Isolation and characterization of cloned human fetal globin genes

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Received 15 August 1979

ABSTRACT

Three clones containing both the human $\gamma_2$ and $\alpha_2$ globin genes have been isolated and characterized from a library of DNA fragments generated by partial Eco RI digestion of cellular DNA using charon 4A phage as vector. Two of the clones (NY 2 and 3) are identical and have an insert of 14.0 kb. The third clone (NY 1) has a 15.4 kb insert by virtue of an extra 1.4 kb Eco RI fragment at its 5' most end. This clone also has a Kpn I site not present in the other two suggesting it is the product of the $\gamma$ gene on the opposite chromosome. Restriction analysis of the three clones indicates that the $G_\gamma$ and $A_\gamma$ genes are linked on a single continuous piece of DNA and are separated by 3.5 kb and each contains at least one large intervening sequence of 0.85 kb between the Bam HI and Eco RI sites. These findings in cloned DNA provide direct evidence for linkage and organization of the $\gamma$ genes in man.

INTRODUCTION

The predominant hemoglobin during human gestation is fetal hemoglobin (HbF, $a_2\gamma_2$), a protein consisting of two $\gamma$ chains, $G_\gamma$ and $A_\gamma$, differing by one amino acid at position 136 (gly-$\alpha$la). After birth, there is a dramatic switch from the production of HbF to adult hemoglobin (HbA, $a_2b_2$). Hemoglobin $A_2$, (HbA2, $a_2b_2$), a minor component, is the other hemoglobin in adult blood. Individuals affected by the $\beta$ thalassemia syndromes, a group of anemias of man, show a marked decrease or a total absence of $\beta$ globin chain production with an increase in $\gamma$ globin. In the severe form of these disorders, $\gamma$ globin expression, although increased, does not compensate for the deficiency of $\beta$ globin chains, and severe anemia results. However, in some rarer forms of these disorders, $\gamma$ globin synthesis can fully compensate for the absence of $\beta$ chains and eliminate the pathological effects of decreased or absent $\beta$ globin production (1). With this in mind, studies of the structure of the $\gamma$ globin genes may provide important insights into the mechanisms by which $\gamma$ globin gene expression is regulated, and may lead to
new approaches to ameliorating the anemia of thalassemia.

By cell fusion and molecular hybridization studies it has been shown that γ-δ-β genes are linked together on chromosome 11 (2). Recently, application of restriction enzyme analysis using the Southern technique has allowed several groups to define in detail the restriction map of the globin genes from cellular DNA of normal and thalassemic individuals, and to correlate deletions in the region of the δ genes with the extent of γ chain compensation in certain disorders (3-6).

The feasibility of isolating unique genes from unfractionated cellular DNA by cloning in phage vectors has permitted us to obtain direct evidence for the linkage of the two human γ globin genes in a manner analogous to that already demonstrated for the human δ and β globin genes (7,8). In this paper we report the isolation and characterization of three clones (NY 1, NY 2, and NY 3), each containing two γ genes on the same DNA fragment in this order: 5'→γ to Aγ→3', separated from each other by approximately 3.5 kb. Each of the two genes is interrupted by at least one intervening sequence (intron) 850 nucleotides long between the Bam HI and Eco RI sites within the structural gene sequences and between codons 100 and 121. These two introns appear to be closely related if not identical, unlike those in the δ and β genes. The three clones extend 2.7 kb from the 3' end of the Aγ gene. Two of them (NY 2 and NY 3) are identical by all structural analysis and will be referred to subsequently as one (NY 2). NY 2 extends 5.2 kb from the 5' end of the Gγ gene, while NY 1 extends 1.4 kb further 5'. We also show a polymorphism in the restriction site of at least one enzyme (Kpn I) demonstrating that the two clones are derived from opposite chromosomes. The clones are all oriented within the charon 4A phage in the same fashion: the 3' end of the cellular DNA is attached to the left arm of the vector, and the 5' end to the right arm.

MATERIALS AND METHODS

a. Construction of a Human Gene Library:

A human gene library was constructed by partially digesting cellular DNA from a patient with β⁺ thalassemia with Eco RI and isolating 15-20 kb DNA fragments. These fragments were ligated to charon 4A DNA and re-infected into E. Coli (7,8). The library was screened with α, β and γ globin cDNA probes. JW 101, 102, and 151 were kindly provided by Dr. John Wilson (9). The clones described in this paper all hybridized to γ cDNA and not to β cDNA. No clones were found containing both γ- and β-like genes. Screening and purification of the clones was accomplished as described previously (7,8).
Purified γ gene-containing clones were grown in a P2 facility as required by the Recombinant DNA Guidelines of the NIH.

b. Restriction Endonuclease Analysis of Cloned DNA:

Agarose gels 0.7 to 1.2% were used to separate restricted DNA fragments. Restriction enzymes were obtained largely from New England Biolabs. Partial digests were avoided by adding 2-3 fold excess of enzyme over that required to completely digest similar amounts of λ DNA. A Hind III digest of λ DNA was used as markers for measuring DNA fragment sizes. Transfer of DNA gels to nitrocellulose filters, hybridization, washing, and radioautographic analysis of filters were as described (4). γ cDNA was prepared by digesting the JW151 plasmid clone with HhaI and separating the 1.6 kb fragment containing the γ globin sequences on a 7.5 - 25% sucrose gradient in 50mM Tris pH 7.4, 0.15M NaCl, 3mM EDTA by centrifugation at 4°C for 20 hrs at 39,000 rpm in a SW41 rotor. To prepare specific 5' and 3' end γ probes (5'), (3') the JW151 plasmid clone was digested with Bam HI which cuts the γ cDNA at position 100. Two fragments 5.0 kb, and 0.9 kb in length were isolated on sucrose gradients. The 0.9 kb fragment was then characterized as being the 3' end probe because it was shortened by subsequent digestion with Eco RI which cuts the γ cDNA at codon 120.

For the preparation of the intron probe clone Hs 51.1 (a gift from Dr. Blattner) was first digested with Eco RI. This clone has been shown to contain a 2.6 kb Eco RI fragment of the γ gene including its intervening sequence (8). This intron, 1.0 kb in length, was isolated by sucrose gradient centrifugation, redigested with Bam HI and repurified on a second sucrose gradient. All the probes were labelled by nick translation as described (11) to a specific activity of 2-4 x 10^8 cpm/μg.

c. Restriction Endonuclease Analysis of Cellular DNA

High molecular weight cellular DNA was extracted as previously described (4,5) from Epstein-Barr virus-transformed lymphocyte cell lines and spleen and white blood cell DNA of individuals without thalassemia as well as from those homozygous for hemoglobin Lepore, θθ thalassemia, hereditary persistence of fetal hemoglobin (HPFH), and θ+ and θ° thalassemia. The DNA was digested, subjected to agarose gel electrophoresis, transferred to nitrocellulose filters, and hybridized as previously described (4,5) to 32P labelled γ cDNA probe (JW151).
RESULTS AND DISCUSSION

a. Eco RI fragments in the γ clones:

The clones are the products of ligating human DNA partially digested with Eco RI to charon 4A phage arms. Complete Eco RI digestion yields the two phage arms (10.5 and 20 kb in size), and other Eco RI fragments corresponding to the cellular DNA inserts (Table I and Figure I). The total length of the inserted cellular DNA in the two clones can be calculated by adding the length of these fragments, and is 15.4 and 14.0 kb in NY 1 and NY 2, respectively.

Analysis of the clones, using the enzyme Kpn I, confirm their size, and indicate that they are the products of two different chromosomes. When NY 2 is digested with Kpn I, blotted and hybridized with the structural γ probe, only one band 22.4 kb long is seen. By contrast NY 1 gives two bands 14.5 kb and 9.3 kb long after Kpn I digestion (Figure 2). These results indicate a polymorphism involving a Kpn I site, and shows that NY 2 and 3 are derived from two different γ loci. The Kpn sites in the two arms of the vector are known to be 7.35 kb and 1.0 kb away from the ends at which cellular DNA is inserted. Therefore, the total size of the clones can be deduced: NY 1 (14.5 + 9.3 - 7.35 - 1.0 or 15.45 kb) and NY 2 (22.4 - 7.35 - 1.0 or 14.05 kb). These sizes are in good agreement with the values derived from the Eco RI fragments.

b. Orientation of Cloned DNA:

We oriented the cellular DNA inserts with respect to the phage arms, by digestion with Bam HI and Bgl II, exploiting the asymmetry of the sites for

<table>
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<tr>
<th>EtBr</th>
<th>(S)</th>
<th>(5')</th>
<th>(3')</th>
<th>(I)</th>
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<tbody>
<tr>
<td>NY1</td>
<td>NY2</td>
<td>20</td>
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<td>10.5</td>
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<td>6.2</td>
<td>6.2</td>
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<td>0.55</td>
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<tr>
<td>0.25</td>
<td>0.25</td>
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Summary of the Eco RI fragments of γ globin clones analyzed by ethidium bromide staining and by hybridization to structural (S), 5' end (5'), 3' end (3') and intron (I) γ probes.
Fig. 1. Eco RI cleavage patterns of the human \(\gamma\) globin gene clones. Ethidium bromide staining of a 1% agarose gel electrophoresis, in Lane 2 clone NY 1; in lane 1 clone NY 2. Lanes 3 and 4 represent the autoradiogram of the gel shown in lane 1 and 2, transferred to a nitrocellulose filter and hybridized to \(P^{32}\)-\(\gamma\) cDNA probe (specific activity: 10\(^{6}\) cpm/\(\mu\)g).

these enzymes within the phage arms (Table II, Figures 2 and 3). The 5.1 kb Bam HI fragment which hybridizes to both 5' and 3' \(\gamma\) cDNA probes, presumably contains the 5' end of one \(\gamma\) gene and the 3' end of the other and links the 18 kb 3' ended, and the 2.8 kb 5' ended Bam HI fragments (Figure 3) indicating that two \(\gamma\) genes are present in tandem in the two clones. An alternative explanation that the 5.1 kb fragment represents one or more complete \(\gamma\) globin genes is unlikely, since analysis of double stranded \(\gamma\) cDNA (9) shows that both \(G_{\gamma}\) and \(A_{\gamma}\) structural genes contain a Bam HI site at codon 100. Total NY 1 cloned DNA was also used as probe to identify charon 4A fragments as well as structural and flanking cellular DNA sequences (Figure 4). In NY 1, one additional Bam HI fragment is found; this fragment is 9.4 kb in size, while in NY 2, it is 8 kb in size, consistent with the map shown in Figure 5.

c. Organization of the Eco RI fragments:

Two of the Eco RI fragments 6.2 and 2.5 kb in size hybridize to 5' ended
Fig. 2. Autoradiogram of restriction fragments of human γ globin gene clones electrophoried in a 0.5% agarose gel, transferred to nitrocellulose filters and hybridized to $^{32}$P-γ cDNA probe. (1) Kpn I digest of NY 2 (2) Kpn I digest of NY 1 (3) Bam HI digest of NY 2 (4) Bam HI digest of NY 1 (5) Bam HI/Bgl II double digest of NY 2 (6) Bam HI/Bgl II double digest of NY 1 (7) Bgl II digest of NY 2.

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<thead>
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<th>Bam HI</th>
<th>Bgl II</th>
<th>Bam HI/Bgl II</th>
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<tr>
<td>18 kb (3',I)</td>
<td>30 kb (3',5',I)</td>
<td>18 kb (3',I)</td>
</tr>
<tr>
<td>5.1 kb (3',5',I)</td>
<td>5.1 kb (3',5',I)</td>
<td></td>
</tr>
<tr>
<td>2.8 kb (5')</td>
<td>2.4 kb (5')</td>
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Summary of the fragments of NY1 and NY2 generated after digestion with Bam HI, Bgl II alone and in combination and hybridization to γ structural sequences. In parenthesis is their characterization after hybridization to 3' end γ probe (3'), 5' end γ probe (5') and Aγ intron probe (I).
Fig. 3. Schematic representation of the restriction fragments generated by digestion with Bam HI (B) and Bgl II (Bg) alone and in combination of a human γ globin gene-containing phage vector. In the scheme the orientation of the inserted cellular DNA is opposite to the 5'→3' direction of transcription of the phage arms. The numbers represent the fragment sizes expressed in kilobases (kb).

and intron probes (Table III). The ordering of the Bam fragments (Figure 3) strongly suggest that the 6.2 kb 5' ended Eco RI fragment (Table I) contains the 5' end of the leftward most γ gene; it is too large to be derived from the 5' end of the right-hand gene without also containing 3' γ sequences. The 18 kb Bam HI fragment was isolated by sucrose gradient centrifugation and digested with Eco RI to yield: 1) A 14.0 kb fragment containing the left phage arm and confirming that this arm is correctly oriented (Fig. 3 and 5) (the right phage arm is only 10.5 kb in total size); 2) A 0.55 kb fragment hybridizing to 3' γ probe, indicating that this 3' fragment is part of the rightward or 3' most γ structural gene (Fig. 5); 3) A 0.85 kb fragment hybridizing only to intron probe, indicating the presence of a large intron within the γ structural sequences between codons 100 and 121; 4) A 2.2 kb fragment, which does not hybridize to either γ cDNA and does not represent phage arm, must be the 3' flanking sequence as shown in Figure 5.

Similarly, the 9.4 kb Bam HI fragment of NY 1 and the 8.0 kb Bam HI fragment in NY 2 were isolated and redigested with Eco RI (Table IV, Figure 5). The fragments generated by the two clones differ by only a 1.4 kb fragment, which does not represent phage arm (since it is not present in the charon 4A arm DNA digest) or structural gene. Since all of the other Bam HI/Eco RI fragments are identical in NY 1 and NY 2, it is most likely an extra Eco RI fragment between the 5' end of the inserted cellular DNA and the right phage arm.
Fig. 4. Analysis of the Bam HI digest of the human $\gamma$ globin gene clones and charon 4A arms. Autoradiogram of a 0.7% agarose gel electrophoresis transferred to nitrocellulose filter and hybridized to $^{32}$P-$\gamma$ cDNA probe (Lanes 1-4) and to $^{32}$P-NY 1 total clone probe (Lanes 5-8) (s.a.: $6 \times 10^7$ cpm/ug) 1 and 5: NY 1; 2 and 6: NY 2; 3 and 7: NY 3; 4 and 8 charon 4A arms.

<table>
<thead>
<tr>
<th>Bam HI</th>
<th>Eco RI</th>
<th>Bam HI/Eco RI</th>
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<tbody>
<tr>
<td>18 kb (3',I)</td>
<td>6.2 kb (5',I)</td>
<td>2.8 kb (5')</td>
</tr>
<tr>
<td>5.1 kb (3',5',I)</td>
<td>2.5 kb (5',I)</td>
<td>1.55 kb (3')</td>
</tr>
<tr>
<td>2.8 kb (5')</td>
<td>1.5 kb (3')</td>
<td>1.5 kb (5')</td>
</tr>
<tr>
<td></td>
<td>0.55 kb (3')</td>
<td>0.85 kb (I)</td>
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<tr>
<td></td>
<td></td>
<td>0.55 kb (3')</td>
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Summary of the Bam and Eco RI $\gamma$ structural fragments hybridized with different probes indicated in parenthesis: 5' end $\gamma$ cDNA (5'), 3' end $\gamma$ cDNA (3') and $\gamma$ intron probe (I).
Fig. 5. Schematic representation of the restriction map of NY 1 and NY 2 generated by Eco RI (E) and Bam HI (B) alone and in combination. The numbers represent the fragment sizes expressed in kilobases (kb). The asterisk at the 5' foremost end of the cellular DNA insert of NY 1 represents the extra 1.4 kb Eco RI piece of this clone with respect to NY 2. In the Bam HI digest this extra 1.4 kb of cellular DNA increases the size of the 8.0 kb Bam HI fragment to 9.4 kb in NY 1.

The other Bam HI/Eco RI fragments generated from the 8.0 and 9.4 Bam fragments are: 1) A 5.0 kb fragment representing the right phage arm since it is not present in the phage DNA digest and does not hybridize to γ cDNA. 2) A 0.25 kb fragment which does not hybridize to γ cDNA and is not phage DNA and must be a 5' flanking sequence as in Figure 5; 3) A 2.3 kb fragment, also contains 5' flanking sequences (Figure 5). The location of the 2.3 and 0.25 kb fragments with respect to each other (Fig. 5) is derived from the facts that (1) the 0.25 kb fragment is present with Eco RI digestion alone as well as with Bam HI/Eco RI double digestion, and (2) the 2.3 kb Bam HI fragment when added to the 2.8 and 0.85 kb Bam HI/Eco RI 5' fragments comprise the 6.2 kb Eco RI fragment (Figure 5). If the position of the 0.25 kb and 2.3 kb fragments were reversed, then the total length of the 5' Eco RI fragment would be too short to account for its measured size.

<p>| TABLE IV |</p>
<table>
<thead>
<tr>
<th>NY1-NY2 (Bam HI 18 kb)</th>
<th>NY1 (Bam HI 9.4 kb)</th>
<th>NY2 (Bam HI 8.0 kb)</th>
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<tbody>
<tr>
<td>14.0 kb (λ L. arm)</td>
<td>5.0 kb (λ R. arm)</td>
<td>5.0 kb (λ R. arm)</td>
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<tr>
<td>2.5 kb (flanking)</td>
<td>2.3 kb (flanking)</td>
<td>2.3 kb (flanking)</td>
</tr>
<tr>
<td>0.55 kb (3')</td>
<td>1.4 kb (flanking)</td>
<td>1.4 kb (flanking)</td>
</tr>
<tr>
<td>0.85 kb (I)</td>
<td>0.25 kb (flanking)</td>
<td>0.25 kb (flanking)</td>
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Summary of the fragments generated by restriction with Eco RI of purified Bam HI fragment. In parenthesis is the nature of the sequences. The 9.4 kb was derived from NY1, the 8.0 kb from NY2. The 18 kb gave identical results in both clones.
The other Bam HI/Eco RI double digest fragments, not represented in the 18 and 8 kb Bam fragments, must be generated from the 5.1 and 2.8 kb Bam fragments. Since the 2.8 kb fragment remains unchanged with double digestion, all of the smaller fragments must be derived from the 5.1 kb fragment. One of these fragments, 1.55 kb, hybridizes to the 5' cDNA probe, and must represent the 5' end of the rightward most \( \gamma \) gene. This is supported by evidence presented earlier locating the 2.8 kb fragment at the 5' end of the leftward most gene (Fig. 3,5). Another fragment, 1.5 kb in size, generated by both Eco RI alone and by Eco RI/Bam HI, hybridizes to the 3' end \( \gamma \) cDNA probe, and is consistent with this being the 3' end of the leftward most gene (Fig. 5). The remainder of the 5.1 kb fragment consists of the 0.75 kb Eco RI fragment, which does not hybridize to \( \gamma \) cDNA, and must be an intragenic sequence (Figure 5), and an intron of 0.85 kb, which is known to be present within the 5.1 kb fragment (Table III & Fig. 3,6). Thus, the 5.1 kb Bam HI fragment contains four Eco RI Bam HI double digest fragments, totalling roughly 4.5 to 4.6 kb, well within the error of measurement of the 5.1 kb fragment.

Data previously discussed suggest that the 2.8 kb Bam HI fragment is derived from the 6.2 kb Eco RI fragment since both contain the 5' end of the leftward most \( \gamma \) gene (Fig. 3,5). This organization is further supported by the finding that the other 5' ended Eco RI fragment is only 2.5 kb long, and could not generate a 2.8 kb Bam HI/Eco RI fragment.

d. The \( \gamma \) Genes Map

The 2.5 kb Eco RI fragment (Table I, Figure 5) has been shown to represent the 5' end of the \( A_\gamma \) gene by partial sequence analysis (10). Since the 5' ended Bam HI/Eco RI fragment of the leftward most gene of both of our clones is 2.8 kb long, it cannot be generated from this 2.5 kb \( A_\gamma \) Eco RI fragment. Thus, the 2.5 kb Eco RI fragment and the \( A_\gamma \) gene must be the rightward most \( \gamma \) gene on our map (Figure 5). The map shown in Figure 5 also indicates that the two \( \gamma \) genes are about 3.5 kb apart.

The analysis of these \( \gamma \) clones shows that the two \( \gamma \) genes are linked in the order 5'-3', \( G_\gamma A_\gamma \) on a single contiguous fragment of DNA. The results thus provide the first direct evidence for physical linkage of the two \( \gamma \) genes. The physical arrangement of the four Eco RI \( \gamma \) globin gene-containing fragments in the order 5'-3'-5'-3' (Table I, Fig. 5) rules out the possibility of a single \( \gamma \) globin gene with multiple intervening sequences to explain the data. The data are consistent with those obtained by restriction endonuclease analysis of unfractionated cellular DNA (12,13).
e. Characterization of The Intervening Sequences of The Two γ Genes:

An intron probe was prepared, as described in the Materials and Methods, from a clone containing only part of the Aγ gene. In one experiment it was hybridized to NY 1 and NY 2 DNA digested with either Bam HI and Eco RI alone and in combination (Figure 6). Two Eco RI and Bam HI bands were found to hybridize with NY 1 (Table III). On the basis of the linear map shown in Fig. 5 one of these bands is from the Gγ gene, and the other from the Aγ gene. The intense hybridization of the Gγ intron with the Aγ intron probe indicates strong homology of the introns of these two γ genes. By contrast, when the γ intron probe was hybridized to HBG1 DNA fragments (8) a clone containing the structural δ and β globin genes, no fragments were visualized, thus demonstrating the lack of significant homology between the introns of fetal and adult β-like genes.

Fig. 6. Analysis of the Eco RI and Bam HI digests alone and in combination of the human γ globin gene clones. Autoradiogram of a 0.75% agarose gel electrophoresis transferred to nitrocellulose filter and hybridized to P2′-Aγ intron probe (s.a: 5 x 10^5 cpm/ g) Lane 1-3, Eco RI digest of the three NY clones, Lines 4-6 Bam HI digest of the three NY clones, lines 7-9 double digest Eco RI/Bam HI of the three NY clones.
To further establish the homology of the $G_Y$ and $A_Y$ intron sequences, we utilized a previously described method, which has been shown to melt out partial hybrids by sequential washes of the nitrocellulose filters with decreasing salt (14). Nitrocellulose strips containing NY 1 cloned DNA digested with Eco RI were prepared, hybridized to $A_Y$ intron probe and then washed sequentially with decreasing salt (Figure 7). The hybrids are very stable; the probe hybridized intensely to the 2.5 kb Eco RI fragment of the $A_Y$ gene as well as to the 6.2 kb fragment from the $G_Y$ gene even at very high stringency, indicating that the two introns are very closely related, if not identical.

Fig. 7. Analysis of the homology of the $G_Y$ and $A_Y$ introns. Eco RI digest of NY 1 clone DNA was electrophorised in 0.8% agarose gel, transferred to nitrocellulose filter, hybridized to $P_i^{32}$-labeled $A_Y$ intron probe and washed at 65°C in decreasing concentration of salt. Lane 1: 0.1 x SSC; lane 2: 0.05 x SSC; lane 3: 0.025 x SSC; lane 4: 0.012 x SSC; lane 5: 0.006 x SSC, lane 6: 0.003 x SSC (1 x SSC: 0.15 M NaCl, 15mM citrate, pH 6.8).
f. Use of Other Enzymes And Restriction Mapping of Cellular DNA:

We have used the same approach described with Eco RI and Bam HI to further characterize our clones with other restriction enzymes. After restriction, the DNA fragments were blotted and hybridized to (S), (5'), (3'), (I), and (C) probes, using charon 4A arms as internal control; the Bam HI fragments isolated by sucrose gradients were further digested with other restriction enzymes; the resulting fragments were analyzed by ethidium bromide staining of agarose gels. Additional restriction enzyme sites were determined by these studies (Figure 8).

Our clones have defined the organization of the two $\gamma$ genes, with respect to each other, but have not been useful in determining the distance between the $\alpha_Y$ and $\delta$ genes. We and others have shown previously by restriction enzyme analysis of cellular DNA that these genes are approximately 15 kb apart (6,13,15). We have also provided evidence that the region either within or 5' to the $\delta$ structural gene, is involved in $\gamma$ globin gene regulation (4,5,16,17). In $\delta\beta$ thalassemia and hereditary persistence of fetal hemoglobin (HPFH), two disorders in which the $\delta$ and $\beta$ structural genes are deleted, $\gamma$ globin biosynthesis compensates for the absent $\beta$ synthesis. By contrast, in $\beta^+$ and $\beta^0$ thalassemia, associated with inadequate $\gamma$ globin

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\begin{align*}
5' & \rightarrow 3' \\
\end{align*}
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**Fig. 8.** Restriction endonuclease sites in NY 1 and NY 2 cellular DNA insert. The locations of the cleavage sites of the following restriction enzymes are represented: Eco RI (E), Bam HI (B), Bgl II (Bgl), Xpn (K), Pst I (P), Xba I (X), Hpa I (H). The direction of transcription is indicated by the arrow at the top of the diagram. The 1.0 kb piece at the bottom represents the scale of the map. The additional Eco RI 5' foremost fragment present in NY 1 is shown separately in the lower left corner. The Kpn I site is present only in one of the two clones (NY 1) and therefore is indicated in parenthesis in the figure.
synthesis and severe anemia, there is preservation of the δ gene, and its adjacent regions. Restriction enzyme analysis of cellular DNA from patients with these disorders, using γ cDNA, shows no differences in γ gene organization or number to account for the marked differences in γ globin biosynthesis (Figure 9).

SUMMARY AND CONCLUSIONS:

We have isolated clones from a library of human genes, which contain γ genes, and have characterized these clones by restriction endonuclease analysis. Each of the clones contains two γ genes linked 5′ to 3′ as \( \Gamma \) to

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Fig. 9. Restriction fragments containing γ globin genes seen in Eco RI cleaved total cellular DNA. 1: normal DNA, 2: HPFH DNA; 3: δδ thalassemia DNA; 4: Lepore DNA; 5: δδ thalassemia DNA; 6: δ+ thalassemia DNA. 20 ug of gel, transferred to nitrocellulose filter and hybridized to P\(^{32}\)-γ cDNA probe (s.a: 2 x 10\(^5\) cpm/ug). In this particular experiment the 0.55 kb band was not visible.
Aγ. The two γ genes are 3.5 kb apart. A detailed physical map of the human fetal genes has been obtained, using the cloned fragments of human DNA. This is the first direct evidence of linkage of the two human γ genes on a single DNA fragment. The map does not vary significantly from those generated, using restriction enzyme analysis of cellular DNA (6,12,13,15). The present studies also extend the gene map of γ fragments 2.8 kb from the 3' end of the Aγ gene and 6.6 kb from the 5' end of the Gγ gene; the embryonic gene(s) may be found in this latter region. Subclones generated from our γ clones should provide suitable probes for exploring this possibility, as well as for further defining the sequences within and flanking the γ globin genes, by direct nucleotide sequencing.

ACKNOWLEDGMENT

We thank Dr. Gek Kee Sim for many helpful discussion, Ms. Pamela Schwarsberg for her technical assistance and most of all Mr. Michael R. Stein for his patience and support. These studies were supported by NIH Grants GM-23143 and AM-25274 and NSF Grant 24120.

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REFERENCES