RAPID

PUBLICATION

Deletion of the A_γ-Globin Gene in G_γ-$\delta\beta$-Thalassemia

STUART H. ORKIN, BLANCHE P. ALTER, and CIGDEM ALTAY, *Division of Hematology and Oncology, Children's Hospital Medical Center and the Sidney Farber Cancer Institute; Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115; and the Pediatric Hematology Unit, Hacettepe University, Children's Hospital Medical Center, Ankara, Turkey*

ABSTRACT In an individual homozygous for G_γ-$\delta\beta$-thalassemia, a physical alteration in γ-globin gene organization was detected by restriction enzyme mapping. The data indicated that the absence of A_γ-globin chains resulted from extension of the DNA deletion from the $\delta\beta$-globin gene region into the γ-globin gene region rather than a functional disturbance of γ-gene expression.

INTRODUCTION

During late gestation in humans a switch from γ- to β-globin chain synthesis occurs accounting for the transition from predominantly hemoglobin F (HbF) ($\alpha_2\gamma_2$) to HbA ($\alpha_2\beta_2$) in erythrocytes (1). Accompanying this developmental switch is regulation of expression of the nonallelic γ-chain loci, designated G_γ and A_γ, to denote the presence of either glycine or alanine at amino acid position 136 of their gene products (2). Although the percentage of G_γ-chains is about 70% in HbF of cord blood samples, it is 50% or less in the small amount of HbF found in adult erythrocytes (3). Acquired and inherited conditions are associated with increases in HbF in adults (1, 4). Most striking among these entities are two rare conditions, $\delta\beta$-thalassemia and hereditary persistence of fetal hemoglobin (HPFH),[1] in which only HbF is present in homozygotes. Usually homozygotes have both G_γ- and A_γ-chains in the normal adult proportions, but occasionally HbF of only one type may be present (1, 3). Huisman and co-workers (3) have proposed that different deletions of DNA within the $\gamma\delta\beta$-globin gene complex may account for the various observed phenotypes.

Deletion of δ- and β-globin gene sequences has been found in DNA of individuals homozygous for $\delta\beta$-thalassemia and HPFH, as studied by liquid hybridization analysis (5–8) and gene mapping (9, 10). In HPFH the δ- and β-genes are entirely deleted (10). Homozygotes for $\delta\beta$-thalassemia appear to be of at least two varieties: one of which has no δ- or β-gene sequences (10) and another in which residual β-like sequences are present (9). We report here that in a homozygote for the former type of $\delta\beta$-thalassemia only G_γ-globin chains are present in HbF, and this is associated with a physical alteration in γ-gene organization in cell DNA.

METHODS

Gene mapping. The methods used have been described elsewhere (10–12). Cell DNA were digested with restriction enzymes, and electrophoresed in agarose. DNA fragments were transferred to filter sheets by the Southern procedure (13) for hybridization with ^{32}P-globin complementary DNA (sp act, 400 cpm/pg) transcribed from HPFH reticulocyte messenger RNA (10, 12). This probe is specific for both α- and γ-globin sequences. Filters were washed under stringent conditions (14) and autoradiographed to reveal the globin-specific DNA fragments.

HbF analysis. Hemolysates were electrophoresed in 12% acrylamide in 6 M urea, 2% Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.), and 5% acetic acid, adapted from the method of Rovera et al. (15). The proportions of G_γ and A_γ globin chains were determined by scanning of gels after staining with Coomassie Blue dye.[2]

Received for publication 7 May 1979 and in revised form 12 June 1979.

[1] *Abbreviations used in this paper*: HPFH, hereditary persistence of fetal hemoglobin; kb, kilobase pairs.

[2] Alter, B. P., S. C. Goff, G. D. Efremov, M. E. Gravely, and T. H. J. Huisman. Globin chain electrophoresis: a new ap-

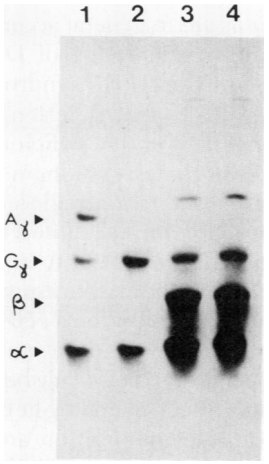

FIGURE 1 Globin chain electrophoresis. Lane 1, HPFH homozygote. Lane 2, δβ-thalassemia homozygote; lanes 3 and 4, mother and father, respectively, of δβ-thalassemia homozygote. Samples of parental hemolysates were intentionally overloaded to permit adequate quantitation of HbF globin chains. The slowly migrating band present in lanes 3 and 4 just above the position of the A_γ chain is carbonic anhydrase.

Patient material. Descriptions of our HPFH and δβ-thalassemia homozygotes have been reported (10). The HPFH homozygote has mild thalassemia by globin chain synthesis studies ($\gamma/\alpha = 0.53$ [10]). The δβ-thalassemia homozygote is an 8-yr-old Turkish male with thalassemia intermedia. Marked globin chain synthesis imbalance is present ($\gamma/\alpha = 0.2$, case 10 of Table III in reference 16). HbF is heterogeneously distributed among the erythrocytes in both parents. By gene mapping the δβ-thalassemia homozygote lacks δ- and β-genes as studied after digestion of DNA with the enzymes Eco RI (10), Hin dIII (10), Pst I, and Xba I (unpublished observations).

DNA was prepared from skin fibroblasts of the δβ-thalassemia homozygote and from peripheral blood leukocytes of the HPFH homozygote or normal individuals as previously described (10).

RESULTS

The δβ-thalassemia homozygote had only G_γ- and α-globin chains in his erythrocytes (Fig. 1). The HbF in the parental erythrocytes was almost exclusively of the G_γ-type as well. These analyses indicate that the homozygote has G_γ-δβ-thalassemia. The elevated HbF in each parent is produced from the chromosome carrying the δβ-lesion. The HPFH homozygote, as in other cases (4, 5, 7), had approximately equal amounts of G_γ- and A_γ-globin chains.

DNA samples were treated with the restriction enzyme Eco RI, and analyzed for the presence of γ-sequences by hybridization with α + γ-^{32}P-complementary DNA. Normal DNA contain four γ-DNA fragments, reported by Little et al. (17) as 6.5, 2.5, 1.65, and 0.65 kilobase pairs (kb) long. The HPFH homozygote DNA had the same γ-fragments as normal DNA (Fig. 2, lane 1), estimated in our gels to be 7.0, 3.0, 1.5, and 0.5 kb long. In contrast to normal and HPFH DNA digested with Eco RI, DNA of the G_γ-δβ-thalassemia homozygote revealed only one of the normal γ-fragments (7.0 kb) (Fig. 2, lane 2). Alteration in the hydridization of γ-DNA fragments in this patient's DNA was not limited to the use of Eco RI. Whereas normal (17) and HPFH DNA contain a single γ-DNA fragment of about 13 kb after digestion with the enzyme Bgl II, the G_γ-δβ-thalassemia homozygote had a new, shorter γ-fragment of about 8 kb that was also present in both parents in addition to a normal Bgl II γ-fragment (Fig. 2, lanes 3–6).

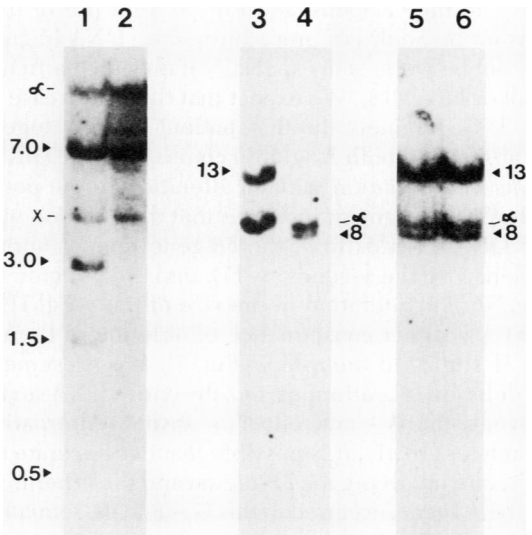

FIGURE 2 Globin gene mapping. DNA samples were treated either with Eco RI (lanes 1 and 2) or Bgl II (lanes 3–6), electrophoresed in agarose, and hybridized with ^{32}P-α + γ-globin complementary DNA. HPFH and G_γ-δβ-thalassemia homozygote DNA are compared in lanes 1 and 2, respectively. HPFH, as well as normal (not shown), DNA contains the γ-specific fragments denoted by the arrowheads, a 22.5-kb α-specific fragment marked "α" (10), and band "X", which is thought to represent a globinlike sequence (possibly "ε") with extensive homology with γ. Minor bands just above and below the 7.0 kb γ-fragment, seen in lane 2, were variably seen in normal, HPFH, and G_γ-δβ-thalassemia DNA in many experiments and are not thought to be globin related. Bgl II-digested normal and G_γ-δβ-thalassemia homozygote DNA are compared in lanes 3 and 4, respectively. Normal, as well as HPFH (not shown), DNA has the 13-kb γ-specific fragment (17). The smaller fragment of about 9 kb hybridizes specifically with α probe and is absent in homozygous α-thalassemia DNA (Orkin, unpublished data). The G_γ-δβ-thalassemia DNA has a new γ-specific fragment at about 8 kb (lane 4). This fragment does not hybridize with α probe alone. Bgl II-treated DNA of the mother and father of the homozygote are shown in lanes 5 and 6.

proach to the determination of the G_γ/A_γ ratio in fetal hemoglobin and to studies of globin synthesis. *Br. J. Haematol.* In press.

DISCUSSION

Our results are best interpreted by reference to the structure of the normal γ-globin gene region reported by Little et al. (17). In normal DNA restriction enzyme sites about the closely linked nonallelic G_γ- and A_γ-loci are arranged as depicted in Fig. 3. Based on the crossover between A_γ- and β-genes leading to the formation of hemoglobin Kenya, the δ- and β-loci can be situated 3′ to the γ-complex (1, 17). Fragments of DNA 3.0 and 0.5 kb long, derived from the A_γ-gene, were absent in Eco RI digests of our G_γ-δβ-thalassemia homozygote DNA. The 1.5-kb fragment, normally derived from the 3′-portion of the G_γ-locus, was not apparent either, although the presence of G_γ-chains indicates that it cannot be missing from the genome. The 3′-G_γ-sequences must reside in a fragment of different size from normal on account of the creation of a new Eco RI restriction site 3′ to the G_γ-locus (see Fig. 3). Because of limitations in the Southern procedure, some DNA fragments may not be well seen, especially if they are particularly small or large (13). We expect that this is the case with the 3′-G_γ-fragment in this patient. Taken together, the absence of both A_γ-globin chains and A_γ-DNA fragments in association with an alteration in the position of the 3′-G_γ-fragment indicates that the deletion within this DNA extends from the δβ-gene region, normally present 3′ to the γ-genes (1, 17), into the γ-region itself (Fig. 3). The alteration in the size of the γ-Bgl II fragment is a direct consequence of deletion of the usual Bgl II site 3′ to the γ-loci (Fig. 3). We presume that the deletion is continuous, i.e., the entire DNA segment between the A_γ- and δ-loci is absent. Alternatively, though less likely, it is possible that two separate deletions, one involving the δβ-region and the other involving the A_γ-gene, occurred in this G_γ-δβ-thalassemia DNA.

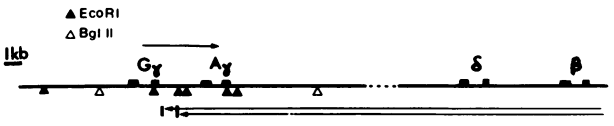

FIGURE 3 Organization of the γ-globin gene region in normal DNA and the deletion in the G_γ-δβ-thalassemia homozygote. Locations of the Eco RI and Bgl II restriction sites about and within the G_γ- and A_γ-loci in normal DNA were deduced by Little et al. (17). The orientation of the γ-gene sequences is indicated by the arrow above the physical map (drawn 5′ to 3′, as the sequences are ultimately represented in messenger RNA). Each globin locus is interrupted by a large intervening sequence preceding the Eco RI site (17). The arrows drawn below the normal map indicate the possible extents of deletion of DNA in the G_γ-δβ-thalassemia homozygote. The deletion originates 3′ to the γ-gene cluster, eliminating the δ- and β-loci (10), and extends to the region flanking the G_γ-gene. At a minimum, the deletion would terminate just to the 5′ side of the first extragenic Eco RI site (right vertical line). At a maximum, the deletion could end just 3′ to the G_γ-gene itself (left vertical line). The extent of the deletion 3′ to the β-gene is unknown.

Our observations are in general accord with the notion that various extents of deletion of DNA occur in the δβ-thalassemias and the HPFH syndromes (3). The data exclude the possibility that deletion of the δβ-region alone is responsible for the deficiency of A_γ-globin chains in our G_γ-δβ-thalassemia homozygote.

It has been proposed that a region of DNA 5′ to the δ-locus is required for normal shutoff of γ-gene expression in the transition from fetal to adult life (1, 3). Its deletion in HPFH might lead to continued γ-gene expression. In our patient with G_γ-δβ-thalassemia this putative control region would be missing. His severe impairment in γ-chain synthesis may be the consequence of a gene dosage effect in that γ-gene sequences required for maximal compensation are deleted. Alternatively, deletion of other critical DNA regions may lead to clinical differences. Whether clear-cut distinctions among disorders associated with maintenance of high levels of γ-gene expression into adult life can rest on physical analysis of DNA alone must await careful correlation of hematologic and molecular data in a wide number and variety of additional patients.

Note added in proof. Recently the intergene distance between the Aγ- and δ-loci has been determined as ≅16 kb by restriction mapping (18, 19). Similar conclusions regarding the deletion in our δβ-thalassemia homozygote were reached by Fretsch et al. (18).

ACKNOWLEDGMENTS

We thank Dr. David G. Nathan for continued support and encouragement, and Dr. Tom Maniatis for helpful discussions.

This work was supported by National Institutes of Health Young Investigator Award, a Basil O'Connor Award to Dr. Orkin and a Research Career Development Award of the National Institutes of Health (HL-00177) to Dr. Alter.

REFERENCES

1. Nienhuis, A. W., and E. J. Benz, Jr. 1977. Regulation of hemoglobin synthesis during the development of the red cell. *N. Engl. J. Med.* **297**: 1318–1328, 1371–1381, 1430–1436.
2. Schroeder, W. A., T. H. J. Huisman, J. R. Shelton, J. B. Shelton, E. F. Kleihauer, A. M. Dozy, and B. Robertson. 1968. Evidence for multiple structural genes for the γ chain of human fetal hemoglobin. *Proc. Natl. Acad. Sci. U. S. A.* **60**: 537–544.
3. Huisman, T. H. J., W. A. Schroeder, G. D. Efremov, H. Duma, B. Meadenovski, C. B. Hyman, E. A. Rachmilewitz, N. Bouver, A. Miller, A. R. Brodie, J. R. Shelton, and G. Appel. 1974. The present status of the heterogeneity of fetal hemoglobin in β-thalassemia: an attempt to unify some observations in thalassemia and related conditions. *Ann. N. Y. Acad. Sci.* **232**: 107–124.
4. Weatherall, D. G., and J. B. Clegg. 1975. Hereditary persistence of foetal haemoglobin. *Br. J. Haematol.* **29**: 191–198.
5. Forget, B. G., D. G. Hillman, H. Lazarus, E. F. Barell, E. J. Benz, Jr., C. T. Caskey, T. H. J. Huisman, W. A. Schroeder, and D. Housman. 1976. Absence of messenger

RNA and gene DNA for β globin chains in hereditary persistence of fetal hemoglobin. *Cell.* **7**: 323–329.
6. Kan, Y. W., J. P. Holland, A. M. Dozy, S. Charache, and H. H. Kazazian. 1975. Deletion of the β globin structural gene in hereditary persistence of foetal haemoglobin. *Nature (Lond.).* **258**: 162–163.
7. Ottolenghi, S., P. Comi, B. Giglioni, P. Tolstoshev, W. G. Lanyon, G. J. Mitchell, R. Williamson, G. Russo, S. Musumeci, G. Schiliro, G. A. Tsistrakis, S. Charache, W. G. Wood, J. B. Clegg, and D. J. Weatherall. 1976. δβ-Thalassemia is due to a gene deletion. *Cell.* **9**: 71–80.
8. Ramirez, F., J. V. O'Donnell, P. A. Marks, A. Bank, S. Musumeci, G. Schiliro, G. Pizzarelli, G. Russo, B. Luppis, and R. Gambino. 1976. Abnormal or absent beta mRNA in beta^0 Ferrara and gene deletion in delta-beta thalassaemia. *Nature (Lond.).* **263**: 471–475.
9. Mears, J. G., F. Ramirez, D. Leibowitz, F. Nakamura, A. Bloom, F. Konotey-Ahulu, and A. Bank. 1978. Changes in restricted human cellular DNA fragments containing globin sequences in the thalassemias and related disorders. *Proc. Natl. Acad. Sci. U. S. A.* **75**: 1222–1226.
10. Orkin, S. H., B. P. Alter, C. Altay, M. J. Mahoney, H. Lazarus, J. C. Hobbins, and D. G. Nathan. 1978. Application of endonuclease mapping to the analysis and prenatal diagnosis of thalassemias caused by globin gene deletion. *N. Engl. J. Med.* **299**: 166–172.
11. Orkin, S. H. 1978. The duplicated human alpha globin genes lie close together in cellular DNA. *Proc. Natl. Acad. Sci. U. S. A.* **75**: 5950–5954.
12. Orkin, S. H., J. Old, H. Lazarus, C. Altay, A. Gurgey, D. J. Weatherall, and D. G. Nathan. 1979. The molecular basis of α-thalassemias: frequent occurrence of dysfunctional α loci among non-Asians with Hb H disease. *Cell.* **17**: 33–42.
13. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**: 503–517.
14. Jeffreys, A. J., and R. A. Flavell. 1977. A physical map of the DNA regions flanking the rabbit β globin gene. *Cell.* **12**: 429–439.
15. Rovera, G., C. Magarian, and T. W. Borun. 1978. Resolution of hemoglobin subunits by electrophoresis in acid urea polyacrylamide gels containing Triton X-100. *Anal. Biochem.* **85**: 506–518.
16. Reyes, G. R., A. Piña-Camara, A. E. Felice, M. E. Gravely, and T. H. J. Huisman. 1978. δβ-Thalassemia in a Mexican family: clinical differences among homozygotes. *Hemoglobin.* **2**(6): 513–529.
17. Little, P. F. R., R. A. Flavell, J. M. Kooter, G. Arnison, and R. Williamson. 1979. The structure of the human foetal globin gene locus. *Nature (Lond.).* **278**: 227–231.
18. Fretsch, E. F., R. M. Lawn, and T. Maniatis, 1979. Characterization of deletions which affect the expression of fetal globin genes in man. *Nature (Lond.).* **279**: 598–603.
19. Tuan, D., P. A. Biro, J. K. deRiel, H. Lazarus, and B. G. Forget. 1979. Restriction endonuclease mapping of the human γ globin gene loci. *Nucleic Acids Res.* **6**: 2519–2544.