A large deletion encompassing the entire α -like globin gene cluster in a family of Northern European extraction

Paolo Fortina, Kathleen Delgrosso, Eric Rappaport, Mortimer Poncz, Samir K. Ballas¹, Elias Schwartz and Saul Surrey*

Division of Hematology, The Children's Hospital of Philadelphia, Departments of Pediatrics and Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 and ¹Cardeza Foundation for Hematologic Research, Department of Medicine, Thomas Jefferson University, Philadelphia, PA 19107, USA

Received July 1, 1988; Revised and Accepted October 26, 1988

ABSTRACT

We describe a new deletional form of α thalassemia segregating in three generations of a family of northern European origin. A full-term female girl had hypochromic, microcytic anemia since early infancy associated with delayed language development, slow growth and weight gain. Hematologic studies suggested the presence of α thalassemia. Gene-blotting studies showed no abnormal α -like globin gene fragments; however, studies of inheritance of informative polymorphic restriction fragments using ζ , α and 3'- α -hypervariable region (3'-HVR) probes showed evidence for an extensive deletion encompassing the entire α -like globin gene cluster. The 3' breakpoint of this deletion maps beyond the 3'-HVR, a region implicated as a hot spot for the generation of other large deletional events within the α -like cluster. The 5' breakpoint maps at least 10 kilobases (kb) 5' to the ζ -globin gene. The minimum size estimate for this deletion is greater than 47 kilobases.

INTRODUCTION

The human α -globin gene cluster encompasses a region of approximately 40 kb located on the short arm of chromosome 16, and contains three functional genes, three pseudogenes, and one gene of undetermined status (θ_1) arranged on the same DNA strand in the order 5'- ζ - $\psi\zeta$ - $\psi\alpha_2$ - $\psi\alpha_1$ - α_2 - α_1 - θ_1 -3' (1-5). In deletion forms of α thalassemia relatively large (> 1 kb) regions of DNA are removed, frequently as a result of unequal homologous recombination events which are potentiated by the duplications in the α -like globin gene cluster in humans (6,7). Homologous recombination is the cause of $-\alpha^{3.7}$ (8), $-\alpha^{4.2}$ (9,10) and $-\zeta$ (11,12) deletions, while $-\alpha^{5.2}$ (13) is due to an illegitimate recombination event. Five more deletions within the α -like cluster remove approximately 20 to 30 kb of DNA each: 1) the 5' breakpoint in $--_{SEA}$ maps close to $\psi\zeta$ while the 3' breakpoint is 1 kb upstream of the 3'-HVR (14,15); 2) $-(\alpha)^{20.5}$ removes $\psi\zeta$, $\psi\alpha_2$, $\psi\alpha_1$, α_2 and the first 56 codons of the α_1 -gene (16); 3) $--_{MED}$ leaves a functional ζ gene intact, but removes $\psi\zeta$, $\psi\alpha_2$, $\psi\alpha_1$, α_2 and α_1 (17); 4,5) both $--_{BRIT}$ and $--_{SA}$ have 3' endpoints which are 3' to θ and 5' to the 3'-HVR, while their 5' endpoints map in the inter- ζ region (IZHVR) (18). Additional deletions with different endpoints have been described in blacks (19) and Chinese (20). Finally, three larger deletions were recently described (21). The entire α -like globin gene cluster is missing in $--_{THAI}$ and $--_{FIL}$. Although the 5' breakpoints have not yet been determined, the 3' breakpoints map just 5' to the 3'-HVR. The third deletion, $(\alpha\alpha)^{RA}$, removes approximately

62 kb with the 3' breakpoint mapping just upstream of $\psi\zeta_1$. With the exception of $(\alpha\alpha)^{RA}$, the 3' endpoints of all of the large deletions are located just upstream of 3'-HVR, suggesting a role for this region in the generation of these deletions (21). In this paper we present hematological profiles and DNA studies of a family of northern European extraction in which the entire α -like cluster is removed. In this family the 3'-deletion breakpoint extends beyond the 3'-HVR and the 5' breakpoint maps more than 10 kb 5' to the ζ -globin gene.

MATERIALS AND METHODS

Family History

The proband (III-1), a 2 $\frac{1}{2}$ -year-old girl of German extraction, was referred to The Children's Hospital of Philadelphia for evaluation of hypochromic microcytic anemia unresponsive to ferrous sulfate therapy. Liver and spleen size were normal. She was the product of an uncomplicated first pregnancy and delivery. Her early life was characterized by anemia, slow growth and weight gain, and delayed language development. Development at present is normal, and growth is in the normal range for age. The probanda's parents, maternal aunt and maternal grandmother had no history of anemia, while the maternal grandfather was noted to be anemic in childhood.

Hematologic and Hemoglobin Studies

Hematologic data were obtained using standard laboratory procedures (22) with an electronic Coulter Counter (Model II Plus). Iron status was evaluated by studies of free erythrocyte protoporphyrin (FEP). Hemoglobin determinations included DEAE cellulose microchromatography for quantitation of Hb A₂ (23) and alkali denaturation for quantitation of Hb F (24). Ratios of α/β globin chain synthesis were determined after incorporation of ^{14}C -leucine by reticulocytes followed by chromatography on carboxymethylcellulose (25).

Restriction Endonuclease Analysis

Leukocyte DNA was isolated from peripheral blood, as described previously (26). A 10 μg aliquot of DNA was digested at 37°C with 20-40 units of each restriction endonuclease including Acc I, Bam HI, Bgl II, Eco RI, Hind III, Hpa I, Pvu II, Rsa I, Sst I and Xba I, using buffer conditions specified by the suppliers. DNA fragments were separated by electrophoresis in agarose gel, transferred to a GeneScreen Plus (New England Nuclear, Boston, MA) nylon membrane, and filters were then hybridized with ^{32}P -labeled probes, washed and processed for autoradiography.

Preparation of Probes

The α_1 -globin gene insert (1.5 kb) was isolated from pBR α_1 (28) after digestion with Pst I; the $\psi\zeta$ fragment (2.0 kb) from pBR ζ (28) after digestion with Hinf I; the 3'-HVR insert (4.0 kb) from p α_3 'HVR.64 (29) using Pst I/Xba I double digestion; the β -IVS2 insert (0.9 kb) from pIVS2 β following Eco RI/Bam HI double digestion; the 1.8 kb 5'- β -gene insert from p1.8 Bam HI after Bam HI digestion; and the fragment designated L1 (0.6 kb) from pJW5

(21) using Eco RI/Bam HI double digestion. A cosmid clone probe, CRI-090 (D16S45, insert size 35 to 40 kb), which maps on the short arm of chromosome 16, was also used in these studies (30). Radiolabeled probes were prepared as previously described (31).

Relative estimates of α - and ζ -globin gene number

The number of ζ - and α -globin genes was estimated by gene-blotting studies following digestion of DNA with Hind III, employing the β -globin gene signal as an internal standard. This enzyme was used since the resulting α -, ζ - and β -gene containing fragments are readily separated by electrophoresis in agarose gels. Genomic blots containing Hind III digests were hybridized with ^{32}P -labeled α - and β - or ζ - and β - gene probes, and intensities of α/β as well as ζ/β bands were compared with normal controls.

RESULTS

Hematologic data on this family are summarized in Figure 1. Hemoglobin levels of the maternal grandfather (I-1), maternal aunt (II-1), proband's mother (II-2) and proband (III-1) were 10.5 to 12.4 g/dl, with a MCV range of 64 to 71.5 fl. Peripheral smears

Figure 1: Hematologic Data.

Inheritance patterns show three generations with affected individuals indicated by solid half circles or squares. Values for Hb (g/dl), MCV (fl) and α/β globin synthetic ratios are located as indicated in the key.

revealed anisopoikilocytosis, microcytosis, and red cell fragmentation, suggestive of thalassemia. Hemoglobin electrophoresis studies showed normal values for Hb A₂ (2.6 to 2.8 %) and Hb F (0.5 to 1.2 %). Globin chain synthesis studies indicated the presence of an α -thalassemia syndrome.

Gene-blotting studies showed normal band patterns after hybridization to ζ , α or 3'-HVR probes (Figure 2). Despite the absence of abnormally sized fragments, analysis of the inheritance of polymorphic restriction bands indicated heterozygosity for a large deletion encompassing the entire α -like globin gene complex in four members of the pedigree. For example, the mother (II-2) has a 2.4 kb α_2 -Rsa I fragment, while the father (II-3) is homozygous for a polymorphic 3.5 kb α_2 fragment (Figure 3A). The proband inherits only the 3.5 kb band (from the father), indicating hemizyosity in both mother and proband for the α_2 gene. An invariant 1.7 kb α_1 -Rsa I fragment is present in all family members but appears to be at half intensity in II-2, III-1, I-1 and II-1, all of whom have decreased Hb and MCV levels.

Variation in length of short repeat sequence elements located between ζ and $\psi\zeta$ (IZHVR) and 3' to θ_1 (3'-HVR) result in polymorphic fragment size after digestion with a variety of restriction enzymes (32,33). Inheritance patterns for polymorphic ζ -like fragments are presented in Figure 3B. The father (II-3) and mother (II-2) have single ζ -Bgl II bands of 11.5 kb and 10.5 kb, respectively, while the proband (III-1) inherits only the 11.5 kb paternal band, consistent with maternal inheritance of DNA lacking the ζ -gene region. The constant band of ~ 12.0 kb in all family members encompassing the $\psi\zeta$, $\psi\alpha_2$, $\psi\alpha_1$ and the α_2 genes, was present at reduced intensity in I-1, II-1, II-2 and III-1.

Analysis of polymorphic Sst I α -gene fragments with the α_1 gene and 3'-HVR probes demonstrates that the deletion extends beyond α_1 (Figure 3C). The mother (II-2) has a single 13.5 kb α_1 fragment and the father (II-3) is heterozygous for 16.5 kb and 15.0 kb fragments, and the proband inherits only the 15.0 kb paternal fragment. None of the large deletions previously described within the α -like cluster extend beyond the 3'-HVR (21). In

Figure 2: Map of Restriction Endonuclease Cleavage Sites in The α -Like Globin Gene Cluster.

The top line shows coordinates in kilobases (kb) with zero indicating ζ -gene mRNA cap site. Functional ζ , α_2 and α_1 genes are shown with black boxes representing mRNA coding and white boxes non-coding intervening sequence regions. Stippled boxes indicate pseudogenes $\psi\zeta$, $\psi\alpha_2$ and $\psi\alpha_1$, while the θ gene is shown as a black box. Hypervariable regions (IZHVR and 3'-HVR) are denoted as zigzag lines, and probes described in the text are listed below as solid bars. For the following regions, the map was constructed from analysis of sequence information on BIONET using the SEQ (version 5.1) DNA analysis program: a) 770 bp 5' from the ζ cap site to 246 bp 3' to the ζ poly A addition site; b) 356 bp 5' from the $\psi\zeta$ cap site to 1525 bp 3' to the α_1 poly A addition site. The remainder of the map was derived from data in reference 21 and our own observations. Regions for which the restriction map is incomplete are indicated by the dashed lines. Endonucleases used for gene-blotting analyses in this family are shown.

Figure 3: Inheritance of Polymorphic ζ , α and 3'-HVR Fragments. Gene-blotting studies are shown for inheritance of polymorphic Rsa I- α_2 (panel A), Bgl II- ζ (panel B), Sst I- α_1 (panel C) and Pvu II-3'-HVR (panel D) fragments.

the current study, inheritance patterns of polymorphic 3'-HVR Sst I (Figure 3C) and the smaller Pvu II fragments (Figure 3D) are consistent with removal of the 3'-HVR region in affected individuals. The enzyme Pvu II cleaves at the boundaries of the 3'-HVR, resulting in the generation of small fragments whose length differences can be readily distinguished by gene-blotting studies. The father (II-3) is heterozygous for 4.3 and 3.0 kb bands, the mother (II-2) has a single 2.1 kb band, and the proband inherits only the paternal 3.0 kb band. The affected maternal aunt (II-1) and unaffected maternal grandmother (I-2) also possess only the 2.1 kb band found in the mother (II-2). However, the unaffected maternal grandmother (I-2) appears to be homozygous and has two copies of this size fragment, based on her increased relative band intensity compared with II-1 and II-2. Although most individuals are heterozygous for length variation in this region (34), homozygosity for fragment length has been noted previously in some individuals at this locus (6). Not included in the Figure, but present on other blots, the affected maternal grandfather (I-1) has a single 2.3 kb band which is not inherited by either of his affected daughters. Thus, the inheritance pattern in three generations is consistent with hemizygosity for the 3'-HVR region in all affected family

Figure 4: Summary of Inheritance of Polymorphic Fragments Within and Flanking The α -Like Globin Gene Complex.

Diagram of α -like globin gene complex with location and sizes of polymorphic fragments is shown in panel A. Inheritance of linked polymorphic fragments and their size in kb is shown in panel B. Note affected individuals in pedigree (darkened half circles or squares) are hemizygous and have only one chromosome containing the normal complement of α -like globin genes.

members. A summary of inheritance of ζ -, α - and 3'-HVR polymorphic restriction fragments in this pedigree is shown in Figure 4.

Additional evidence for deletion of the entire α -like cluster in this family is provided

Figure 5: Decreased Relative α -Like Globin Gene Number in Affected Family Members.

Genomic DNA was digested with Hind III and blots were hybridized with either mixed radiolabeled α and β (panel A) or ζ and β (panel B) probes. Note decreased signal intensities of Hind III α (3.7 kb)/ β (7.8 kb) in A, and ζ (13.5 kb)/ β (7.8 kb) in B for affected individuals. Panel C shows Bam HI blots hybridized with radiolabeled β and L1 probe which maps ~ 10 kb 5' to the ζ -globin gene. Again, note decreased L1/ β intensity in affected family members.

from estimates of relative band intensities indicating a lower copy number for normal and polymorphic fragments in I-1, II-1, II-2 and III-1. Measurements of relative α/β and ζ/β band intensities from gene-blotting analyses in this family are consistent with decreased α - (Figure 5A) and ζ -globin gene number (Figure 5B) in I-1, II-1, II-2 and III-1. In addition, a nonpolymorphic Bam HI gene fragment of 6.6 kb of one-half intensity was found in all affected individuals using a unique sequence probe (L1) (Figure 5C) which maps about 10 kb 5' to ζ (21). When the same filter was then washed and rehybridized with a 1.8 kb Bam HI fragment containing the 5' portion and flanking sequence region of the β -globin gene, the expected 1.8 kb internal control band was present in equal intensity in both normal and affected individuals, indicating that the deletion extends at least 10 kb 5' to the ζ gene.

We have used a cosmid probe (CRI-090) mapping approximately 8 centimorgans from the α -globin cluster (30) in an effort to try to establish an upper size limit for this deletion. When genomic DNA was digested with Eco RI and probed with this cosmid for single-copy sequences (35), similar band intensities and fragment sizes were seen in all family members compared with normal unrelated controls (data not shown). These results indicate that there are two copies of this locus in affected as well as unaffected individuals, and that the deletion does not encompass this region. Chromosomal studies have not been obtained on this family.

DISCUSSION

A summary of the forms of α -thalassemia deletions in humans is presented in Figure 6. This study defines a new deletion greater than 47 kb in length present in three generations of a northern European family which encompasses the entire α -globin gene cluster. This deletion is associated in heterozygotes with hypochromia, microcytosis and a decreased α/β globin chain synthetic ratio. No other well-documented clinical disorders have been noted in this family, but in early infancy the probanda demonstrated transiently delayed language development and slow growth.

This phenotype is very different from the previously described unusual type of α^0 thalassemia associated with both Hb H (formation of β_4 tetramers due to expression of only one of the four normal α -globin genes) and mental retardation (36,37). In the family of northern European extraction we have studied, the deletion segregates in three generations of the family and the proband has reached normal developmental levels after the initial delay. The other affected members also have only mild clinical symptoms and are of normal intelligence.

Since no abnormal α -like globin gene bands were observed in the family we studied, the presence of a large deletion was inferred from three lines of evidence. First, none of the individuals with microcytosis is heterozygous for any restriction endonuclease cleavage site commonly known to be polymorphic within or flanking the α -like cluster. Second, the proband inherits only the paternal pattern of polymorphic restriction enzyme cleavage sites,

implying maternal inheritance of a large deletion encompassing the entire cluster. Third, estimates of α/β and ζ/β gene ratios was consistent with the presence of one half the normal complement of α -like genes in affected family members.

Recently, the breakpoints of a number of the large deletions within the human α -like globin gene cluster have been described (21). Several of the deletions are similar in size, ranging from 20 to 30 kb, with similarities in the positioning of their 5' and 3' endpoints. The similar size (20-30 kb) seen in this group of deletions involving the α -like globin gene cluster (21) and also of those (~ 100 kb in size) involving the β -like globin gene cluster (38) has led to the theory that these deletional events may have occurred during DNA replication, due to removal of a fixed size DNA loop whose endpoints are defined by nuclear matrix attachment sites (39). In the family we have studied, the greater than 47 kb size of the deletion precludes the removal of a single 20-30 kb DNA loop within the α -like cluster, although removal of multiple loop structures has been hypothesized to account for larger deletions (21).

In addition, the location of at least one breakpoint in each of the 20-30 kb deletions within the α -gene cluster occurs within or near an Alu repeat sequence, suggesting a role for this sequence in the generation of these deletional events. The 3' endpoints of 8 of these large deletions also map within an 8 kb region mapping immediately upstream to the 3'-HVR, suggesting that the tandem repeat array in this region leads to frequent recombinational events. None of these previously described deletions extend beyond the 3'-HVR.

Our findings in this northern European family demonstrate that this deletion extends beyond the 3'-HVR. Identification of breakpoint-fragment clones and DNA sequence analysis across the 5' and 3' junctions in this deletion may provide insight into the generation of very large deletional events. In addition, a molecular analysis of the 3' breakpoint in this family may be helpful in generating clones for chromosome walking 3' to the HVR. Previous attempts at isolating probes 3' to this region by conventional cloning techniques have been unsuccessful (21), perhaps due to recombinants involving the 3'-HVR. In fact, we often experience instability of insert size when growing the 3'-HVR plasmid subclone in bacteria. Finally, analysis of positions and sequences of the 5'- and 3'- deletion breakpoints in this family may also be informative in establishing a more precise map for evaluation of a variety of other genetic disorders linked to the α -like globin gene cluster, including polycystic kidney disease (40) and some forms of mental retardation (36,37).

Figure 6: Summary of Large Deletions Within and Encompassing The α -Like Globin Gene Cluster in Humans.

Map of the α -like globin gene cluster with position and size of the various deletions (solid bars under map) associated with α thalassemia is shown. Unfilled regions at end of deletions represent possible location of breakpoint, while a question mark indicates that the position of the endpoint is not known. Names of various deletions are as previously described (41).

ACKNOWLEDGMENTS

This work was supported in part by grants from The National Institutes of Health (DK 16691 and HL 28157), from UNICO National and by a fellowship from The Cooley's Anemia Foundation. The authors thank Drs. D. Higgs (MRC Molecular Haematology Unit, John Radcliffe Hospital, Headington, Oxford, UK), G. Saglio (Dipartimento di Scienze Biomediche ed Oncologia Umana, Universita' di Torino, Italy) and T. Keith (Collaborative Research, Inc. Bedford, MA, USA) for supplying us with plasmid (3'-HVR and L1) and cosmid (CRI-090) clones. We also thank Emi Asakura for help in the preparation of this manuscript.

The data base used for DNA analysis was the most recent (January 1988) version of the National Institutes of Health DNA sequence library (GenBank). Computer resources used to conduct our studies were provided in part by the National Institutes of Health sponsored Bionet TM National Computer Resource for Molecular Biology (NIH 1U41 RR-01685).

*To whom correspondence should be addressed

REFERENCES

1. Deisseroth, A., Nienhuis, A., Turner, P., Velez, R., Anderson, W.F., Ruddle, F., Lawrence, J., Creagan, R., Kucherlapati, R. (1977) *Cell* 12, 205-218.
2. Lauer, J., Shen, C.-K.J., Maniatis, T. (1980) *Cell* 20, 119-130.
3. Proudfoot, N.J., Gill, A., Maniatis, T. (1982) *Cell* 3, 553-563.
4. Hardison, R.C., Sawada, I., Cheng, J.F., Shen, C.-K.J., Schmid, C.W. (1986) *Nucl. Acids Res.* 4, 1903-1911.
5. Marks, J., Shaw, J.P., Shen, C.-K.J. (1986) *Nature* 321, 785-788.
6. Nicholls, R.D., Jonasson, J.A., McGee, J.O.D., Patil, S., Ionasescu, V.V., Weatherall, D.J., Higgs, D.R. (1987) *J. Med. Genet.* 24, 39-46.
7. Higgs, D.R., Hill, A.V.S., Bowden, D.K., Weatherall, D.J. Clegg, J.B. (1984) *Nucl. Acids Res.* 12, 6965-6977.
8. Embury, S.H., Miller, J.A., Dozy, A.M., Kan, Y.W., Chan, V. (1980) *J. Clin. Invest.* 66, 1319-1325.
9. Embury, S.H., Lebo, R.V., Dozy, A.M., Kan, Y.W. (1979) *J. Clin. Invest.* 63, 1307-1310.
10. Orkin, S.H., Old, J., Lazarus, H., Altay, C., Gurgey, A., Weatherall, D.J., Nathan, D.G. (1979) *Cell* 17, 33-42.
11. Winichagoon, P., Higgs, D.R., Goodbourn, S.E.Y., Lamb, J., Clegg, J.B., Weatherall, D.J. (1982) *Nucl. Acids Res.* 10, 5853-5858.
12. Rappaport, E., Schwartz, E., Poncz, M., Surrey, S. (1984) *Biochem. Biophys. Res. Commun.* 25, 817-823.
13. Pressley, L., Higgs, D.R., Aldridge, B., Metaxatou-Mavromati, A., Clegg, J.B., Weatherall, D.J. (1980) *Nucl. Acids Res.* 8, 4889-4898.
14. Pressley, L., Higgs, D.R., Clegg, J.B., Weatherall, D.J. (1980) *Proc. Natl. Acad. Sci. USA* 77, 3586-3589.
15. Winichagoon, P., Higgs, D.R., Goodbourn, S.E.Y., Clegg, J.B., Weatherall, D.J., Wasi, P. (1984) *EMBO J.* 3, 1813-1818.
16. Nicholls, R.D., Higgs, D.R., Clegg, J.B., Weatherall, D.J. (1985) *Blood* 65, 1434-1438.
17. Orkin, S.H., Michelson, A. (1980) *Nature* 286, 538-540.
18. Higgs, D.R., Ayyub, H., Clegg, J.B., Hill, A.V.S., Nicholls, R.D., Teal, H., Wainscoat, J.S., Weatherall, D.J. (1985) *Brit. Med. J.* 290, 1303-1306.

19. Felice, A.E., Cleek, M.P., McKie, K., McKlie, V., Huisman, T.H.J. (1984) *Blood* 63, 1253-1257.
20. Chan, V., Chan, T.K., Cheng, M.Y., Kan, Y.W., Todd, D. (1986) *Br. J. Haematol.* 64, 97-105.
21. Nicholls, R.D., Fischel-Ghodsian, N., Higgs, D.R. (1987) *Cell* 49, 369-378.
22. Dacie, J.V., Lewis, S.M. (1985) In *Practical Hematology*, 5th edn, J. & A. Churchill, London.
23. Huisman, T.H.J., Schroeder, A.N., Brodie, S.M. (1975) *J. Lab. Clin. Med.* 86, 700-702.
24. Pembrey, M.E., McWade, P., Weatherall, D.J. (1972) *J. Clin. Pathol.* 25, 738-740.
25. Schwartz, E. (1974) *Sem. Hematol.* 11, 549-567.
26. Poncz, M., Solowiejczyk, D., Harpel, B., Mory, Y., Schwartz, E., Surrey, S. (1982) *Hemoglobin* 6, 27-36.
27. Southern, E.M. (1975) *J. Mol. Biol.* 98, 503-517.
28. Lauer, J., Shen, C.-K.J., Maniatis, T. (1980) *Cell* 20, 119-130.
29. Nicholls, R.D., Hill, A.V.S., Clegg, J.B., Higgs, D.R. (1985) *Nucl. Acids Res.* 13, 7569-7578.
30. Donis-Keller, H., Green, P., Helms, C., Cartinhour, S., Weiffenbach, B., Stephens, K., Keith, T.P., Bowden, D.W., Smith, D.R., Lander, E.S., Botstein, D., Akots, G., Rediker, K.S., Gravius, T., Powers, J.A., Watt, D.E., Kauffman, E.R., Bricker, A., Phipps, P., Muller-Kahle, H., Fulton, T.R., Ng, S., Schumm, J.W., Braman, J.C., Knowlton, R.G., Barker, D.F., Crooks, S.M., Lincoln, S.E., Daly, M.J., Abrahamson, J. (1987) *Cell* 51, 319-337.
31. Summers, J. (1975) *J. Virol.* 15, 946-953.
32. Goodbourn, S.E.Y., Higgs, D.R., Clegg, J.B., Weatherall, D.J. (1983) *Proc. Natl. Acad. Sci. USA* 80, 5022-5026.
33. Wainscoat, J.S., Higgs, D.R., Kanavakis, E., Cao, A., Georgiou, D., Clegg, J.B., Weatherall, D.J. (1983) *Am. J. Hum. Genet.* 35, 1086-1089.
34. Jarman, A.P., Nicholls, R.D., Weatherall, D.J., Clegg, J.B., Higgs, R.D. (1986) *EMBO J.* 5, 1857-1863.
35. Litt, M., White, R.L. (1985) *Proc. Natl. Acad. Sci. USA* 82, 6206-6210.
36. Weatherall, D.J., Higgs, D.R., Bunch, C., Old, J.M., Hunt, D.M., Pressley, L., Clegg, J.B., Bethlenfalvay, N.C., Sjolin, S., Koler, R.D., Magenis, E., Francis, J.L., Bebbington, D. (1981) *N. Engl. J. Med.* 305, 607-612.
37. Bowcock, A.M., Van Tonder, S., Jenkins, T. (1984) *Br. J. Haematol.* 56, 69-78.
38. Vanin, E., Henthorn, P., Kioussis, D., Grosveld, F.G., Smithies, O. (1983) *Cell*, 35, 701-709.
39. Small, D., Nelkin, B., Vogelstein, B. (1982) *Proc. Natl. Acad. Sci. USA* 79, 5911-5915.
40. Reeders, S.T., Breuning, M.H., Davies, K.E., Nicholls, R.D., Jarman, A.P., Higgs, D.R., Pearson, P.L., Weatherall, D.J. (1985) *Nature* 317, 542-544.
41. Higgs, D.R., Weatherall, D.J. (1983) *Curr. Topics Hematol.* 4, 37-97.