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A gene controlling fetal hemoglobin expression in adults is not linked to the non-α globin cluster

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The possible linkage between a gene causing heterocellular hereditary persistence of fetal hemoglobin (HPFH) and human non-α globin loci has been studied in a large Sardinian family. In this family a homozygous β°-thalassemic patient was found, with an unusually mild form of this disease, which was ascribed to the co-existence of a gene causing heterocellular HPFH. DNA polymorphisms in the non-α globin cluster were analyzed by restriction enzyme digestion with *Hinc*II, *Hind*III and *Bam*HI and with ε-, γ- and β-globin probes; the pattern of inheritance of these polymorphisms indicates that the HPFH gene is transmitted with one β°-thalassemic gene in a single instance, with the second β°-thalassemic gene in three instances and with a normal β-globin gene in two cases. These data indicate that this HPFH gene is not linked to the non-α globin gene cluster, in contrast to previous observations with different HPFH genes, and suggest that this gene might code for diffusible substances acting, directly or indirectly, on γ-globin gene expression.

Key words: linkage analysis/DNA polymorphisms/gene regulation

Introduction

In humans, inherited persistence of fetal hemoglobin is a very heterogeneous group of conditions, characterized by variably decreased (or absent) synthesis of adult δ- and β-globin chains, and increased expression in adult life of fetal hemoglobin (HbF) (containing $^{G}\gamma$- and/or $^{A}\gamma$-globin chains), which appears to be present in all erythrocytes [pancellular hereditary persistence of HbF (pancellular HPFH) and δβ-thalassemia] (Weatherall and Clegg, 1981). In all types so far analyzed at the molecular level, the defect consists either in the deletion of DNA segments in the non-α globin cluster (Orkin *et al.*, 1978; Mears *et al.*, 1978; Ottolenghi *et al.*, 1979, 1982; Fritsch *et al.*, 1979; Tuan *et al.*, 1979, 1980; Bernards *et al.*, 1979; Bernards and Flavell, 1980; Jones *et al.*, 1981) or in mutations (so far unidentified) linked to the same group of genes (Bernards and Flavell, 1980; Tuan *et al.*, 1980; Ottolenghi *et al.*, 1982; Basley *et al.*, 1982; Jones *et al.*, 1982). More importantly, when the proper genetic analysis could be carried out, it was apparent that the genetic determinant(s) for this group of disorders only affects globin genes *cis* to the defect (Weatherall and Clegg, 1981).

In addition to these rare forms, HbF synthesis may persist at slightly raised levels in the so called heterocellular HPFH, in which HbF appears to be confined to only a relatively small proportion of erythrocytes (F cells), and the activity of adult δ- and β-globin genes is essentially normal (Weatherall and Clegg, 1981). Although the phenotypic difference might suggest a different mechanism for this group of disorders, linkage analysis of polymorphic restriction endonuclease sites in the non-α globin gene cluster suggested that the genetic determinants of this condition lie either within or only at some distance from the ϵ-$^{G}\gamma$-$^{A}\gamma$-δ-β globin cluster on chromosome 11 (Jones *et al.*, 1981; Old *et al.*, 1982). We show here that a determinant of the HbF level in a family with heterocellular HPFH (Cappellini *et al.*, 1981) is not linked to the non-α globin cluster.

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Results

A large Sardinian family was described a few years ago (Cappellini *et al.*, 1981), showing the interaction of β°-thalassemia and heterocellular HPFH. Four generations were studied; in the second one, a homozygous β°-thalassemic patient was detected, with a very mild form of this otherwise lethal disease. The reason for this unusual clinical picture was ascribed to the co-existence of a gene for heterocellular HPFH (which is known to ameliorate β-thalassemia by stimulating γ-globin synthesis, thereby decreasing the α/non-α globin synthetic ratio), which was transmitted to all three offspring in the third generation and to a child in the fourth generation together with a β°-thalassemia gene, and to a second child in the fourth generation together with a normal β-globin gene. This was interpreted as evidence of a possible crossing-over event leading to the segregation of the HPFH gene from the β°-thalassemic gene. To test this hypothesis and to try to localize the site of the putative crossing-over, we examined polymorphic restriction endonuclease sites in the non-α globin cluster to identify the haplotypes linked to β°-thalassemia and possibly to HPFH. The analysis was carried out with three different restriction enzymes, which yielded four informative polymorphisms allowing detailed analysis of the DNA region spanning ~60 kb pairs from a *Hinc*II site situated ~4 kb 5′ to the ε-globin gene to a *Bam*HI site ~20 kb 3′ to the β-globin gene. In this family, with an ε-globin probe and *Hinc*II, polymorphic fragments of either 8 or 3.7 kb (Antonarakis *et al.*, 1982) are detected (Figure 1); with a γ-globin probe and *Hind*III (Figure 2), $^{G}\gamma$-globin fragments of 8 or 7.2 kb and $^{A}\gamma$-globin fragments of 3.5 or 2.7 kb (Jeffreys, 1979) are seen; with a β-globin probe and *Bam*HI (Figure 3) fragments of either 22 or 8.3 kb are revealed (Kan *et al.*, 1980). The presence of the polymorphic restriction site (+) is indicated by the smaller fragment; the absence (−) by the larger one.

As shown in Figure 4, the β°-thalassemic homozygote, HPFH heterozygote in the second generation (II 2) is heterozygous for two different haplotypes (ϵ-$^{G}\gamma$-$^{A}\gamma$-β: − + − +/− − − +); the study of his three offspring (III 2; III 3; III 4) shows that the β°-thalassemia gene is transmitted in two cases (III 2 and III 4) in conjunction with the − + − + haplotype, and in the remaining one (III 3) with the − − − + haplotype, although all three siblings are also HPFH carriers. In the successive generation, the β°-

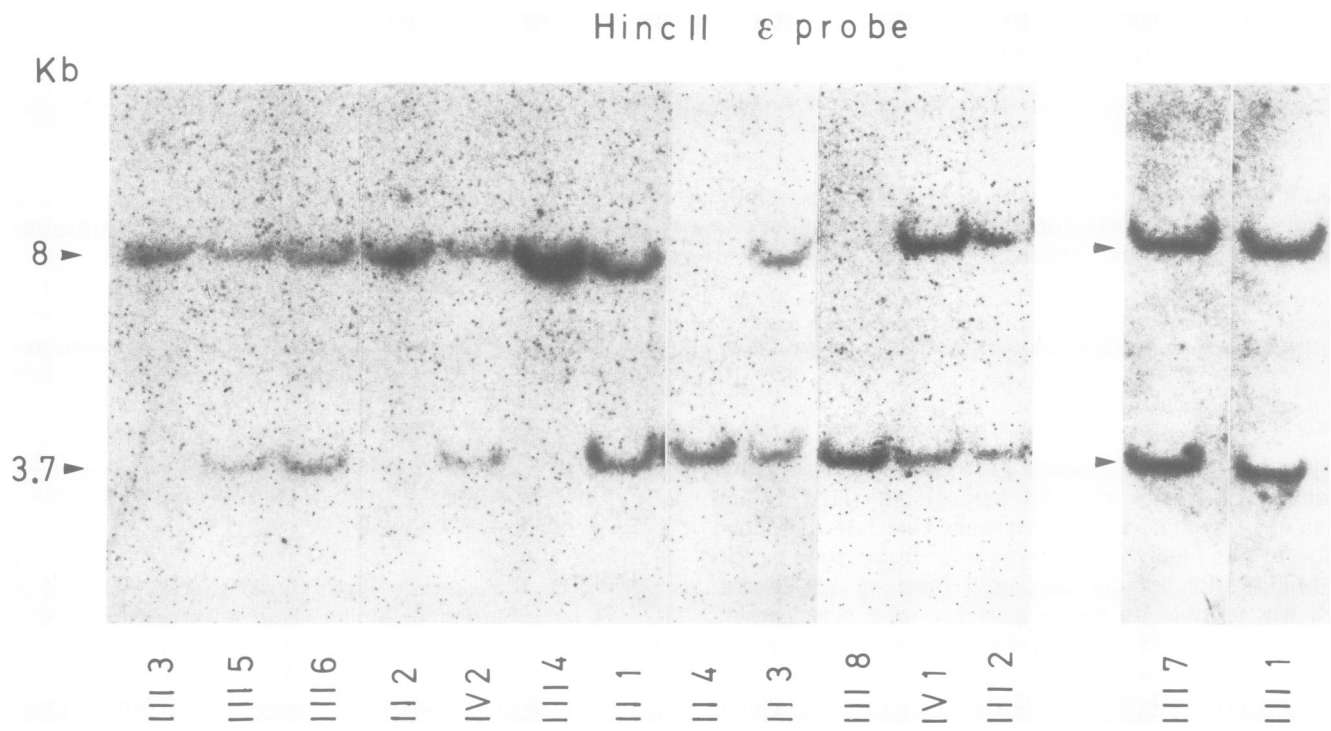

Fig 1. *Hinc*II polymorphisms detected with ϵ-globin probe. Fragment sizes are indicated in kilobase pairs (kb). The 8-kb band indicates the absence, and the 3.7-kb band the presence of the polymorphic *Hinc*II site.

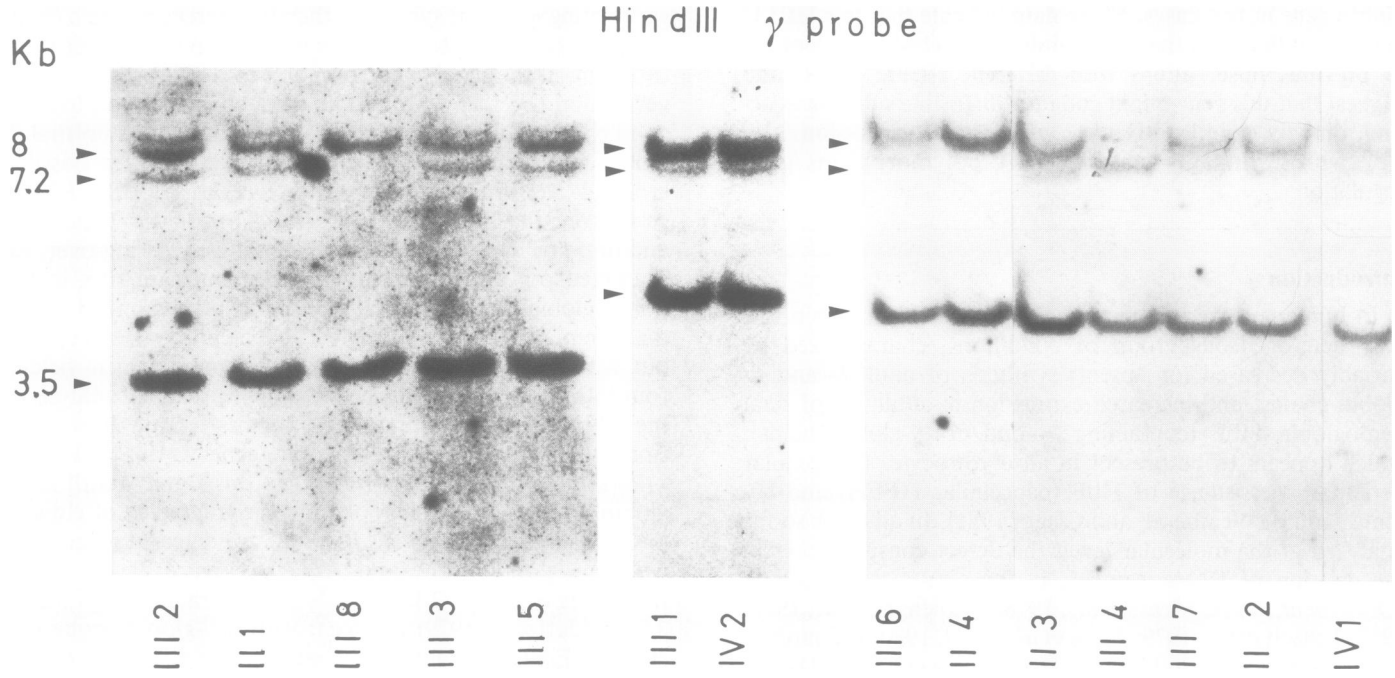

Fig. 2. *Hind*III polymorphisms detected with γ-globin probe. The 8-kb band indicates the absence, and the 7.2-kb band the presence of the polymorphism in the $^{G}\gamma$-globin gene; the 3.4-kb band indicates the absence of the polymorphism in the $^{A}\gamma$-globin gene (a 2.7-kb band is expected in the presence of the polymorphic site).

thalassemia gene is transmitted to only one offspring (IV 2) in association with the expected haplotype (− + − +) and with HPFH, while HPFH alone is transmitted to the second sibling (IV 1) in association with the normal β-globin gene and its haplotype (+ − − +). In summary, the HPFH determinant is transmitted in association with a normal β-globin gene (in one case), a β°-thalassemic gene (in two cases), and a different β°-thalassemic gene (in one case). These data, suggesting the absence of linkage between HPFH determinant and non-α globin cluster, are further supported by the analysis carried out in a second branch of the family stemming from a sister (II 4) of the homozygous β°-thalassemic patient, and showing slightly increased HbF and F cell levels, compatible with the diagnosis of heterocellular HPFH. This

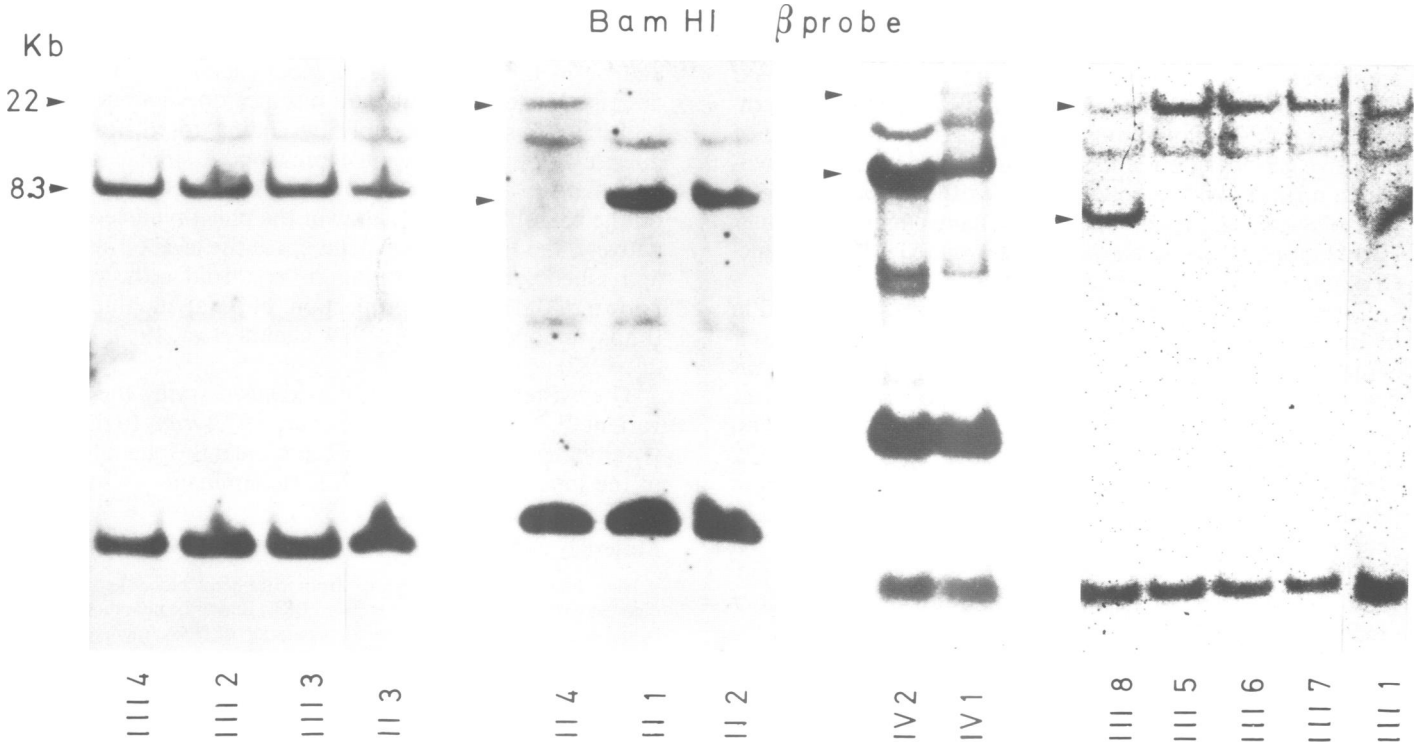

Fig. 3. *Bam*HI polymorphisms detected with β-globin probe. The 22-kb band indicates the absence, and the 8.3-kb band the presence of the polymorphic site. Polymorphic bands are indicated by arrows; additional non-polymorphic δ- and β-globin bands are detected.

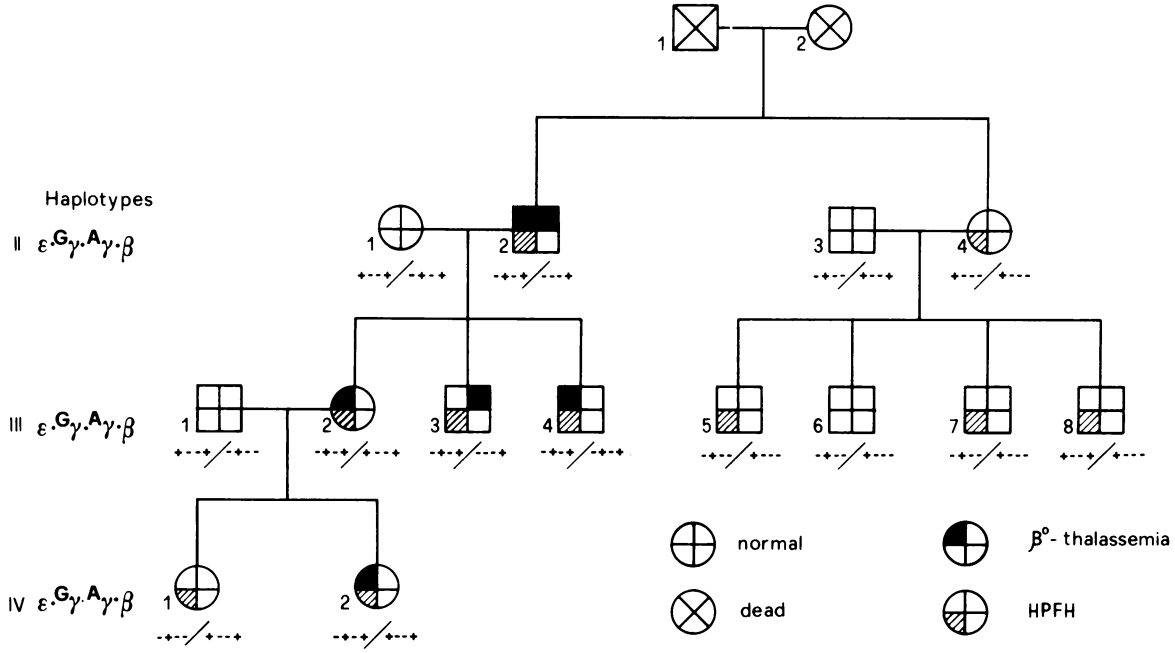

Fig. 4. Inheritance of β°-thalassemia, heterocellular HPFH and restriction enzyme polymorphisms in the family under study. Presence (+) and absence (−) of polymorphic sites are shown and refer to ε- $^{G}\gamma$- $^{A}\gamma$- and β-globin sites, respectively. The assignment of haplotypes to specific chromosomes is discussed in the text.

subject was homozygous for the + − − − haplotype, which is different from those associated with the β°-thalassemic genes in her brother (II 2, − + − +/− − − +) and cannot be explained simply by a crossing-over occurring between the ε-$^{G}\gamma$-$^{A}\gamma$-cluster and the β-globin gene. Thus, a total of three out of six informative subjects demonstrate absence of linkage between HPFH and the β-globin gene (the haplotypes of the four offspring of II 4 were not informative due to the homozygous haplotype of the mother).

Discussion

The study of heterocellular HPFH is important for both practical and theoretical reasons. This condition in fact is

relatively common (up to 1% carriers in European populations) and, when in association with β-thalassemia or HbS, considerably alleviates the clinical symptoms (Weatherall and Clegg, 1981). From the theoretical standpoint, it has been emphasized that this condition, characterized by the absence of extensive gene deletions and of unbalanced globin chains synthesis, might provide a better model for the understanding of physiological regulation of globin chains than pancellular HPFH and δβ-thalassemia, usually associated with unbalanced globin synthesis (Old *et al.*, 1982).

Classical genetic analysis suggested that gene(s) involved in heterocellular HPFH are linked to the non-α globin cluster (Weatherall and Clegg, 1981); this provisional evidence was recently confirmed by the application of restriction enzyme analysis of DNA polymorphisms in two different families, one of British and the other of Indian origin (Old *et al.*, 1982); in the latter, the analysis of one possible recombinant out of a total of nine informative subjects, suggested that the genetic determinant of HPFH, although linked to the non-α globin cluster, is outside the region spanning the ε-β globin genes. On the basis of this evidence and other considerations (Jones *et al.*, 1982; Old *et al.*, 1982), the authors favored the hypothesis that a mutation causing heterocellular HPFH may be active in *cis*, possibly by causing a delay in the propagation of a change in chromosome structure necessary for globin gene expression.

The previous phenotypic analysis (Cappellini *et al.*, 1981) of our family had demonstrated the association of the HPFH determinant with the β°-thalassemic gene in four out of five individuals and with a normal β-globin gene in the fifth one; this result could be explained either by non-linkage between the HPFH and the β-globin gene, or by a crossing-over segregating the HPFH gene from the linked β°-thalassemic gene. This uncertainty can now be resolved by direct analysis at the DNA level. The scheme shown in Figure 4 was constructed on the basis of the existence in the family of three individuals (III 2; III 3; III 4) whose six haplotypes can be defined without ambiguity, and is the only one compatible overall with the inheritance of these haplotypes in the family. In particular, in contrast with the previous phenotypic studies, which suggested the possible existence of linkage between the HPFH determinant and a specific β°-thalassemic locus, our analysis clearly shows that this is not the case: in fact, in III 2 and III 4, the HPFH gene must be inherited with the − + − + haplotype, while in III 3 with the − − − + haplotype (indeed, if the − − − + haplotype of III 3 were not linked to the paternal II 2 β°-thalassemic gene, and were instead inherited from the mother, II 1, in association with the normal β-globin gene, the resulting haplotypes in the mother − − − + / + + − + would then be incompatible with the known haplotypes of III 4, a − + − + homozygote). Thus, we end up with two instances (III 3; IV 1) out of five (III 2; III 3; III 4; IV 1; IV 2) of non-linkage between HPFH and β-globin locus in this branch of the family; this figure might increase to three out of six possibly informative haplotypes if the raised F cell and HbF levels of II 4 (+ − − − homozygote) are due to the same HPFH gene.

These data strongly indicate that this HPFH gene is either on a different chromosome or at a great distance on the same chromosome from the β-globin locus. This conclusion has the important implication that this HPFH gene is likely to act in a different way from most, and possibly all, of the other HPFH genes described so far; at variance with this group of genes, which are adjacent to the non-α globin locus (and might therefore affect the structure of chromatin inside and/or in the vicinity of the globin loci), the determinant described here should act at a distance on γ-globin genes on both chromosomes, possibly by coding for diffusible products affecting globin gene expression. A similar suggestion was made several years ago by Martinez and Colombo (1974) on the basis of a family study at the phenotypic level. Alternatively, the HPFH gene might act at the level of maturation and kinetics of proliferation of erythroid cells, which are known to affect the proportion of fetal globin synthesis (Papayannopoulou *et al.*, 1979; Gianni *et al.*, 1980; Peschle *et al.*, 1982).

The existence of HPFH individuals carrying the common variant $^{A}\gamma^{Sardinia}$ gene (Saglio *et al.*, 1979) *trans* to the normal $^{A}\gamma$-globin gene will allow confirmation at the phenotypic level of the *trans* effect of the HPFH determinant.

Materials and methods

High mol. wt. DNA prepared from peripheral blood leukocytes was digested with restriction enzymes (from BRL) under the conditions specified by the manufacturer. Electrophoretic separation of DNA fragments, transfer to nitrocellulose sheets, hybridization and washing procedures were according to Jeffreys and Flavell (1977) and Ottolenghi *et al.* (1979). Gamma globin probe was plasmid pHγG1 (Little *et al.*, 1978), a gift from R.Williamson and P.Little; ε- and β-globin probes were kindly provided by T.Maniatis (β *Pst*I 4.4-kb fragment and ε *Bam*HI 0.7-kb fragment were subcloned in pBR322; Proudfoot *et al.*, 1980).

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