β-Globin gene inactivation by DNA translocation in γβ-thalassaemia

D. Kioussis, E. Vanin*, T. deLange†, R. A. Flavell‡ & F. G. Grosveld

Laboratory of Gene Structure and Expression, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK
* Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706, USA
† Laboratory of Biochemistry, Jan Swammerdam Institute, University of Amsterdam, PO Box 60.000, 1005 GA Amsterdam, The Netherlands
‡ Biogen Inc., 14 Cambridge Center, Cambridge, Massachusetts 02142, USA

The β-globin gene present on the deletion locus in a Dutch γβ-thalassaemic patient was found to be identical to the normal β-globin gene with respect to DNA sequence and its transcription in HeLa cells. DNase I sensitivity and methylation experiments show that the affected β-globin gene is present in an inactive configuration in vivo. *This is the result of a translocation of a normally inactive locus next to the β-globin gene on the affected chromosome, or the deletion of sequences which are normally required for the maintenance of the active state.*

THE human β-globin related polypeptide chains are encoded by a cluster of genes, located on chromosome 11 in the order 5′-ε-γ^G-γ^A-δ-β-3′. These genes are expressed differentially during development from the embryonic (ε) and fetal (γ^G, γ^A) to the adult stage (δ, β)[1]. Several well-defined defects in the functioning of these genes have been described at the molecular level, which either affect the structure or the amount of β-globin protein. The latter class of defects can be subdivided into two groups, the β^+-thalassaemias, which are characterized by a reduced amount of β-globin protein, and the β^0-thalassaemias, in which the β-globin protein is absent in homozygotes. The defect present in several cases of β^+-thalassaemia is caused by a point mutation, which results in the aberrant splicing of part of the β-globin precursor mRNA to a non-functional mRNA[2–4]. Most commonly, the defect in β^0-thalassaemias is caused by point mutations or deletions resulting in either early termination[5–7], or the absence of a functional mRNA. In most of these cases the γ-globin genes remain functional and active in fetal life and, in some cases, even during adult life (HPFH)[8,9]. However, in a small group of disorders, known as γβ-thalassaemias, the ε- and the γ-genes are also affected. These thalassaemias[10,11] are characterized by a severe anaemia in newborn heterozygotes as a result of the reduction of the ratio of γ/α globin synthesis to about 0.5. As γ-chain synthesis ceases in the course of normal development, the disease develops into a mild β-thalassaemia with the unusual phenomenon that the HbA_2 ($\alpha_2\delta_2$) levels are normal, rather than elevated as in the classical β-thalassaemia[10].

Three such cases have been reported. The first case of γβ-thalassaemia[12] to be described was later shown to have a deletion of a large segment of chromosome 11, including the ε-γ^G-γ^A and δ-globin genes and the 5′ end of the β-globin gene. In a Dutch[11] case, however, the entire β-globin gene and its normal 5′ and 3′ flanking sequences are still present, whereas the γ- and δ-globin genes are deleted. Although at different positions on the β-locus, the generation of these deletions may be the result of a common underlying mechanism, since the same DNA sequences were found to be juxtaposed to the β-gene in these unrelated cases[13]. A similar phenomenon is observed in the generation of some HPFH genotypes[13]. In a third case of γβ-thalassaemia, all the globin genes have been deleted (H. H. Kazazian, personal communication).

The Dutch γβ-thalassaemia is particularly interesting in that although the patients have two complete copies of the β-globin gene, they still have the haematological symptoms characteristic of a heterozygous β^0-thalassaemia. It is surprising that the apparently normal β-globin gene in the Dutch γβ-thalassaemia is inactive in adult life. Several possible explanations have been given for this phenomenon[11]; the thalassaemia might be the result of a double mutation, that is, the largest deletion in the ε-γ-δ region might be irrelevant to the β-thalassaemia, but a second, smaller deletion or a point mutation, near or in the β-globin gene, may render it inactive. Alternatively, the disease may be the result of the absence of some, or all, of the deleted DNA sequences lying far from the β-globin gene. Finally, the deletion might juxtapose the β-globin gene to a chromatin structure which is not compatible with gene expression in erythropoietic cells.

The first explanation implies that the β-globin gene region on the mutant chromosome would still be properly regulated, and that a region(s) upstream from the β-globin gene does not play a primary role in its expression. In contrast, the latter explanations imply that the upstream sequences are important in the regulation of β-globin expression. Consequently, a further analysis of this γβ-thalassaemia could provide a better understanding of the mechanisms involved in the regulation of β-globin expression.

Sequence of the two allelic β-globin genes

A cosmid library was constructed using the DNA isolated from the blood of a Dutch γβ-thalassaemic patient[14]. Four β-globin positive clones were isolated and analysed by restriction enzyme mapping. Three of the clones contained the mutant 4.2 kilobase (kb) *Eco*RI fragment, while the fourth clone contained the normal 5.2 kb *Eco*RI fragment which is the 5′ end of the β-globin gene from the apparently unaffected chromosome (Fig. 1). Comparison of the restriction fragments from all the clones with known restriction maps[11,14] of the mutant and normal DNA from the patient and normal individuals established that none of the cosmid recombinants were rearranged (data not shown). The *Bgl*II fragments containing the β-globin gene from two of the cosmid recombinants, cosmid 5γβ from the mutant chromosome and cosmid 6N from the normal chromosome, were subcloned for sequence analysis. The three exons, the promoter regions and all the exon–intron boundaries of each of the genes were sequenced by the method of Maxam and Gilbert[15]. Both genes showed a sequence identical to that of the normal β-globin gene[16] in these regions, that is from −100 upstream from the cap-site through the three coding segments to a position 50 base pairs (bp) downstream from the poly(A) addition site (data not shown). These data thus exclude the possibility that a point mutation in the coding sequence is responsible for the β-thalassaemic phenotype.

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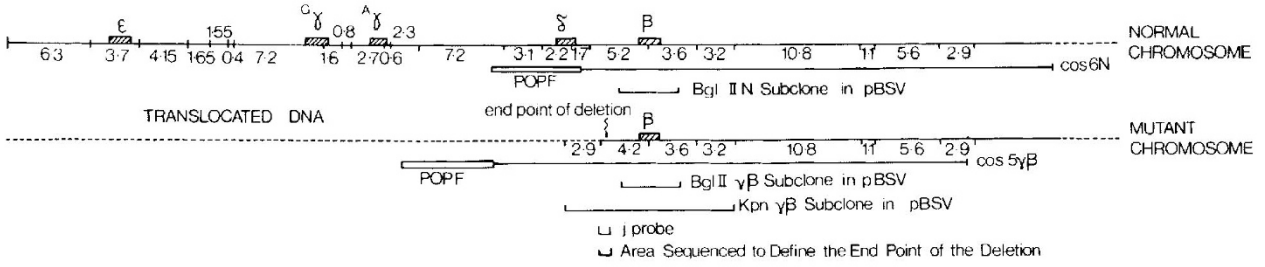

Fig. 1 Physical map of the normal and $\gamma\beta$-thalassaemic β-globin region. Each map shows the position of the genes in relation to the *Eco*RI restriction map. The relevant clones (cos 6N and cos 5$\gamma\beta$) were isolated from a cosmid library constructed from blood DNA of a $\gamma\beta$-thalassaemic patient and the cosmid vector pOPF[14]. The brackets underneath indicate the fragments which were subcloned in the expression vector pBSV[17] and the location of the junction probe (j). The arrow indicates the deletion end point in the affected locus.

Transcription of allelic β-globin genes

The fact that the DNA sequence of the affected gene was found to be identical to normal does not exclude the possibility that the gene is transcriptionally non-functional. To test the transcription of the genes, subclones in the transient expression vector pBSV[17] were introduced into HeLa cells by $CaPO_4$ precipitation[18] and the RNAs from these cells were analysed for the presence of β-globin mRNA using S_1 nuclease mapping[19].

The following pBSV recombinants were constructed (Fig. 1): (1) A 4.7 kb *Bgl*II fragment from both the normal and the mutant chromosome which contains the β-globin gene and about 3 kb of flanking sequences, was cloned in the *Bam*HI site of pBSV. (2) A 14 kb *Kpn*I fragment from the mutant chromosome (from cosmid clone $\gamma\beta$4) was cloned into the *Kpn*I site of pBSV. This fragment contains, in addition to the above sequences, flanking DNA in both 5′ and 3′ direction. At the 5′ end of this fragment there is 2.5 kb of the flanking DNA that has been transposed next to the β-globin gene by the deletion. Insertion into the *Bam*HI or *Kpn*I site of pBSV does not affect the efficiency of transcription. A fourth plasmid containing the *Bgl*II β-globin fragment from a normal individual was used as a control.

Figure 2*a* shows the protected fragments after S_1 digestion, when a 5′ end labelled 1,200 bp *Cvn*I probe from the 5′ end of the β-gene is used. In all cases, the same amount of a 68 bp fragment is protected, which represents the 5′ end of the β-globin mRNA. A similar result is found when a 3′ end labelled 700 bp *Eco*RI–*Msp*I fragment is used as a probe for the 3′ end of the mRNA (Fig. 2*b*). In all cases, a 212 bp fragment is protected. The 5′ and 3′ ends of the RNA transcribed from the mutant gene are therefore indistinguishable from normal β-globin mRNA. In addition, each of the splice junctions of the mRNA was analysed as previously described[3]. Again, no differences were found between mature β-globin mRNA and the RNA of each of the transformants (data not shown). These results show that by these methods both the normal and the mutant gene from the patient are transcribed equally to give an mRNA indistinguishable from β-globin mRNA from reticulocytes. In addition the level of transcripts found are the same for the β-globin from the 'mutant' and normal chromosomes. Therefore, the transcription and sequence data indicate that the phenotype observed in the patient cannot be caused by a promoter defect, a 'splice' mutation, or a mutation in the coding sequence.

DNase I sensitivity of $\gamma\beta$ DNA sequences

Another explanation for the thalassaemic phenotype is the possibility that the deletion in the mutant chromosome would have brought in juxtaposition to the β-globin gene a region of the chromatin that is not expressed in erythropoietic cells, and that proximity of such a region would result in a failure to activate

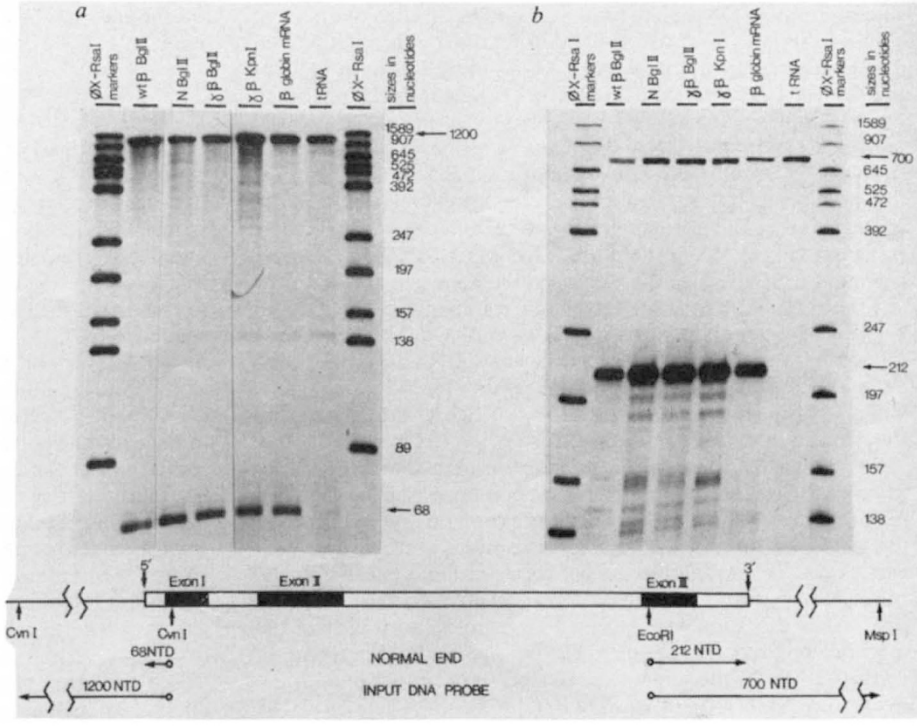

Fig. 2 Transient expression of the normal and $\gamma\beta$-thalassaemic β-globin in HeLa cells. *a*, The 5′ end analysis of the subclones indicated in Fig. 1, plus two controls, a β-globin subclone from normal DNA and *in vivo* produced mRNA from reticulocytes. *b*, The 3′ end analysis for the same samples. The labelled marker is ΦX174X *Rsa*1 and the numbers are the length of the marker fragments in nucleotides. The lower part of the figure shows the 5′ labelled 1,200-nucleotide probe and the 3′ labelled 700 nucleotide probe which contain respectively 68 nucleotides from the 5′ end and 212 nucleotides from the 3′ end of the gene which are protected against S_1 nuclease digestion of the RNA·DNA hybrids.
Methods: The *Bgl*II and *Kpn*I restriction fragments indicated in Fig. 1 were subcloned in the expression vector pBSV[17] by standard procedures. 15 μg from each of these subclones were mixed with 25 μg of salmon sperm DNA and used to transform half confluent 100 mm dishes of HeLa cells by the calcium phosphate method[18]. After 16 h the medium was changed, the cells grown for 36 h, collected and the RNA isolated by the LiCl–urea method[30]. The β-globin present in the HeLa RNA was detected by S_1 mapping[19] and end labelled DNA probes[31].

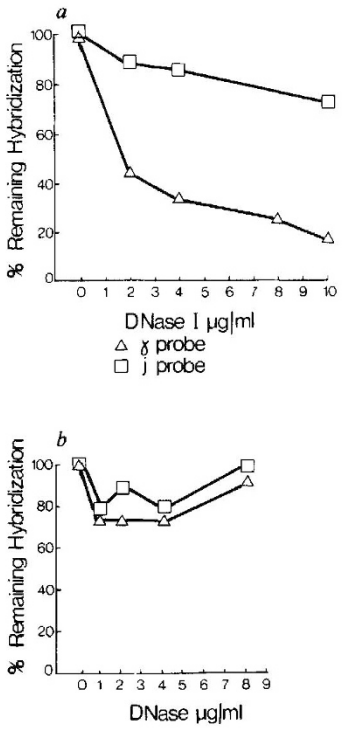

Fig. 3 DNase I sensitivity of the globin locus and the transposed sequences. *a*, Plot for 18-week fetal liver DNA; *b*, 18-week fetal brain DNA. The γ-probe hybridization is indicated by triangles, the junction-probe by squares.
Methods: Nuclei were purified from fetal brain and liver tissue as described elsewhere[32] with the following modifications. The tissue was homogenized in buffer A (60 mM KCl 15 mM NaCl, 0.5 mM dithiothreitol (DTT), 0.1 mM phenylmethyl sulphenyl fluoride, 15 mM Tris-HCl, *p*H 7.5, 0.25 M sucrose and 1 mM EDTA) using a Dounce homogenizer. The homogenate was filtered through cheesecloth and centrifuged at 5,000 r.p.m. for 10 min. The cell pellet was washed twice and lysed in 10 volumes of buffer A by the addition of Nonidet P-40 to 0.25%. Pelleted nuclei were washed twice in buffer A. The normal liver nuclei further purified on a sucrose gradient[27]. 10^8 nuclei per ml were suspended in 60 mM KCl, 15 mM NaCl, 0.5 mM DTT, 3 mM $MgCl_2$, 25 mM Tris-HCl *p*H 7.5 and digested with DNase I (Sigma) for 1 min at 37 °C. The digestion was stopped by the addition of EDTA to 10 mM and SDS to 0.5%. The DNA was further purified by standard procedures and digested with *Eco*RI. 10 μg of each DNA sample (different DNase I concentrations) was electrophoresed through a 0.5% agarose gel. After blotting the filters were hybridized with a 0.6 kb γ-globin fragment or a 0.5 kb junction probe (Fig. 1). The resulting bands on the autoradiographs were scanned using a Joyce–Loebl densitometer. The band intensities were normalized and plotted in relation to the non DNase I treated standard DNA sample.

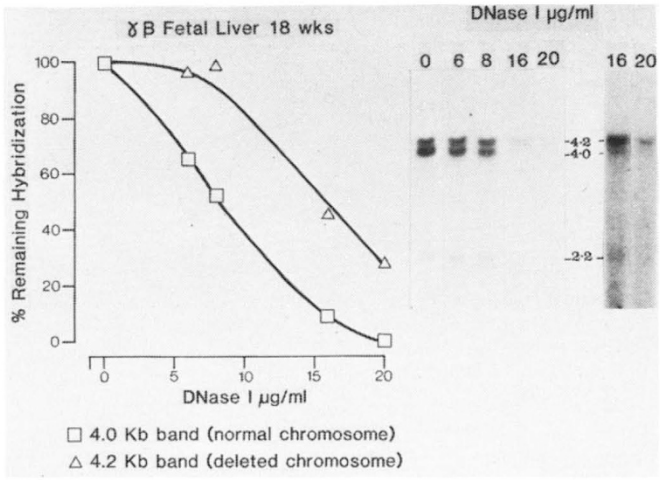

Fig. 4 The DNase I sensitivity of the normal and the mutant β-globin genes. Nuclei and DNA samples were isolated from an 18-week fetal liver of a γβ-thalassaemic patient and treated essentially as described elsewhere[26], before double digestion with *Eco*RI and *Hin*III. The autoradiograph shows the lanes obtained with the 5′β-globin probe (Fig. 1) and an increasing concentration of DNase I. The numbers indicate the length of δ and β-DNA fragments. The plot was obtained from the blot, as described in Fig. 3. A longer exposure of the 16 and 20 μg DNase I lanes is shown on the right.

the β-globin gene. Since transcriptional activity of a region of the genome has been associated with an increased sensitivity to DNAse I[20] and a hypomethylation of the DNA[21–23] these assays were applied to the rearranged β-globin locus. We tested the susceptibility to DNase I of fetal liver and brain chromatin, from a normal fetus (18 weeks old) and of fetal liver chromatin from a γβ-thalassaemic individual (18 weeks old). Southern blots of the DNase I treated and *Eco*RI or *Eco*RI/*Hin*III restricted DNA samples were hybridized to the following three probes: (1) An *Eco*RI–*Sph*I DNA fragment (j probe in Figs 1 and 5) from the junction point where the normal β-globin locus joins the juxtaposed DNA; this probe recognizes a 0.9 kb *Eco*RI fragment from the normal chromosome, and the 4.2 kb *Eco*RI fragment (Fig. 1) from the mutant locus. (2) A 1.8 kb *Bam*HI fragment from the 5′ end of the β-globin gene which recognizes a 4.0 kb *Eco*RI/*Hin*III 5′ β-globin fragment and a 2.25 kb δ-globin fragment in normal DNA, and a 4.2 kb *Eco*RI 5′ β-globin fragment from the mutant locus. (3) As a control probe a 0.6 kb *Eco*RI fragment from the 3′ end of the γ^A globin gene, which hybridizes to the 0.6 kb and 1.6 kb *Eco*RI fragments from the γ^A and γ^G genes, respectively.

The similarity in size of the 0.6 kb γ^A fragment and the 0.9 kb junction fragment allows a reliable comparison of the DNase I sensitivity of the two regions of the chromatin. As expected in the nuclei of the normal fetal liver samples (the tissue which expressed the fetal globin genes) the γ-globin genes from the normal liver were found to be very sensitive to DNase I (Fig. 3*A*). After digestion with DNase I 20 μg ml^{-1}, only 18% of the original material remained in the 0.6 kb *Eco*RI fragment. In contrast, 80% of the junction sequences (j probe) in the normal liver were still present in the 0.9 kb *Eco*RI fragment. The chromatin from fetal brain of the same age showed a comparable resistance to DNase I digestion for both loci (γ-globin and junction DNA, Fig. 3*B*). These data show that the juxtaposed (junction) DNA is resistant to DNase I in erythropoietic, and at least one non-erythropoietic tissue. If we assume that the 'inactive' configuration is transferred to the β-globin gene in the mutant locus, it predicts that the mutant locus would be less sensitive to DNase I than the normal locus in the γβ-thalassaemia patient. This is confirmed when the 5′ β-globin probe is hybridized to *Eco*RI/*Hin*III restricted DNA from DNase I treated fetal liver nuclei of a patient (Fig. 4). Both the 4.0 kb β-globin and the 2.25 kb δ-globin fragments from the normal locus are much more sensitive to DNase I than the 4.2 kb β-globin fragment from the mutant locus. The fact that the 4 and 2.25 kb fragments, which are smaller than the 4.2 kb fragment show a higher DNase I sensitivity excludes the possibility of differential DNase I sensitivity due to varying fragment lengths.

Methylation of γβ DNA sequences

In addition to an increased DNase I sensitivity, transcriptionally active areas of the genome have been shown to exhibit hypomethylation when compared with their non-active counterparts[21]. DNA from fetal liver and brain of a normal individual, and DNA from fetal liver of a γβ-thalassaemic patient were digested with *Msp*I and *Hpa*II to establish the extent of methylation of the β locus. Although both these enzymes recognize the sequence CCGG, *Hpa*II will not cleave the sequence C^mCGG or $^mC^mCGG$, while *Msp*I will not cleave the sequence ^mCCGG or $^mC^mCGG$[22]. Since most of the methylated C residues (^mC) occur in the dinucleotide ^mCG in eukaryotic DNA, the difference in the cleavage pattern of these two enzymes provides a measure for the extent of methylation in a particular region of the DNA[24]. Southern blots of the DNA digests were hybridized to the same probes as in the DNase I experiments. Figure 5 shows that the β-probe yields the hypomethylation pattern described previously[21] for the fetal liver, in contrast to a hypermethylation pattern in fetal brain. This is consistent with the fact that active globin genes are hypomethylated in erythroid tissues (fetal liver).

Hybridization of the β-probe to the fetal liver DNA yields a 20 kb *Hpa*II band and some high molecular weight fragments

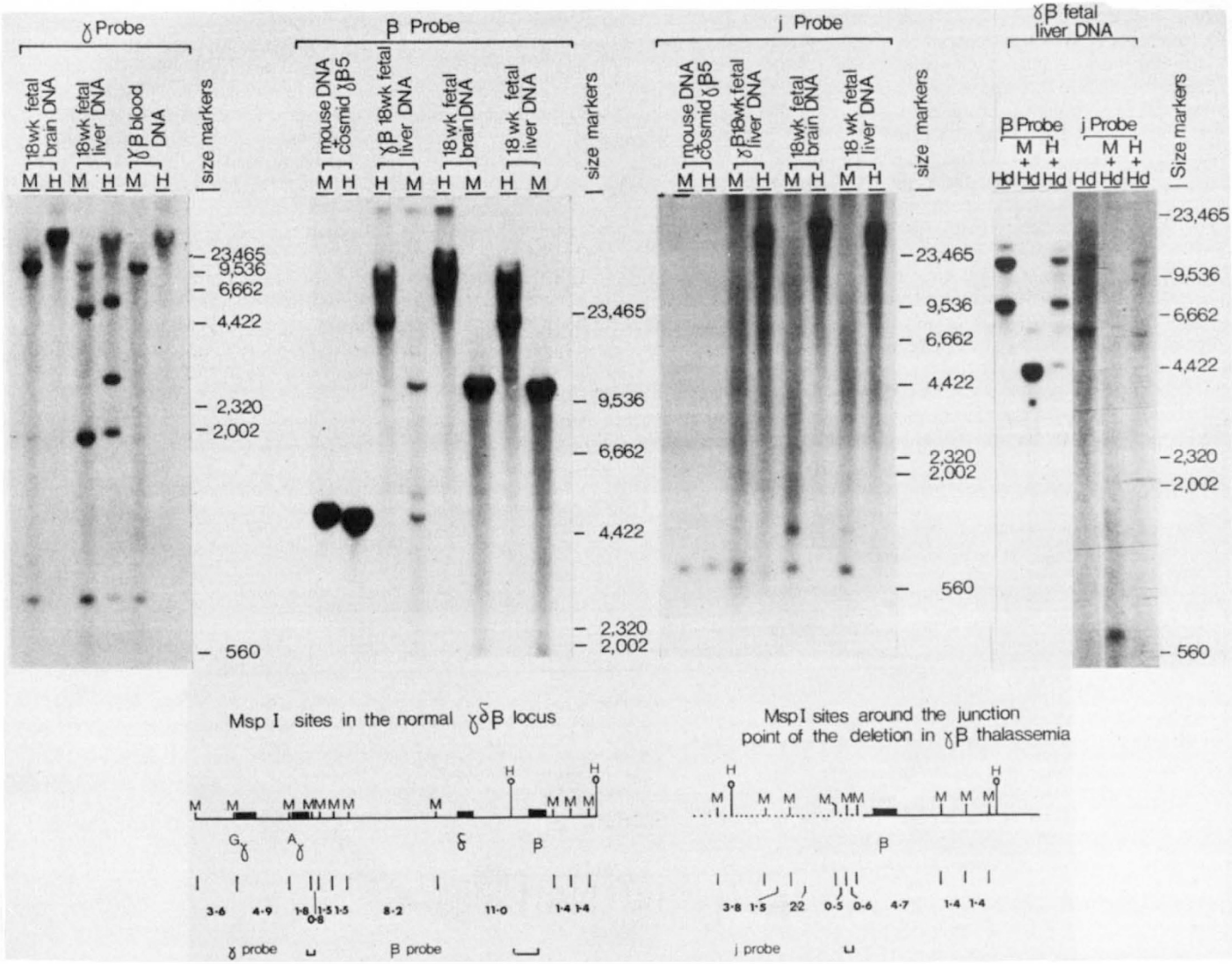

Fig. 5 Methylation of the translocated and normal $\gamma\beta$-thalassaemia locus. DNA was isolated from 18-week fetal liver of a normal and a $\gamma\beta$-thalassaemic patient and brain of a normal 18-week fetus. 10 μg of DNA was digested with *Msp*I or *Hpa*II (and *Hin*III in the double digests), electrophoresed through a 0.5% agarose gel and blotted. The filters were hybridized to: a 0.6 kb 3′ γ-globin fragment, a 1.8 kb 5′ β-globin fragment and a 0.2 kb junction probe. The numbers indicate the fragment size of a λ X *Hin*dIII marker. The control lanes contained 10 μg of mouse DNA mixed in with 1 ng of cosmid DNA.

presumably derived from the non-erythropoietic cells present in fetal liver: the 20 kb *Hpa*II band is created by cleavage of the *Hpa*II–*Msp*I sites on the 3′ side of the γ^A gene and on the 3′ side of the β-gene (M9 or M10 to M15 respectively in ref. 21). In fetal brain DNA, however, all of the signal has shifted to the high molecular weight range (Fig. 5, β-probe). This indicates (as previously described[21]) that the *Hpa*II sites at the 3′ sites of the β-globin gene are hypomethylated in erythroid tissue. Hybridization to the fetal liver DNA of the patient (Fig. 5, β-probe) shows two complete digest bands with *Msp*I, an 11.0 kb band for the normal locus and a 4.7 kb band for the mutant locus (see maps Fig. 5). Despite the fact that all digests are controlled by an internal marker, a partial *Msp*I digest band of 5.3 kb is visible, which is probably caused by the failure of *Msp*I to cut certain C^mCGG sites[25]. The *Hpa*II digest shows the normal 20 kb band and a high molecular weight signal, indicating that the normal locus is hypomethylated. Hybridization with the junction probe shows the expected 0.6 kb band of a complete *Msp*I digest, and a 1.1 kb partial digest band (for the same reasons as described above). The *Hpa*II digest of all the tissues examined fails to detect any low molecular weight band, indicating that all the *Msp*I sites in the $\gamma\beta$-thalassaemia locus are methylated (Fig. 5, j probe). A *Hin*III/*Hpa*II double digest experiment (Fig. 5, $\gamma\beta$ fetal liver DNA) indeed shows that all the *Hpa*II/*Msp* sites both at the 5′ and the 3′ side of the gene (7 sites) are methylated, because the junction probe detects only the uncleaved *Hin*III fragments in the double digest. Compared with the normal locus (β probe) these results show that certainly the methylation pattern at the 3′ side of the gene has been changed (there are no sites to measure in the normal locus at the 5′ side of the gene). Consequently, the junction DNA is hypermethylated in the erythroid and non-erythroid tissues examined and retains this pattern (including the β-gene) when it is juxtaposed to the 5′ side of the β-gene (Fig. 5). From both the DNase I sensitivity and methylation data, we conclude that the β-globin gene at the mutant locus is present in a transcriptionally inactive form.

Discussion

Van der Ploeg *et al.*[11] have suggested four different possibilities to explain the inactivation of the β-globin gene *in vivo* in the Dutch case of $\gamma\beta$-thalassaemia: (1) a second mutation (for example, a stop codon or splice mutation) unrelated to the deletion, (2) inactivation of the transcriptional unit by the deletion (such as a defect in promoter sequences), (3) a *trans* effect caused by the deletion of a regulating component which affects both the normal and mutant locus and (4) a long-range *cis* effect involving either the deletion of regulatory sequences, or the transposition of inactive sequences resulting in a 'position effect'. The sequence and transcription data presented here exclude the

first two of these explanations, since both the primary sequence and the transient expression of the mutant gene are the same as for the normal gene. Our sequence data, however, do not exclude changes in the region upstream from −100, which might affect the expression of the gene *in vivo*[26,27,33]. To this we can add that the presence of translocated sequences *per se* next to the 5′ regions of the β-globin gene does not have a negative effect on the expression of the gene: the *Kpn* subclone which contains 2.5 kb of sequences from the translocated DNA is accurately and efficiently transcribed in our experimental system, although we cannot exclude that translocated sequences even further upstream might have such an effect.

The third explanation which postulates the lack of a *trans*-acting component originating in the deleted region of the chromosome in the γβ-thalassaemia, leaving both β-genes active, but at a reduced efficiency, was described as unlikely[11], but could not be excluded. This explanation predicts that both the β-globin genes of the patient would be in a transcriptionally 'active' state. Our data clearly contradict this prediction, since they show the normal locus to be in an 'active' state, and the affected locus to be in an 'inactive' state.

This observation clearly favours the fourth explanation which postulates a *cis* influence of sequences far from the β-globin gene. Whether this *cis* effect is exerted by the removal of regulating sequences, or the addition of actively suppressing sequences upstream from the β-globin gene is at present unclear. Any of these possibilities could block the normal progression of globin gene expression during normal erythropoiesis[28], or alter the ability of this chromosomal region to be expressed, for example, by the use of a different replication origin[29]. Either way, in both cases, the net result is a position effect similar to those found in *Drosophila*.

The transposed DNA in γβ-thalassaemia is normally found in an area of the chromatin which is DNase I insensitive and hypermethylated. Consequently, after the deletion the affected β-globin gene is present in a chromatin domain that is not expressed in erythroid tissue, resulting in the silencing of the β-globin gene.

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1. Weatherall, D. J. & Clegg, J. B. *Cell* **29,** 7–9 (1982).
2. Westaway, D. & Williamson, R. *Nucleic Acids Res.* **9,** 1777–1788 (1981).
3. Busslinger, M., Moschonas, N. & Flavell, R. A. *Cell* **27,** 289–298 (1981).
4. Spritz, R. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **78,** 2455–2459 (1981).
5. Chang, J. C. & Kan, Y. W. *Proc. natn. Acad. Sci. U.S.A.* **76,** 2886–2889 (1979).
6. Moschonas, N. *et al. Nucleic Acids Res.* **9,** 4391–4407 (1981).
7. Orkin, S. H. & Goff, S. C. *J. biol. Chem.* **256,** 9782–9784 (1981).
8. Fritsch, E. F., Lawn, R. M. & Maniatis, T. *Nature* **279,** 598–603 (1979).
9. Bernards, R. & Flavell, R. A. *Nucleic Acids Res.* **8,** 1521–1534 (1980).
10. Kan, Y. W., Forget, B. G. & Nathan, D. G. *New Engl. J. Med.* **286,** 129–134 (1972).
11. van der Ploeg, L. H. T. *et al. Nature* **283,** 637–642 (1980).
12. Orkin, S. H., Goff, S. & Nathan, D. G. *J. clin. Invest.* **67,** 879–884 (1981).
13. Vanin, E., Henthorn, P., Kioussis, D., Grosveld, F. & Smithies, O. *Cell* (in the press).
14. Grosveld, F. G. *et al. Nucleic Acids Res.* **10,** 6715–6732 (1982).
15. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65,** 499–560 (1980).
16. Lawn, R. M., Efstratiadis, A., O'Connell, C. & Maniatis, T. *Cell* **21,** 647–651 (1980).
17. Grosveld, G. C., deBoer, E., Shewmaker, C. K. & Flavell, R. A. *Nature* **295,** 120–126 (1982).
18. Wigler, M. *et al. Cell* **16,** 777–785 (1979).
19. Berk, A. J. & Sharp, P. A. *Cell* **12,** 721–732 (1977).
20. Weintraub, H. & Groudine, M. *Science* **93,** 848–858 (1976).
21. van der Ploeg, L. H. T. & Flavell, R. A. *Cell* **19,** 947–958 (1980).
22. McGhee, J. D. & Ginder, G. D. *Nature* **280,** 419–420 (1979).
23. Mandel, J. L. & Chambon, P. *Nucleic Acids Res.* **7,** 2081–2103 (1979).
24. Waalwijk, C. & Flavell, R. A. *Nucleic Acids Res.* **5,** 3231–3236 (1978).
25. Busslinger, M., deBoer, E., Wright, S., Grosveld, F. G. & Flavell, R. A. *Nucleic Acids Res.* **11,** 3559–3569 (1983).
26. Stalder, J., Groudine, M., Dodgson, J. B., Engel, J. D. & Weintraub, H. *Cell* **19,** 973–980 (1980).
27. McGhee, J. D., Wood, W. I., Dolan, M., Engel, J. D. & Felsenfeld, G. *Cell* **27,** 45–55 (1981).
28. Jones, R. W. Old, J. M., Trent, R. J., Clegg, J. B. & Weatherall, D. J. *Nucleic Acids Res.* **9,** 6813–6825 (1981).
29. Smithies, O. *J. cell. Physiol.* Suppl. **1,** 137–143 (1982).
30. Auffrey, C. & Rougeon, F. *Eur. J. Biochem.* **107,** 303–314 (1980).
31. Weaver, R. F. & Weissmann, C. *Nucleic Acids Res.* **6,** 1175–1193 (1979).
32. Wu, C., Bingham, P. M., Livak, K. J., Holmgren, R. & Elgin, S. C. R. *Cell* **16,** 797–806 (1979).
33. McKnight, S. L. *Cell* **31,** 355–365 (1982).

LETTERS TO NATURE

Can pregalactic stars or black holes generate an IR background?

B. J. Carr*†, J. McDowell* & H. Sato†

* Institute of Astronomy, University of Cambridge, Madingley Road, Cambridge CB3 0HA, UK
† Research Institute for Fundamental Physics, Kyoto University, Kyoto 606, Japan

Matsumoto *et al.* report[1] that they have detected an IR background in the waveband 2–5 μm which has a density $\Omega_R \sim 10^{-4}h^{-2}$ in units of the critical density ($\rho_{crit} = 5\times10^{-30}h^2$ g cm^{-3} with $H_0 = 50h$ km s^{-1} Mpc^{-1}) and an approximately black-body spectrum with a temperature of about 1,500 K. They claim that the intensity is too high to be explained by zodiacal light, interplanetary dust, low mass halo stars or galactic emission, and suggest that it is derived from a generation of pregalactic (population III) stars. We argue here that this is possible only if the stars began forming at a redshift exceeding 40 with a density parameter $\Omega_* \sim 1$ and a mass in the range 10^2–10^5 $M_\odot$. With such a high density, they could avoid over-enriching the background medium with heavy elements only if they collapsed to black holes after their nuclear burning phase[2]. These holes may also have contributed to the IR background, provided they formed optically thick accretion disks. However, we argue that holes could generate the entire background only if they have a density parameter $\Omega_B \sim 0.1$ and a mass in the range 10^6–$10^8 M_\odot$, in which case they would be too large to have stellar precursors.

We first assume that the stars are all VMOs with a mass M exceeding 200 $M_\odot$, in order to collapse to black holes[3,4] and thereby avoid over-enrichment; in this case, their luminosity is $L = 1.3\times10^{38}\,(M/M_\odot)$ erg s^{-1} and their surface temperature is $T_s \simeq 10^5$ K. If they all form at the same redshift z_*, their radiation will also be generated at that epoch providing their main-sequence time ($t_{MS} \simeq 2\times10^6$ yr) is less than the expansion time, which requires $z_* < 300h^{-2/3}$. This means that the background radiation from the stars should just have a redshifted black-body spectrum with present temperature $T_{bb}^{obs} \simeq 10^5\,(1+z_*)^{-1}$K, providing there is no absorption by grains or neutral hydrogen. The integrated energy density of the background is

$$\Omega_R = 0.004\left(\frac{f_b X_0}{0.6}\right)\Omega_*(1+z_*)^{-1} \qquad (1)$$

in units of the critical density, where the coefficient is the product of the efficiency of energy release in burning hydrogen to helium (0.007) and the fraction f_b of the hydrogen mass burnt to helium, and X_0 is the initial hydrogen abundance ($1 \geq X_0 \geq 0.75$); $f_b X_0 \simeq 0.6$ for a VMO with $X_0 = 0.75$ (ref. 3). We will describe the spectrum in terms of the density parameter $\Omega_R(\nu) \equiv 4\pi\nu i(\nu)/\rho_{crit}c^3$ (where $i(\nu)$ is the intensity per unit frequency interval) because this best emphasizes the energetic requirements of our model. The predicted spectrum is then

$$\Omega_R = 6.5\times10^{-4}\left(\frac{f_b X_0}{0.6}\right)\left(\frac{\Omega_*}{1+z_*}\right)\left(\frac{x^4}{e^x-1}\right) \qquad (2)$$

where $x \equiv h\nu(1+z_*)/kT_s$; this peaks at $\nu = 8.1\times10^{15}\,(1+z)^{-1}$ Hz, corresponding to $\lambda = 3.7\times10^{-6}(1+z_*)$ cm.

Equation (2) is compared with the data in Fig. 1. If we assume $X_0 = 0.75$, a representative fit is shown by curve *a* in Fig. 1 and

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