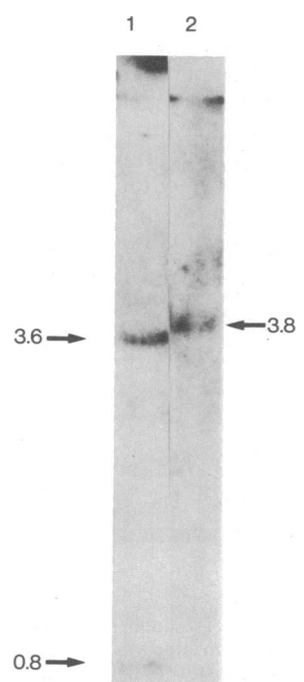

FIG. 4. *Eco*RI treatment of isolated 4.4- and 3.8-kb *Pst* I fragments. *Pst* I-digested DNA of β^0-thalassemic patient 1 was subjected to preparative electrophoresis to isolate DNA fractions 4.4 and 3.8 kb long (see text). The isolated DNA samples were treated with *Eco*RI, reelectrophoresed, and hybridized with β-globin cDNA. Lane 1, 4.4-kb *Pst* I DNA; lane 2, 3.8-kb *Pst* I DNA. Approximately 1.5 μg of DNA was applied to each lane. Internal λ DNA was included in the samples to ensure complete *Eco*RI digestion and to serve as molecular markers.

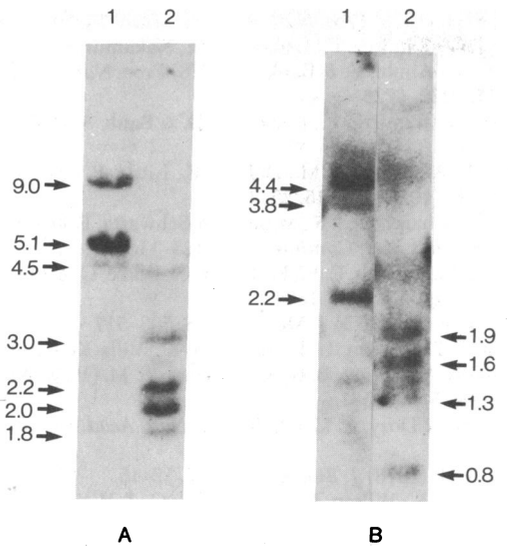

FIG. 5. Absence of intragenic *Eco*RI and presence of intragenic *Bam*HI sites in partially deleted β^0-thalassemia locus. (*A*) Lane 1, *Bgl* II digest; lane 2, *Bgl* II + *Eco*RI digest. (*B*) Lane 1, *Pst* I digest; lane 2, *Pst* I + *Bam*HI digest. Approximately 15 μg of total genomic DNA was applied per lane; β-globin [^{32}P]cDNA was used as probe. Faint band in *Pst* I and *Pst* I + *Bam*HI lanes at about 1.4 kb is not β- or δ-specific. It is variably seen in many experiments with β-globin cDNA as probe and is present in hereditary persistence of fetal hemoglobin DNA (unpublished observations). If no *Bam*HI site were present intragenically in the 3.8-kb *Pst* I fragment, yet one was present 5′ extragenic to the gene (see Fig. 1), the presence of a new fragment at about 2.5 kb would be anticipated. No band in this region has been observed. Normal DNA yields an identical *Pst* I + *Bam*HI pattern (not shown).

the 3.8-kb *Pst* I fragment was cleaved (Fig. 5). This cleavage would be expected to produce a new fragment 0.6 kb shorter than the normal 3′ *Bam*HI plus *Pst* I fragment (see Fig. 1), but in several experiments no fragments other than those normally present were observed. However, this result is not surprising, since the predicted fragment would carry the partial gene deletion itself and our β-globin cDNA probe would, therefore, have few, if any, sequences with which to hybridize. Double digestion with *Bgl* II and *Bam*HI confirmed the presence of a *Bam*HI site in the abnormal locus (not shown).

The range of possible locations of the deletion in the β-globin gene in our unusual patients with β^0-thalassemia between (a) and (b) is depicted in Fig. 1.

DISCUSSION

The restriction mapping experiments reported here document the existence of a β^0-thalassemia locus in which an extensive deletion of β-globin 3′ gene sequences has occurred. R. A. Flavell *et al.*[§] have studied material of patient 3 and arrived at a similar conclusion. The presence of structurally different loci in each of three patients with thalassemia illustrates directly that individuals "homozygous" for β-thalassemia may, in fact, have two different types of thalassemia defects.

The finding of a partial gene deletion in association with β-thalassemia was unexpected. Because most individuals with β-thalassemia appear to have grossly normal β-globin gene organization (this paper; ref. 12; [§]), the identification of this distinctive abnormality in three unrelated patients, all of Indian origin, raises the possibility that it may be found with appreciable frequency among other thalassemics of particular geographic or racial backgrounds. Another example of the concentration of a characteristic at the DNA level within a particular population group is the discovery of polymorphism for *Hpa* I sites that is linked to the sickle gene in blacks (18).

The precise boundaries of the deletion described here remain to be defined. The gene sequence 3′ to the large intervening sequence is 0.25 kb long [codons 105–146 plus 135 bp of the 3′ noncoding region (20)]. Deletion of β-globin 3′ gene sequences, including the intragenic *Eco*RI site at codons 121/122, suggests that this entire segment is most likely absent in the partially deleted gene. The presence of a short, residual stretch derived from this region cannot be excluded by our data, however. The physical mapping indicates that an additional 0.35 kb of DNA is deleted, and this must therefore involve either the flanking region 3′ to the gene or intervening sequences 5′ to codon 105 (or both). The possible extremes are illustrated in Fig. 1. In case (a), the deletion would include the former, whereas in case (b) it would involve the latter. Placement of the 0.6-kb deletion between these extremes is also consistent with our data. These uncertainties may be investigated further by additional restriction mapping with probes for intervening or flanking sequences (15) or by analysis of cloned genomic sequences. Because the order of *Pst* I, *Bgl* II, and *Xba* I (unpublished) sites 3′ to the β-globin gene is preserved in the abnormal DNAs, the deletion is clearly entirely internal within *Pst* I and *Bgl* II sites rather than the result of a more extensive deletion that introduced new restriction sites from distant DNA regions.

More extensive deletions, 4–5 kb at a minimum, are found in individuals homozygous for α-thalassemia (9, 10, 22, 23), δβ-thalassemia (5, 9, 12, 24), hereditary persistence of fetal hemoglobin (9, 12, 25, 26), and hemoglobin Lepore (12, 13, 27). In at least certain patients with δβ-thalassemia complete deletion of both β- and δ-globin genes has occurred (9). In others, however, residual β-globin gene-like material, presumably derived from the δ locus, is found (12), and so partial deletion of a globin gene, perhaps analogous to that described here, may have taken place. The unequal crossingover event responsible for creation of the fusion gene of hemoglobin Lepore (27) deletes 3′-δ and 5′-β sequences plus intergenic DNA (12, 13). More limited deletions than those seen in our three Indian patients may well exist in other patients with β-thalassemias, but might be considerably more difficult to detect with restriction mapping methods applied to date. Further study of the apparently "normal" β loci in β-thalassemias by sequence analysis of cloned genomic segments (15) should reveal the full spectrum of genetic defects.

No β-globin mRNA sequences are detectable in erythroid RNA of patient 2 (unpublished observations). Appropriate material from patients 1 and 3 has yet to be analyzed. We tentatively conclude that the partial deletion prevents this locus from contributing to β-globin mRNA sequences detectable by molecular hybridization. However, abortive transcripts might be produced, and so it may be worthwhile to examine the erythroid cell nuclear RNA of such patients very closely for the presence of labile RNA sequences derived from the 5′-region of the β-globin gene. Indirect evidence for instability of nuclear β-globin RNA sequences in β^+-thalassemias has been reported by Nienhuis *et al.* (28). Alternatively, the deletion within the 3′ region might affect transcription of the gene itself in some as yet unspecified manner. Studies of β-globin RNA metabolism in such patients may shed light on nuclear degradative mechanisms responsible for dealing with defective gene transcripts.

The prevalence of this partially deleted locus among

[§] Flavell, R. A., Grosveld. G. C., Grosveld, F. G., Bernards, R., Kooter, J. M., de Boer E. & Little, P. F. R. (1979) *From Gene to Protein: Information Transfer in Normal and Abnormal Cells*, Miami 11th Winter Symposium (Univ. of Miami School of Medicine, Miami, FL), in press.

β^0-thalassemics overall is not known. Without question it is uncommon. However, the identification of this locus in three unrelated patients should encourage an extensive survey of other individuals, particularly of Indian background. One practical consequence of the identification of this defect in families with thalassemia would be potentially safer and simpler prenatal diagnosis (9). For selected families, the absence of the abnormal 3.8-kb *Pst* I fragment in amniotic cell DNA could be used to exclude severe thalassemia in an at-risk fetus.

We thank Drs. B. Modell and G. Mann for splenic tissue of patients 2 and 3. We are grateful to Ms. Domenica Paci for technical assistance, and to Catherine Lewis for preparation of the manuscript. This work was supported by National Institutes of Health Grant AM-15322, by a National Institutes of Health Young Investigator Award HL-21428, a Medical Foundation, Inc., Fellowship, and a Basil O'Connor Starter Research Award to S.H.O., and by the Medical Research Council.

1. Weatherall, D. J. & Clegg, J. B. (1972) *The Thalassaemia Syndromes* (Blackwell, Oxford), 2nd Ed.
2. Old, J. M., Proudfoot, M. J., Wood, W. G., Langley, J. I., Clegg, J. B. & Weatherall, D. J. (1978) *Cell* **14,** 289–298.
3. Benz, E. J., Jr., Forget, B. G., Hillman, D. G., Cohen-Solal, M., Pritchard, J. & Cavallesco, C. (1978) *Cell* **14,** 299–312.
4. Tolstoshev, P., Mitchell, J., Lanyon, G., Williamson, R., Ottolenghi, S., Comi, S., Giglioni, B., Masera, G., Modell, B., Weatherall, D. J. & Clegg, J. B. (1976) *Nature (London)* **259,** 95–98.
5. Ramirez, F., O'Donnell, J. V., Marks, P. A., Bank, A., Musumeci, S., Schiliro, G., Pizzarelli, G., Russo, G., Luppis, G. & Gambino, R. (1976) *Nature (London)* **263,** 471–475.
6. Temple, G. F., Chang, J. C. & Kan, Y. W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3047–3051.
7. Godet, J., Verdier, G., Nigon, V., Belhani, M., Richard, F., Colonna, P., Mitchell, J., Williamson, R. & Tolstoshev, P. (1977) *Blood* **50,** 463–470.
8. Ramirez, F., Starkman, D., Bank, A., Karem, H., Cividalli, G. & Rachmilewitz, E. A. (1978) *Blood* **52,** 735–739.
9. Orkin, S. H., Alter, B. P., Altay, C., Mahoney, J., Lazarus, H., Hobbins, J. C. & Nathan, D. G. (1978) *N. Engl. J. Med.* **299,** 166–172.
10. Orkin, S. H. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5950–5954.
11. Mears, J. G., Ramirez, F., Leibowitz, D., Nakamura, F., Bloom, A., Konotey-Abulu, F. & Bank, A. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1222–1226.
12. Mears, J. G., Ramirez, F., Leibowitz, D. & Bank, A. (1978) *Cell* **15,** 15–23.
13. Flavell, R. A., Kooter, J. M., DeBoer, E., Little, P. F. R. & Williamson, R. (1978) *Cell* **15,** 25–41.
14. Surrey, S., Chambers, J. S., Muni, D. & Schwartz, E. (1978) *Biochem. Biophys. Res. Commun.* **83,** 1125–1131.
15. Lawn, R. M., Fritsch, E. F., Parker, R. C., Blake, G. & Maniatis, T. (1978) *Cell* **15,** 1157–1174.
16. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
17. Wilson, J. T., Wilson, L. B., deRiel, J. K., Villa-Komaroff, L., Estratiadis, A., Forget, B. G. & Weissman, S. M. (1978) *Nucleic Acids Res.* **5,** 563–581.
18. Kan, Y. W. & Dozy, A. M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5631–5635.
19. Orkin, S. H. (1978) *J. Biol. Chem.* **253,** 12–15.
20. Marotta, C. A., Wilson, J. T., Forget, B. G. & Weissman, S. M. (1977) *J. Biol. Chem.* **252,** 5040–5053.
21. Roberts, R. (1976) *CRC Crit. Rev. Biochem.* **4,** 123–154.
22. Ottolenghi, S., Lanyon, W. G., Paul, J., Williamson, R., Weatherall, D. J., Clegg, J. B., Pritchard, J., Pootrakul, S. & Boon, W. H. (1974) *Nature (London)* **251,** 389–392.
23. Taylor, J. M., Dozy, A. M., Kan, Y. W., Varmus, H. E., Lie-Injo, L. E., Ganesan, J. & Todd, D. (1974) *Nature (London)* **251,** 392–393.
24. Ottolenghi, S., Comi, P., Giglioni, B., Tolstoshev, P., Lanyon, W. G., Mitchell, G. J., Williamson, R., Russo, G., Musumeci, S., Schiliro, G., Tsistrakis, G. A., Charache, S., Wood, W. G., Clegg, J. B. & Weatherall, D. J. (1976) *Cell* **9,** 71–80.
25. Kan, Y. W., Holland, J. P., Dozy, A. M., Charache, S. & Kazazian, H. H., Jr. (1975) *Nature (London)* **258,** 162–163.
26. Forget, B. G., Hillman, D. G., Lazarus, H., Barell, E. F., Benz, E. J., Jr., Caskey, C. T., Huisman, T. H. J., Schroeder, W. A. & Housman, D. (1976) *Cell* **7,** 323–329.
27. Baglioni, C. (1962) *Proc. Natl. Acad. Sci. USA* **48,** 1880–1886.
28. Nienhuis, A. W., Turner, P. & Benz, E. J., Jr. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3960–3964.