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Genetics

Different 3′ end points of deletions causing δβ-thalassemia and hereditary persistence of fetal hemoglobin: Implications for the control of γ-globin gene expression in man

(repetitive DNA sequence/DNA polymorphism/gene cloning/Southern blot)

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ABSTRACT **DNA at the end point of the gene deletion associated with one form of hereditary persistence of fetal hemoglobin (HPFH) was cloned and used as a probe in gene mapping experiments to analyze the extent and approximate 3′ end points of various deletions associated with HPFH and δβ-thalassemia. The deletions in the two known forms of deletion-type HPFH were shown to be considerably more extensive than in the two cases of δβ-thalassemia studied. The overall extents of the deletions in the two types of HPFH were quite similar in both cases and the 3′ end points were located at a minimum distance of ≈52 and 57 kilobases from the 3′ extremity of the β-globin gene. In contrast, the 3′ end points of the deletions in the two forms of δβ-thalassemia were located ≈5 and 10 kilobases to the 3′ side of the β-globin gene. The extent of these deletions and the nature of the DNA brought into the vicinity of the γ-globin genes by the deletions may therefore be a more important influence on the phenotype of the deletions than the specific nature of the DNA sequences that are deleted within the non-α-globin gene cluster as a result of the mutations.**

In an effort to understand the molecular mechanisms that may be associated with the control of human globin gene expression, we studied the disorder hereditary persistence of fetal hemoglobin (HPFH), which provides an especially interesting system for study. In individuals with HPFH, expression of the γ-globin genes of fetal hemoglobin persists at a high level into adult life, instead of being suppressed, as in normal individuals, to <1% of the total globin gene output after the first 6 months of life (for review, see ref. 1). A common type of HPFH found in Blacks is associated with an extensive deletion involving the entire adult δ- and β-globin genes (2–5). The deletions of the δ- and β-globin genes, however, do not seem to constitute the basis for the persistent γ-globin gene expression in HPFH, because, in other disorders, such as δβ-thalassemia, the Hb Lepore syndrome, and one form of β^0-thalassemia, the δ- and β-globin genes are totally or partially deleted (3–14), but the γ-globin genes are not expressed at the same high or uniform levels as in HPFH.

Initial attention focused on the different 5′ end points of the deletions in HPFH and δβ-thalassemia (3, 13–18) and provided some support for the original hypothesis proposed by Huisman *et al.* (19) that the inter-γδ-globin gene DNA might contain regulatory sequences, the deletion of which would result in persistent γ-globin gene expression into adult life. However, in certain forms of δβ-thalassemia, the entire inter-γδ-globin gene DNA is deleted (3, 11, 14), but the deletions do not result in the HPFH phenotype. Thus it has not been possible to elaborate a single model, consistent with all of the known deletion mutants, that adequately explains the persistent γ-globin gene expression in HPFH on the basis of differential deletion of inter-γδ-globin gene DNA sequences.

Alternatively, differences in the size of the deletions or in the nature of the DNA sequences brought by the deletion events into the vicinity of the γ-globin genes from the DNA normally flanking the 3′ extremity of the β-globin gene may be more important factors in the generation of the HPFH vs. thalassemia phenotype in these deletion mutants. As a further step toward elucidating the various factors that may influence γ-globin gene expression in the deletion mutants, we have cloned and analyzed the DNA that is located at the 3′ deletion end point in one form of HPFH.

MATERIALS AND METHODS

Genomic DNA Samples. The sources of the total cellular DNA used in the gene mapping studies were as follows: U.S. Black HPFH (HPFH-1), a lymphoblastoid cell line (LAZ-149) that has been the subject of numerous studies (2, 3, 17, 20); a lymphoblastoid cell line (LAZ-177) from a second U.S. Black patient with HPFH (21), referred to as HPFH-2 in a previous study (3); Ghanaian HPFH, a lymphoblastoid cell line GM-2064, also the subject of previous studies (17, 18); Sicilian δβ-thalassemia, a lymphoblastoid cell line GM-2267, the subject of previous reports (3, 4, 8); and Turkish δβ-thalassemia, cultured fibroblasts or peripheral blood leukocytes from the patient described by Orkin *et al.* (5, 11) and called δβ-thal-2 in a previous study (3). "Normal DNA" consisted of DNA extracted from peripheral blood leukocytes, placenta, or spleen of various nonthalassemic individuals or individuals with nondeletion types of β^0- or β^+-thalassemia.

Molecular Hybridization Probes. Molecular hybridization probes not previously reported were as follows: pRK29, a subclone in pBR322 of a 1.2-kilobase (kb) *Eco*RI fragment derived from the 3′-flanking DNA, ≈18 kb to the 3′ side of the β-globin gene, provided to us by R. Kaufman; pψβ1-5, a 4-kb *Bgl* II fragment containing the ψβ1-globin gene and adjacent DNA, cloned by us in the *Bam*HI site of pBR322; $p^G\gamma$1.6, the 1.6-kb 3′ *Eco*RI fragment of the $^G\gamma$-globin gene subcloned by us in pBR328 from the recombinant pBRHγG-1 (22); and p51.1, the 2.7-kb 5′ *Eco*RI fragment of the $^A\gamma$-globin gene, subcloned in pBR322 (23), provided by O. Smithies. The 1.0-kb *Bam*HI/*Eco*RI

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Abbreviations: HPFH, hereditary persistence of fetal hemoglobin; kb, kilobase(s).

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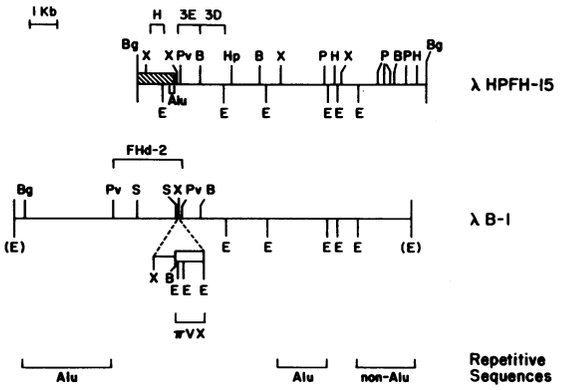

FIG. 1. Restriction endonuclease maps of cloned DNA segments from the region of the 3′ end point of the HPFH deletion. Clone λ HPFH-15 contains the 11-kb *Bgl* II fragment isolated from the U.S. Black individual with homozygous HPFH-1. Cross-hatched bar represents DNA from the normal inter-γδ-globin gene region containing the RI H sequence. Clone λ B1 was isolated from a library of normal human DNA (25) by using the π VX recombination-screening technique of Seed (26) with the *Xba* I/*Bam*HI fragment (3E) inserted into the π VX miniplasmid. Insert represents sequences of the π VX vector retained in the cloned DNA. E, authentic *Eco*RI sites; (E), *Eco*RI sites derived from linkers used in cloning (25); Bg, *Bgl* II; X, *Xba* I; Pv, *Pvu* II; B, *Bam*HI; Hp, *Hpa* I; P, *Pst* I; H, *Hin*dIII; S, *Sac* I.

HPFH-3D and the 0.8-kb *Xba* I/*Bam*HI HPFH-3E DNA fragments derived from the 3′ end point of the HPFH-1 deletion and the 2.5-kb *Pvu* II fragment (FHd2) from adjacent normal DNA, were subcloned in pBR328 (see Fig. 1 for relative positions).

RESULTS

Cloning and Characterization of DNA at the End Point of the HPFH-1 Deletion. The end point of the HPFH-1 deletion was cloned by constructing a bacteriophage λ library from *Bam*HI-digested Charon 28 DNA (24) ligated to 10- to 12-kb size-selected *Bgl* II-digested DNA of an individual with HPFH-1 (20) and by screening with the RI H probe from normal inter-γδ-globin gene DNA (3, 25). Previous gene mapping experiments showed that the RI H probe hybridized to a fragment of ≈11 kb in *Bgl* II digests of HPFH-1 DNA (2, 17), including ≈9.5 kb of new DNA brought into the vicinity of the γ-globin genes by the gene deletion. The restriction endonuclease map of the inserted DNA in one of the isolated recombinants (designated λ HPFH-15) is shown in Fig. 1.

To isolate DNA sequences within normal DNA that are deleted in the region of the 3′ end point of the HPFH-1 deletion, we used the screening technique of Seed (26) for isolation of specific DNA sequences by recombination in *Escherichia coli*. The normal DNA library of Lawn *et al.* (25) was screened with a π VX miniplasmid containing the HPFH-3E DNA fragment (Fig. 1). Of six clones that were isolated and shown to contain sequences complementary to HPFH-3E DNA, only one, designated λ B1 (Fig. 1), had a restriction endonuclease map that was not in conflict with the map of normal DNA obtained by Southern gel analysis of total normal human DNA.

Table 1. Gene mapping results in HPFH

Probe	Enzyme digest	DNA fragment size, kb: Nondeletion control	U.S. HPFH-1	Ghana HPFH
pHPFH-3D	*Hin*dIII	12	14	16
	Bgl II	16	11	16
	*Eco*RI	11	2.2	11.5
	Hpa I	≈22	ND*	14
	Pst I	13	10	14
	Bcl I	2.6	7.5	2.6
	Taq I	8.7	7	8.7
	Sac I	10.5	12	10.5
	*Eco*RI/*Bgl* II	7.5	ND	7.5
	*Eco*RI/*Hin*dIII	8.8	ND	10.5
	*Eco*RI/*Pst* I	9.3	ND	10.5
	*Eco*RI/*Hpa* I	11	ND	11
pHPFH-3E	*Bam*HI	≈25	ND*	14
pFHd2	*Bam*HI	≈25	ND	ND

ND, not determined.
* Thirteen-kilobase fragments were obtained by Fritsch *et al.* (3) using the RI H probe.

The location and nature of repetitive DNA sequences within λ HPFH-15 and λ B1 were investigated by blotting various restriction endonuclease digests of the clones and hybridizing them to nick-translated total human DNA (27) or to cloned DNA fragments containing various specific types of repetitive DNA sequences. In addition to the *Alu* I family repetitive DNA sequence derived from inter-γδ-globin gene DNA in clone λ HPFH-15 (16), repetitive DNA sequences were identified at three more sites within the two clones (Fig. 1). A nick-translated probe specific for *Alu* I family repetitive DNA sequences hybridized to two of the three additional DNA regions (Fig. 1). The third region of repetitive DNA sequence located near the 3′ extremity of λ HPFH-15 and encompassed by a 2-kb *Eco*RI/*Hin*dIII fragment did not hybridize to the *Alu* I family probe or to probes for five distinctive repetitive DNA sequences, including members of the *Kpn* I family of repetitive DNA sequences (28) located in the non-α-globin gene cluster and flanking the 5′ extremity of the $^{G}\gamma$-globin gene and the 3′ extremity of the β-globin gene (29, 30). This repetitive DNA therefore appears to constitute yet another distinctive class of repeated DNA elements in the human genome.

Gene Mapping of the 3′ End Points of the Deletions in HPFH. The pHPFH-3D clone was used as a probe in gene mapping experiments with total cellular DNA from normal individuals and from individuals with various types of HPFH and δβ-thalassemia. The size of the various restriction endonuclease fragments obtained with different enzymes in these syndromes is listed in Table 1. DNA from individuals with two forms of δβ-thalassemia yielded fragments, with the first five en-

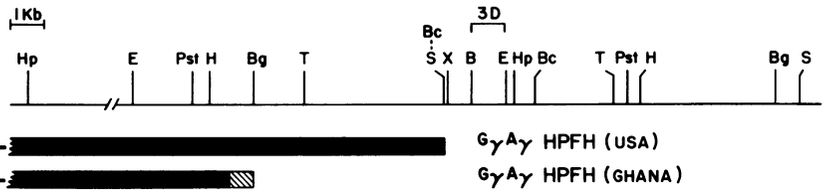

FIG. 2. Restriction endonuclease sites in the region of DNA at the 3′ end point of the deletions associated with HPFH. Solid bars indicate the region of DNA deleted in the two cases of HPFH; cross-hatched area represents the region of uncertainty in the precise breakpoint of the deletion as defined by gene mapping alone. Bc, *Bcl* I; T, *Taq* I. Remaining abbreviations are as in Fig. 1.

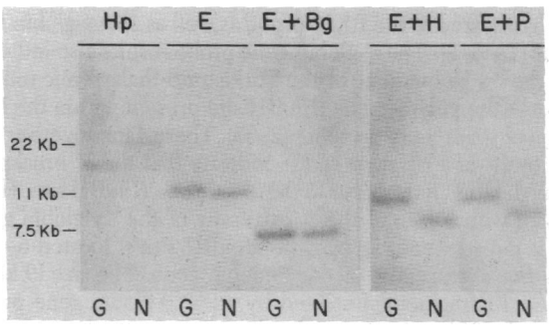

FIG. 3. Autoradiograph of hybridization of digests of normal and Ghanaian HPFH DNAs with the pHPFH-3D probe derived from the 3′ end point of the HPFH-1 deletion. Gene mapping procedures were carried out as described (2, 17). G, Ghanaian HPFH; N, nondeletion control. Remaining abbreviations are as in Fig. 1.

zymes listed in Table 1, of the same size as those obtained with digests of normal DNA. These results indicated that the deletions in these cases of $\delta\beta$-thalassemia did not extend as far to the 3′ side as those in the cases of HPFH. On the other hand, DNA from the individuals with the two types of Black HPFH gave many fragments of sizes different from one another and from normal (Table 1).

A map of restriction endonuclease cleavage sites in normal DNA (Fig. 2) was deduced based on the gel blotting studies listed in Table 1 and on knowledge of the precise locations of cleavage sites in the cloned DNA fragments indicated in Fig. 1. In contrast to DNA from the U.S. Black HPFH (HPFH-1), DNA from the Ghanaian HPFH yielded fragments of the same size as normal DNA with the enzymes *Bgl* II, *Bcl* I, *Sac* I, and *Taq* I (Table 1) as well as with *Eco*RI/*Bgl* II (Fig. 3). However, fragments of a different size from normal were obtained with *Pst* I and *Hin*dIII (Table 1) as well as with double digests using these enzymes together with *Eco*RI (Fig. 3). These results are consistent with the conclusion (Fig. 2), that the 3′ end point of the Ghanaian HPFH is located approximately 6 kb to the 5′ side of that of HPFH-1, for the following reasons. All of the restriction endonuclease sites generating fragments of the same size in Ghanaian and normal DNA are located at or to the 3′ side of the *Bgl* II site that maps, in normal DNA, to a point ≈5.5 kb to the 5′ side of the HPFH-1 3′ end point (Figs. 1 and 2). On the other hand, abnormally sized fragments are generated in Ghanaian HPFH DNA with *Hin*dIII and *Pst* I and the sites for both of these enzymes are located between 1 and 2 kb to the 5′ side of the *Bgl* II site (Fig. 2). With *Hpa* I and *Eco*RI, for which the sites are located further to the 5′ side, different sized fragments were also obtained between normal and Ghanaian DNA, although in the case of *Eco*RI, the difference in size of the fragments was only slight (Fig. 3; Table 1).

Gene mapping studies with the HPFH-3D probe also allowed a more precise determination of the genotype of a third

Table 2. Gene mapping results in $\delta\beta$-thalassemia

Probe	Enzyme digest	DNA fragment sizes, kb: Nondeletion control	Sicilian	Turkish
pRK29	*Hin*dIII	13.5, 15.5, or 13.5 and 15.5	≈30	11.5
	Bgl II	2.5	ND	2.5
	Kpn I	18	18	ND
	Hpa I	≈24	≈24	≈24
	Pst I	9	9	9
	*Bam*HI	16	16	ND
pψβ1-5	*Hin*dIII	18	≈30	ND
β	*Hin*dIII/*Pst* I	2.3	9.2*	ND
5′-γ (p51.1)	*Hin*dIII	7.2, 7.8, or 7.2 and 7.8; 2.7, 3.4, or 2.7 and 3.4	7.8 and 3.4	7.8 and 11.5
3′-$^{G}\gamma$ ($p^{G}\gamma$1.6)	*Eco*RI	1.6	ND	9

ND, not determined.

* Bernards *et al.* (8) obtained a 3.35-kb fragment in a *Hin*dIII/*Pst* I digest of DNA from the same patient's lymphoblastoid cell line. Although the reason for the discrepancy is not clear, our *Hin*dIII mapping data in this patient agree with that of Ottolenghi *et al.* (13) who studied an unrelated individual with Sicilian $\delta\beta$-thalassemia.

Black individual with homozygous HPFH (21). This individual was designated HPFH-2 in a previous study (3) and was thought to be homozygous for the same gene deletion as that in HPFH-1. DNA from this individual digested with *Hin*dIII, *Bgl* II, and *Eco*RI and hybridized to the HPFH-3D probe yielded two sets of fragments: one consistent with the HPFH-1 deletion, the other consistent with the Ghanaian HPFH deletion (data not shown). This individual is therefore doubly heterozygous for both forms of deletion type HPFH found in Blacks.

Gene Mapping of the 3′ End Points of the Deletions in $\delta\beta$-Thalassemia. To map the 3′ end points of the two $\delta\beta$-thalassemia deletions we used the probe pRK29 that is derived from normal 3′-flanking DNA, located ≈18 kb downstream from the normal β-globin gene, as shown in Fig. 4. The sizes of the various restriction endonuclease fragments detected in the normal and in the two $\delta\beta$-thalassemia DNAs are listed in Table 2. In summary, both normal and abnormally sized fragments were detected by this probe in different enzyme digests of the $\delta\beta$-thalassemia DNAs.

Polymorphism of a *Hin*dIII Site in 3′-Flanking DNA of β-Globin Gene. An unexpected result of our gene mapping studies was the finding of heterogeneity among normal (nondeletion) DNA samples when *Hin*dIII-digested DNA was hybridized to the pRK29 probe. Fragments of either 13.5 or 15.5 kb (alone or in combination) were detected in DNA samples from different individuals (Fig. 5). Based on the known normal restriction endonuclease map for *Hin*dIII sites in this region of DNA (30), these results could be best interpreted by the pres-

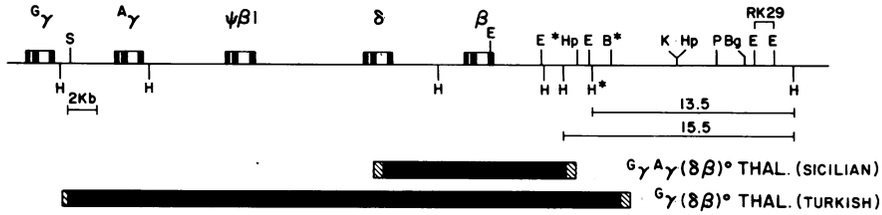

FIG. 4. Restriction endonuclease sites in the regions of DNA at the end points of two $\delta\beta$-thalassemia deletions. Restriction endonuclease sites to the 3′ side of the β-globin gene have been mapped by others (30, 31). Asterisks indicate sites that have been shown to be polymorphic, resulting in restriction fragment length polymorphisms in digests of DNAs of different individuals (32, 33). K, *Kpn* I. Remaining abbreviations and symbols are as in Figs. 1 and 2.

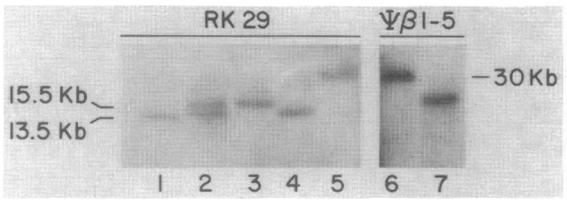

FIG. 5. Autoradiographs of hybridizations of *Hin*dIII digests of various DNA samples with the pRK29 and pψβ1-5 probes. The conditions were the same as in Fig. 3. Lanes 5 and 6, Sicilian δβ-thalassemia; other lanes, nondeletion control samples.

ence of a polymorphism for one of the *Hin*dIII sites (indicated by asterisk in Fig. 4) located in the 3′-flanking β-globin gene DNA.

It is noteworthy that this polymorphism occurs in close proximity to two other known polymorphic restriction endonuclease sites (also indicated by asterisks in Fig. 4) for the enzymes *Hpa* I (32) and *Bam*HI (33). These sites are located in a region of DNA that contains repetitive DNA sequences (29, 30) of the *Kpn* I family (28), and such repetitive sequences may be somewhat prone to variability between individuals. We found no linkage disequilibrium between the polymorphism of the *Hin*dIII site and that of the nearby *Bam*HI site or that of an *Ava* II site within the large intron of the β-globin gene (34) (data not shown).

3′ End Point of Sicilian δβ-Thalassemia. *Hin*dIII digests of DNA from the individual with Sicilian δβ-thalassemia yielded a fragment of ≈30 kb after hybridization to the pRK29 probe (Fig. 5). A fragment of the same size was also detected in a parallel digest of the same DNA hybridized to a probe derived from the ψβ1-globin gene, whereas nondeletion DNA yielded an 18-kb fragment (Fig. 5). These results are consistent with the conclusion that the same *Hin*dIII fragment in Sicilian δβ-thalassemia DNA hybridizes to both ψβ1 and RK29 probes. Based on the knowledge that the 5′ end point of this deletion occurs within the large intron of the δ-globin gene (3, 7), the 3′ end point of the deletion must occur at a point beyond the 5′ *Hin*dIII site of the 15.5-kb fragment detected in normal DNA by the RK29 probe (Fig. 4). On the other hand, detailed mapping data by others (3, 13, 18), using γ- and δ-globin gene probes, are consistent with preservation of the nearby (3′) adjacent *Hpa* I, *Eco*RI, and *Bam*HI sites—i.e., sites in a region of DNA that includes the polymorphic *Hin*dIII site (H*, in Fig. 4). This H* site must therefore be absent by polymorphism rather than by deletion in Sicilian δβ-thalassemia.

Taken together, the various data are consistent with the conclusion that the 3′ breakpoint of the Sicilian δβ-thalassemia occurs at a point 5–5.5 kb to the 3′ side of the β-globin gene, between the *Hin*dIII and *Hpa* I (*Hp) sites as shown in Fig. 4.

3′ End Point of Turkish δβ-Thalassemia. In the case of the Turkish δβ-thalassemia, gene mapping experiments using the pRK29 probe together with γ-globin gene probes also permitted rather precise delineation of the deletion end points. *Hin*dIII-digested DNA from this patient yielded a fragment of 11.5 kb that hybridized to the RK29 probe as well as to a γ-globin gene probe (Table 2). The γ-globin gene probe results also indicated that the ^{G}γ-globin gene of the Turkish δβ-thalassemic individual lacks the polymorphic *Hin*dIII site present within the large intron of some γ-globin genes (2, 35). These data, together with the results of Fritsch *et al.* (3), indicate that the 5′ breakpoint of the deletion must occur to the 3′ side of a *Hin*dIII site, flanking and within 1 kb of the 3′ extremity of the ^{G}γ-globin gene, and it must extend beyond the *Hin*dIII site(s) located 5–6 kb from the 3′ extremity of the β-globin gene. The size (9 kb) of the *Eco*RI fragment, detected by a 3′-^{G}γ-globin gene probe, places the 3′ breakpoint of the deletion ≈9–10 kb from the 3′ extremity of the β-globin gene (Fig. 4). Additional mapping data (Table 2) are also consistent with this conclusion.

Extent of the Globin Gene Deletion in HPFH. By using the RK29 and HPFH-3D probes in gene mapping experiments with normal DNA, we have estimated the minimum extent of the deletion in HPFH, and this consists of at least 60 kb of DNA deleted beyond the 3′ end of the β-globin gene (Fig. 6). When normal DNA is digested with *Hpa* I, gel blotting experiments using the HPFH-3D and RK29 probes reveal hybridization to large fragments of >20 kb in both cases. However, the fragments are not of identical size. The fragment revealed by the RK29 probe is slightly larger than that revealed by the HPFH-3D probe: ≈24 kb vs. ≈22 kb, respectively (Tables 1 and 2), as confirmed by sequential rehybridization of the same filters to the two probes. The mapping results using *Bam*HI and the RK29, FHd2, and HPFH-3E probes (Tables 1 and 2) also exclude overlap of the two large *Hpa* I fragments (Fig. 6). Because the *Hpa* I site 5′ to the RK29 probe and the site 3′ to the HPFH-3D probe have been mapped (refs. 30 and 31 and Fig. 1, respectively), the amount of DNA that can be mapped between these two probes is increased by ≈30 kb beyond that which has already been isolated in recombinant DNA clones.

DISCUSSION

We have analyzed the 3′ breakpoints and overall extent of the DNA deletions that are associated with two types of δβ-thalassemia and two types of HPFH. Our results show that, in the two types of δβ-thalassemia studied, the 3′-breakpoints are located ≈5 and 10 kb to the 3′ side of the β-globin gene. The overall extent of the deletions therefore comprise ≈13 kb for the Sicilian ^{G}γ^{A}γ δβ-thalassemia and 40 kb for the Turkish ^{G}γ δβ-thalassemia. In the case of the two forms of Black HPFH, the deletions extend much further to the 3′ side of the β-globin gene, but their 3′ end points occur within ≈6 kb of each other, as shown by gene mapping experiments using a probe isolated from the 3′ breakpoint of one of the two forms of HPFH. The minimum length of DNA deleted to the 3′ side of the β-globin gene is ≈52 and 57 kb in the two forms of HPFH. The total amount of DNA deleted in these cases of HPFH therefore comprises a minimum distance of at least 70 kb and possibly much more. Furthermore, the total lengths of the deletions are virtually identical in both forms of HPFH because both the 5′ breakpoints and 3′ breakpoints are staggered by approximately the same distance of 5–6 kb in each case. Analysis of DNA of

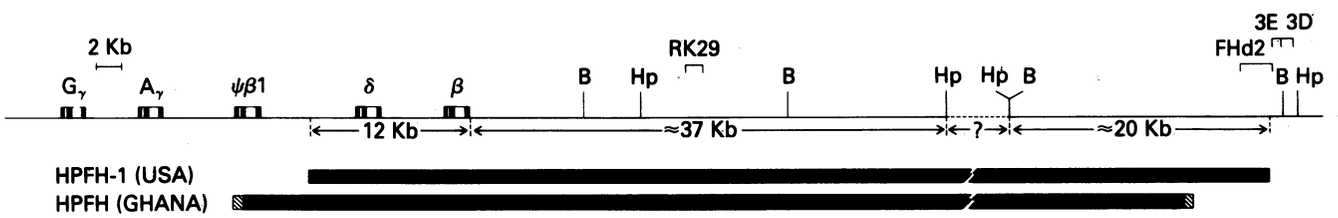

FIG. 6. Minimum estimate of the total extent of the two deletions associated with HPFH. Abbreviations and symbols are as in Figs. 1 and 2.

a third Black individual (U.S. HPFH-2) with the phenotype of homozygous HPFH revealed the presence of double heterozygosity for the two forms of HPFH deletions. Vanin *et al.* (36) have obtained similar results in their studies of the Ghanaian HPFH and U.S. HPFH-2.

The comparative analysis of these various deletion syndromes suggests that the principal factor responsible for the generation of the HPFH phenotype vs. the $\delta\beta$-thalassemia phenotype may not necessarily be the nature of the inter-$\gamma\delta$-globin gene DNA sequences that are deleted as a result of the 5′ breakpoints of the deletions. Perhaps more relevant factors that cause the different phenotypes consist of either the total lengths of the deletions themselves or the nature of the DNA sequences that are brought by the deletion into the vicinity of the γ-globin genes. It is possible, as originally suggested by Bernards and Flavell (18), that very large deletions such as those associated with HPFH disrupt chromatin structure in a manner that results in continued expression of the γ-globin genes, whereas shorter deletions associated with $\delta\beta$-thalassemia have no such effect or a much lesser effect. However, the recent finding by O. Smithies and colleagues (personal communication) that a third type of $\delta\beta$-thalassemia, originally described by Jones *et al.* (14), is associated with a deletion of approximately the same size as that in the two types of HPFH makes it unlikely that deletion size alone is the critical determinant that causes the phenotype differences associated with the various globin gene deletion syndromes.

Alternatively, it is possible that the DNA that is brought into the vicinity of the γ-globin genes from the region of the 3′ end point of the deletion may contain sequences, such as enhancer elements (37, 38), that facilitate constitutive expression of the γ-globin genes to which they have become juxtaposed. In nondeletion types of HPFH and $\delta\beta$-thalassemia (1, 13, 17, 18), however, different molecular mechanisms must be operative.

One additional conclusion can be drawn from our results with regard to the nature of the recombination event that caused the deletion in the case of HPFH-1. The region of normal DNA at the 3′ breakpoint of this deletion does not contain any *Alu* I repetitive DNA sequences, whereas the 5′ breakpoint of the deletion occurs at the midpoint of one of the *Alu* I repetitive DNA sequences situated 5′ to the δ-globin gene (16). The minimum distance between the 3′ breakpoint of the HPFH deletion and any neighboring *Alu* I family repetitive DNA sequences is $\approx$2.5 kb on the 5′ side and $\approx$4 kb on the 3′ side. It therefore appears that the recombination event that created the HPFH deletion was associated with illegitimate recombination between *Alu* I family repetitive DNA at the 5′ side of the δ-globin gene and non-*Alu* (nonrepetitive) DNA, far to the 3′ side of the β-globin gene.

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