Organization of the α-Globin Genes in the Chinese α-Thalassemia Syndromes

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ABSTRACT The α-thalassemia syndromes are a group of inherited anemias, the clinical severity of which has been shown to increase with the number of α-globin structural genes deleted. Employing restriction endonuclease gene mapping, we defined the organization of the α-globin genes in cellular DNA from Chinese subjects with various α-thalassemia syndromes. The four α-globin genes of normals are at two loci located on a 23.0-kilobase pair (kb) Eco RI fragment. In deletion type hemoglobin-H disease the 5' α-globin locus is deleted and the single 3' α-globin locus is found on a 19.0-kb Eco RI fragment. In α-thalassemia-2 there are two α-globin genes on a 23.0-kb Eco RI fragment and one on a 19.0-kb fragment. In α-thalassemia-1 and the nonelevation type of hemoglobin-H disease the two α-globin genes are at two loci on one chromosome and none reside on the other chromosome.

INTRODUCTION

The α-thalassemia syndromes are a group of inherited anemias usually caused by deletion of the α-globin structural genes (1, 2). In the Asian population, the clinical severity of the α-thalassemia syndromes increases with the number of the α-globin genes deleted. Nonthalassemic individuals have four copies of α-globin genes per diploid genome, whereas α-thalassemia trait (α-thalassemia-1) subjects have two, deletion type of hemoglobin-H disease subjects have one, and homozygous α-thalassemia (hydrops fetalis) subjects have none (3). It is known indirectly from family studies that silent carrier (α-thalassemia-2) subjects have three α-globin genes (4). An exception to this pattern is the nonelevation type of hemoglobin-H disease, in which two α-globin genes are present, but which is clinically as severe as the deletion type of hemoglobin-H disease where only one α-globin gene persists (5).

We used the restriction endonuclease mapping technique of Southern (6) to define these syndromes further. In this method, cellular DNA is first digested with one or more restriction endonucleases that cleave DNA at a specific nucleotide sequence. The DNA fragments are separated according to size by agarose gel electrophoresis and transferred from the gel onto nitrocellulose filters. The filters are hybridized with a radioactive DNA sequence that is complementary to the α-globin gene. The α-globin gene-containing DNA fragments can then be detected by autoradiography. With this method we define the α-globin genes in the α-thalassemia syndromes.

METHODS

DNA was prepared from leukocytes, placentas, cultured fibroblasts, and cultured Epstein-Barr virus-transformed B lymphocytes (7) of normal α-thalassemia-2, α-thalassemia-1, deletion type of hemoglobin-H disease, nonelevation type of hemoglobin-H disease (5), and hydrops fetalis subjects, as described (8). Aliquots of 7-10 μg DNA were digested at 37°C for 4-7 h with the restriction enzymes Eco RI, Hpa I, Hin dIII, or their combination, according to conditions specified for each enzyme by the manufacturer (New England Biolabs, Beverly, Mass.).

The digested DNA samples were electrophoresed and transferred onto nitrocellulose filters (Millipore Corp., Bedford, Mass. or Schleicher & Schuell, Inc., Keene, N. H.) by a method modified from Southern (6, 8). The filters were hybridized and autoradiographed and the complementary DNA (cDNA) prepared as described (8).

3'-specific, 5'-specific, and total α-globin gene probes were prepared from hybrid plasmid JW 101 that contains a synthetic copy of the human α-globin gene in pMB 9 (9). The plasmid was cultured and isolated (10) according to NIH guidelines. For total α-probe a 1.6-kilobase pair (kb) DNA fragment con-

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taining all the α-globin cDNA sequences was excised by digestion with Mbo II according to the conditions specified by the manufacturer (New England Biolabs) and isolated by agarose gel electrophoresis (11). To prepare 3'- and 5'-specific globin gene probes, we digested the JW 101 plasmid with Hin dIII and isolated by agarose gel electrophoresis (11) the 0.5-kb fragment that contained the 3' α-globin gene sequences and the 5.2-kb fragment which contained the 5' α-globin gene sequences.2 These plasmid fragments were labeled with 32P by nick translation to a 1–4 × 106 sp act cpm/μg DNA. The 200-μl reaction mixture contained 0.5 μg plasmid DNA and 12.5 U DNA polymerase I (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) in 50 mM Tris-HCl, pH 7.8, 5 mM MgCl2, 10 mM 2-mercaptoethanol, 50 μg/ml gelatin, 5 μM [α-32P]dCTP and [α-32P]dTTP (New England Nuclear, Boston, Mass. or Amersham Corp., Arlington Heights, Ill.) and 15 μM unlabeled dATP and dGTP, and was incubated at 22°C for 1–4 h. The reaction mixture was made 20 mM with EDTA and the radioactive DNA separated from unincorporated nucleotides by chromatography on Sephadex G-50 (Pharmacia Fine Chemicals, Piscataway, N. J.).

RESULTS

When normal DNA was digested with Eco RI, (Fig. 1A) a DNA fragment of ≈23.0 kb fragment containing the α-globin genes was generated. The same size fragment was also obtained from digestion of the DNA of α-thalassemia-1 and the nondeletion type of hemoglobin-H disease. This DNA fragment contained both α-globin loci (12). Thus, nonthalassemic subjects had such a fragment on both chromosomes, whereas Chinese subjects with α-thalassemia-1 and the nondeletion type of hemoglobin-H disease had these two loci on a single chromosome. In the deletion type of hemoglobin-H disease, the single remaining α-globin gene was found on a fragment about 19.0 kb in length. In α-thalassemia-2 both the 23.0- and 19.0-kb fragments were present, a result compatible with the presence of two α-loci on one chromosome and one on the other. Both these fragments contained 5' and 3' sequences (Table I). We did not detect any DNA that reacted with the α-probe in homozygous α-thalassemia.

When normal DNA was digested with the enzyme Hpa I, two α-specific fragments, 14.5 and 4.1 kb in length, were generated (Fig. 1B). Each contained both 5' and 3' sequences (Table I), and therefore the complete α-globin gene. Both fragments were seen in DNA from the α-thalassemia-2, α-thalassemia-1 and the nondeletion type of hemoglobin-H disease. In contrast, only the 14.5-kb fragment was found in the deletion type of hemoglobin-H disease. We observed some variation in the size of the larger fragments, a result most likely due to a polymorphism and apparently unrelated to thalassemia.3

Digestion of the normal DNA with Hin dIII yielded three α-globin gene-containing fragments, 17.0, 4.5, and 3.7 kb in length (Fig. 1C). Because Hin dIII is known to cut within the α-globin structural gene sequences (between the codons corresponding to amino acids 90 and 91) (13), the finding of three fragments confirmed the duplication of the α-loci. Table I shows that the 17.0 kb fragment hybridized only with the 5' probe, the 4.5-kb fragment hybridized only with the 3' probe, and the 3.7-kb fragment hybridized with both probes. This result is compatible with the interpretation that the 3.7-kb fragment contains the 3' end of the 5' α-locus, the DNA in the intergenic region, and the 5' end of the 3' α-locus. This fragment could be called the “bridging fragment” and defines the distance between the two α-loci. In α-thalassemia-2, α-thalassemia-1, and the nondeletion type of hemoglobin-H disease the same three fragments were seen. In the deletion type of hemoglobin-H disease a DNA fragment of about the same size as the bridging fragment was missing and only a 17.0-kb fragment containing the 5' α-sequences and a 4.5-kb fragment containing the 3' α-sequences was seen.

To clarify the nature of the deletion, a series of double digests was performed to locate these restriction sites relative to one another. These filters were hybridized with 5' specific, 3' specific, or total α-probes to establish the

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2 Forget, B. Personal communication.
TABLE I
Sizes and Orientation of α-Specific DNA Fragments 
Generated by Various Restriction Endonucleases

<table>
<thead>
<tr>
<th>Enzymes*</th>
<th>Fragment size (kb)</th>
<th>Normal DNA</th>
<th>Deletion type DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eco RI</td>
<td>23.0</td>
<td>5' + 3'</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>19.0</td>
<td>—</td>
<td>5' + 3'</td>
</tr>
<tr>
<td>Hpa I</td>
<td>4.1</td>
<td>5' + 3'</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>14.5</td>
<td>5' + 3'</td>
<td>—</td>
</tr>
<tr>
<td>Hin dIII</td>
<td>17.0</td>
<td>5'</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3.7</td>
<td>5' + 3'</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>3'</td>
<td>—</td>
</tr>
<tr>
<td>Hin dIII + Eco RI</td>
<td>17.0</td>
<td>5'</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3.7</td>
<td>5' + 3'</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>3'</td>
<td>—</td>
</tr>
<tr>
<td>Eco RI + Hpa I</td>
<td>4.1</td>
<td>5' + 3'</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>5' + 3'</td>
<td>5' + 3'</td>
</tr>
<tr>
<td>Hin dIII + Hpa I</td>
<td>3.4</td>
<td>5'</td>
<td>—</td>
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<tr>
<td></td>
<td>0.7</td>
<td>3'</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>5'</td>
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</tr>
<tr>
<td></td>
<td>4.5</td>
<td>3'</td>
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</tbody>
</table>

* The enzyme or combination of enzymes employed to generate the α-specific DNA fragments whose size is listed. 

The orientation of the DNA fragments as determined by hybridization with the 3'- and 5'-specific α-probes.

5'-3' orientation of each fragment, and the results are shown in Table I and in Fig. 2. The Hin dIII + Eco RI digest showed the size and orientation of the 17.0- and 3.7-kb Hin dIII fragments to be unchanged, but the 3'-4.5-kb Hin dIII fragment was cleaved by Eco RI to a 1.8-kb fragment. The Hpa I + Eco RI digest revealed the 4.1-kb Hpa I fragment to be unchanged, but the 14.5-kb Hpa I fragment was cleaved by Eco RI to a 4.8-kb fragment that contained both 5' and 3' sequences. The Hin dIII + Hpa I digest showed the 3'-4.5-kb Hin dIII fragment to be unchanged, the 5'-17.0-kb Hin dIII fragment to be cleaved by Hpa I to a 3.4-kb fragment and the 3.7-kb Hin dIII bridging fragment to be cleaved by Hpa I to a 0.7-kb fragment that hybridized only with the 3' probe and a 3.0-kb fragment that hybridized only with the 5' probe. In deletion type hemoglobin-H disease the 3.4- and 0.7-kb fragments were missing. Because these two deleted fragments were derived from the 4.1-kb or 5' Hpa I fragment, it was the 5' α-globin gene that had been deleted. The absence of the 3.7-kb fragment on the single Hpa I digest indicates that one of the two Hpa I sites located on either side of the 5' α-globin gene was included in the deletion.

**DISCUSSION**

In this paper we have extended our preliminary observations on the organization of the α-globin structural genes in normal and the Chinese α-thalassemia syndromes (14). Our results were in agreement with those of Orkin (12) on the arrangement of the two normal α-globin loci and that the distance from the Hin dIII site of one locus to that of the other was 3.7 Kb. In addition, by using specific 5' and 3' sequences as probes, we unambiguously establish the 5' and 3' orientation of the α-loci.

These results also establish the nature of the deletion that result in the single α-globin locus in deletion type hemoglobin-H disease. Gene mapping showed that a piece of DNA that contains the 5' α-globin locus is deleted. The deletion results in the loss of the 4.1-kb Hpa I and the 3.7-kb Hin dIII fragments seen in normals.

These findings provide a method for detection of the α-thalassemia-2 gene. Thus, the previously hematologically undetectable silent carrier state can be diagnosed by the findings of two α-globin fragments on Eco RI digests and could be represented by the genotype (-α/aa). Also, the two molecular arrangements giving rise to the α-thalassemia-1 phenotype could also be distinguished. If both α-globin genes on the same chromosome are deleted (-/aa), Eco RI and Hpa I will show the normal pattern. On the other hand, if the α-thalassemia-1 phenotype is a result of the deletion of the two 5' α-globin loci, (homozygous α-thalassemia-2 or -α/-α), Eco RI digest will show only the 19.0-kb band and Hpa I only the 14.5-kb band. The finding of the -/αα genotype in Chinese α-thalassemia-1 subjects explains the occurrence of homozygous α-thalassemia in this group. This disease is not found in the black population because the α-thalassemia-1 phenotype is a result of the deletion of the two α-globin loci on opposite chromosomes (-α/-α) (15).

These results also confirm the findings previously

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shown by liquid hybridization that both α-globin genes loci are intact in the nondeletion type of hemoglobin-H disease (5). Our results from mapping with these and other enzymes have not detected any difference between this genotype and the normal. However, possible differences may be found by more extensive restriction mapping, or by cloning these genes for further structural and functional analysis.

The findings described have already been shown to be of practical clinical value. The sensitivity of this technique allows it to be used as a more rapid means of prenatal diagnosis (16). The detection of the hemoglobin-H genotype in a patient with sickle cell trait permitted the correct diagnosis that had previously not been made because of the paucity of β-globin tetramers (17).

We have studied with single enzyme digests several Chinese patients with deletion type hemoglobin-H disease and obtained results similar to those of this individual. However, even within the same racial group, different mutational events may have occurred. Orkin et al. (18) obtained a different restriction pattern from our study in a Chinese subject with hemoglobin-H disease. Also, in the Mediterranean population with hemoglobin-H disease, heterogeneity in α-globin gene numbers and restriction patterns have also been detected (18, 19). It thus seems likely that, as in β-thalassemia, the mechanism that leads to abnormal gene function in α-thalassemia is heterogeneous.

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REFERENCES


