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Genetics

A naturally occurring deletion in the mouse *Hbb^s* β-globin gene cluster

(embryonic β-globin/pseudogene/*Hbb-bh0* gene/DNA rearrangements)

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ABSTRACT **A restriction fragment size difference of 1.9 kilobases exists between the C57BL/10 (*Hbb^s*) and BALB/c (*Hbb^d*) β-globin gene clusters in the vicinity of the *Hbb-y* and *Hbb-bh0* genes. This length difference is the result of a deletion that removes the majority of the C57BL/10 *Hbb-bh0* gene. The *Hbb-bh0* deletion appears to be widespread among *Hbb^s*-carrying mice. In addition to the deletion, 119 base pairs of DNA of unknown origin are found in C57BL/10 DNA at the point of discontinuity. The existence of this deletion is inconsistent with reports of transcripts homologous to the third exon of the *Hbb-bh0* gene in both C57BL/10 embryos and in a cell line carrying the *Hbb^s* haplotype. We propose that the *Hbb-bh0* gene is a recently disabled pseudogene.**

Vertebrate erythroid differentiation is controlled by a complicated series of gene switches responsible for the changes in erythrocyte cell populations and hemoglobin chain synthesis. During the embryonic development of the mouse, nucleated erythroid cells of yolk-sac origin produce two β-globin-like chains, y and z; the α-globin-like chain, x; and the adult α-globin (1). These proteins are the subunits of the embryonic hemoglobins EI (x_2y_2), EII (α_2y_2), and EIII (α_2z_2) (1–3). EI and EIII constitute the early embryonic hemoglobins, which by day 12 of gestation are replaced by the major embryonic hemoglobin EII (4).

The genes encoding the β-globin-like chains of these hemoglobin tetramers lie within 65 kilobases (kb) of DNA (5, 6). The order of genes that comprise the murine β-globin complex is 5′ *Hbb-y, Hbb-bh0, Hbb-bh1, Hbb-bh2, Hbb-bh3, Hbb-b1, Hbb-b2* 3′; for simplicity, we shall refer to these genes herein as *y, bh0, bh1, bh2, bh3, b1*, and *b2*. The *y* gene encodes the embryonic β-globin-like y protein (7), *bh1* encodes the minor early embryonic β-globin-like z protein (8), *bh2* and *bh3* are pseudogenes (9, 10), and *b1* and *b2* encode the adult β-globin chains (11).

The function of *bh0* is not clear. However, it is more similar to *bh1* than to any other gene in the cluster. Throughout their coding sequences, *bh0* and *bh1* are 93% homologous (12). No protein corresponding to the *bh0* gene has been identified.

Genetic variants of the y and β-globin proteins have been identified through the use of starch gel electrophoresis (13, 14). The inheritance of these electromorphs defines three allelic complexes or haplotypes: *Hbb^d*, *Hbb^p*, and *Hbb^s*. At the DNA level, these three haplotypes are similar. In each haplotype, the same set of genes and pseudogenes is separated by stretches of intergenic DNA of comparable length. In two regions only have exceptions to this general colinearity been described (15–17). In each case, the length difference is attributable to the integration of repetitive elements rather than to the rearrangement of intergenic DNA *per se*. Here we report an unexpected difference in the β-globin cluster organization between the *Hbb^s* and *Hbb^d* haplotypes. The *Hbb^s* complex carries a 2.0-kb deletion, which has removed the majority of the *bh0* gene. This finding is inconsistent with earlier reports of bh0 transcripts from mouse embryos and from murine erythroleukemia (MEL) cells, each carrying the *Hbb^s* haplotype (8, 18). We propose that even in the *Hbb^d* haplotype, the intact *bh0* gene is effectively a pseudogene and that its deletion from the *Hbb^s* haplotype illustrates the contraction of a gene family and one fate of a dispensable gene.

MATERIALS AND METHODS

Materials. Restriction enzymes, *Escherichia coli* DNA polymerase I holoenzyme and Klenow fragment, and sequencing primers were purchased from Boehringer Mannheim. Phage T4 DNA polymerase, terminal deoxynucleotidyl-transferase, and oligonucleotides were purchased from International Biochemicals (London). Dideoxynucleoside triphosphates were purchased from P-L Biochemicals. [α-^{32}P]dCTP was obtained from New England Nuclear.

Cloning and DNA Sequence Analysis. C57BL/10 *Eco*RI restriction fragments H, P, and E (Fig. 1) were subcloned from the genomic clone BA12 (19) into the M13 vector mp8. The P subclone is designated C505. The BALB/c clone pHE100, containing the *Eco*RI fragment B (see Fig. 1), was a gift from S. Hardies. Fragments to be sequenced were subcloned into mp8 or mp19 and deleted further as necessary by using the "Band-Aid" method (20). The sequence of these clones was determined by the dideoxy chain-termination method (21).

Hybridization Probes, Nick-Translation, and Hybridization. Hybridization probes were prepared either by electroelution from an acrylamide gel or by dissolving a low-melting agarose gel, followed by extractions with phenol and chloroform and precipitation with ethanol. Fragments were nick-translated to specific activities of 1–3 × 10^8 cpm/μg as described by Maniatis *et al.* (22). Hybridizations were carried out at 42°C in 50% formamide/10% dextran sulfate/0.9 M NaCl/0.09 M sodium citrate, pH 7, essentially as described by Wahl *et al.* (23).

Preparation of Genomic DNA. Mice of the inbred strains AU/Ss, BALB/c, C57BL/10, SWR, SM, CE, DBA/2, CBA, SJL, LP, and CE were purchased from The Jackson Laboratory. High molecular weight DNA was prepared from four male mice of each strain (after removal of skin and digestive tract) as described by Blin and Stafford (24).

RESULTS

The C57BL/10 β-Globin Gene Cluster Carries a Deletion of *bh0*. Restriction maps of the C57BL/10 and BALB/c em-

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Abbreviations: bp, base pair(s); kb, kilobase(s); IVS, intervening sequence; MEL cells, murine erythroleukemia cells.

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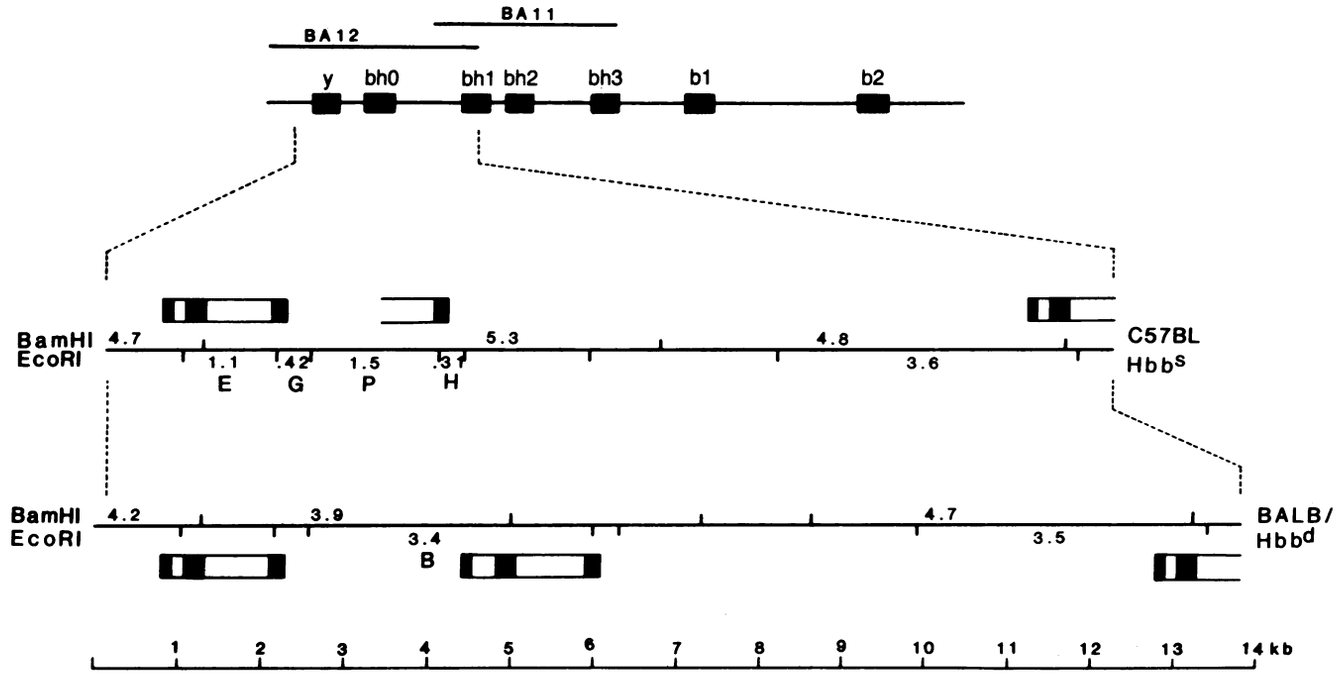

FIG. 1. The embryonic region of the β-globin gene cluster. (*Upper*) The entire 65-kb β-globin gene cluster. The genomic segments carried by the C57BL/10 clones BA11 and BA12 are indicated as bars above the β-globin gene cluster. (*Lower*) *Bam*HI and *Eco*RI restriction maps of the Hbb^d and Hbb^s embryonic β-globin gene region. In this and subsequent figures, restriction fragment lengths are given in kb and exons are shaded.

bryonic β-globin gene regions are illustrated in Fig. 1. The *Eco*RI maps are similar throughout the 14 kb with one major exception. The BALB/c fragment B is 3.4 kb long, whereas the C57BL/10 fragment P, in the corresponding position, is only 1.5 kb long. In addition, the BALB/c fragment B hybridized weakly to a nick-translated adult β-globin cDNA clone (25), whereas the P fragment did not (data not shown).

We established the allelic relationship of the BALB/c and C57BL/10 fragments B and P by hybridizing Southern blots of pHE100 (containing the *Eco*RI fragment B) digested with *Hin*dIII, *Hin*dIII/*Eco*RI, *Hin*dIII/*Bgl* II, and *Hin*dIII/*Acc* I with nick-translated fragment P subclone C505 (data not shown). This clone hybridized to both the left and right ends of fragment B (Fig. 2) but not to the interior 1000-base-pair (bp) *Bgl* II/*Hin*dIII or 620-bp *Hin*dIII fragments. Since these internal fragments contain the *bh0* first and second coding blocks, as well as 1000 bp of 5′ flanking DNA (12), the difference in length between fragments B and P is most simply explained as a deletion that includes the first and second coding blocks of the *bh0* gene. The 590-bp *Hin*dIII/*Bgl* II fragment at the left in Fig. 2 and the 390-bp *Hin*dIII/*Acc* I fragment at the right hybridized weakly and flank the nonhybridizing bands, suggesting that the deletion endpoints map within these fragments.

Characterization of the *bh0* Deletion. To determine the distribution of the deletion among other inbred strains and to confirm that the C57BL/10 genomic clone BA12 was not rearranged, we digested genomic DNA from several strains (Fig. 3) with *Bam*HI and hybridized Southern blots with nick-translated C57BL/10 fragment E (Fig. 1). The probe overlaps the *Bam*HI site in the *y* gene and so should hybridize to two *Bam*HI fragments, one containing the 5′ portion of *y* and the other containing the 3′ part of *y* as well as the DNA spanning the *bh0* deletion. As expected from the genomic clone restriction maps, the probe hybridized to a 5.3-kb C57BL/10 genomic fragment and a 3.9-kb BALB/c genomic fragment (Fig. 3, lanes f and e, respectively). The second hybridizing band in each lane in Fig. 3 contains the 5′ portion of the *y* gene. All Hbb^d strains tested (lanes a–e), as well as the Hbb^p strain (lane g), show hybridization patterns identical to that of BALB/c. Similarly, all hybridization patterns of Hbb^s-carrying strains (lanes f and h–l) are characteristic of

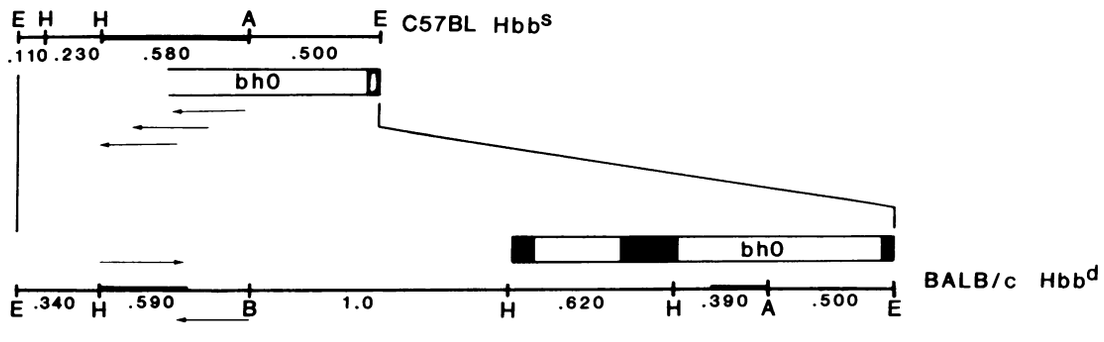

FIG. 2. Sequencing strategy. The restriction maps of the C57BL/10 1.5-kb *Eco*RI fragment P (*Upper*) and BALB/c 3.4-kb *Eco*RI fragment B (*Lower*) are depicted. (Not all sites are shown.) The sequencing strategy is denoted by arrows above and below each fragment map. Thick lines denote the regions whose sequences are shown in Fig. 5.

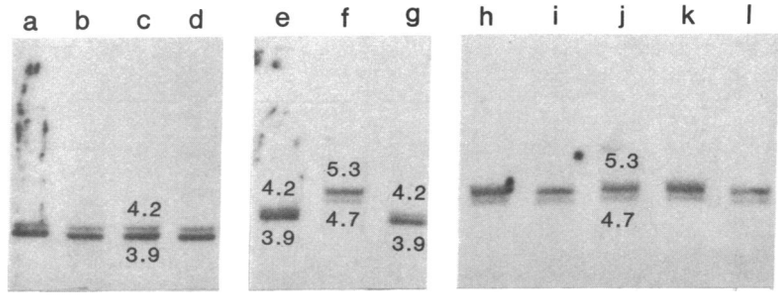

FIG. 3. Distribution of the *bh0* deletion among inbred mice. Genomic DNA (10 μg) was digested with *Bam*HI, run on 0.7% agarose gels, and blotted. Lanes a–d were electrophoresed slightly longer than lanes e–l. (*Left*) Hbb^d haplotype DNAs are in lanes a–d: a, CBA; b, DBA; c, LP; and d, BALB/c. (*Center*) Reference haplotypes are in lanes e–f: e, BALB/c (Hbb^d); f, C57BL/10 (Hbb^s); and g, AU/Ss (Hbb^p). (*Right*) Hbb^s haplotype DNAs are in lanes h–l: h, CE; i, SM; j, SWR; k, SJL; and l, C57BL/10. The blot was hybridized with a nick-translated C57BL/10 *Eco*RI fragment E.

the *bh0* deletion. These data indicate that the *bh0* deletion is limited to and widespread among inbred lines carrying the Hbb^s haplotype.

A nick-translated BALB/c 620-bp *Hin*dIII fragment, mapping within the region spanned by the deletion, was hybridized to *Eco*RI and *Bam*HI digests of genomic DNA from the prototype strains C57BL/10 (Hbb^s), BALB/c (Hbb^d), and AU/Ss (Hbb^p) (Fig. 4). This probe contains the BALB/c *bh0* first and second coding blocks and is expected to hybridize to both the BALB/c *bh0* gene as well as to the homologous *bh1* gene. In lanes with BALB/c and AU/Ss DNA, the probe hybridized to *Bam*HI and *Eco*RI fragments corresponding to the *bh0* gene and to the *bh1* gene (Fig. 4, lanes a, b, e, and f). In contrast, in lanes with C57BL/10 DNA, only *bh1*-containing fragments hybridized. Therefore, the *bh0* first and second coding blocks were not translocated.

Sequence of the Deletion Endpoints. We determined the DNA sequence of the BALB/c 590-bp *Hin*dIII/*Bam*HI fragment and C57BL/10 580-bp *Hin*dIII/*Acc* I fragment. From the published BALB/c *bh0* sequence (12), we know that the 390-bp *Hin*dIII/*Acc* I fragment contains the 5′ portion of the *bh0* second intervening sequence (IVS2). In Fig. 5, the C57BL/10 sequence from the 580-bp *Hin*dIII/*Acc* I fragment is aligned with the corresponding sequences from the BALB/c chromosome (contained within the 590-bp *Hin*dIII/*Bgl* II fragment and the *bh0* IVS2). From the allelic *Hin*dIII sites, the BALB/c and C57BL/10 sequences are homologous for 260 bp. Throughout this region, only one base substitution and an 8-bp length difference are noted. The 8-bp length difference is located within a tract of alternating $(A\text{-}C)_n$ located 194 bp to the right of the shared *Hin*dIII site. In BALB/c the alternating segment is 13 dinucleotides long, whereas in C57BL/10 it is only 9. The alternating $(A\text{-}C)_n$ tract is imbedded within a longer region of alternating purine-pyrimidine $(R\text{-}Y)_n$. Thirty-one bases to the right of the $(R\text{-}Y)_n$ region, the BALB/c and C57BL/10 sequence homology abruptly ends. At this point, the C57BL/10 sequence becomes A+T-rich and is not homologous to any sequences

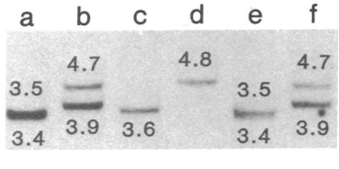

FIG. 4. Search for the *bh0* deleted sequences. Genomic DNA was digested with *Eco*RI or *Bam*HI, run on 0.7% agarose gels, and blotted. Lanes: a and b, BALB/c (Hbb^d) DNA digested with *Eco*RI and *Bam*HI, respectively; c and d, C57BL/10 (Hbb^s) DNA; e and f, AU/Ss (Hbb^p) DNA. The blot was hybridized with a nick-translated BALB/c 620-bp *Hin*dIII fragment containing the Hbb^d bh0 first and second coding blocks.

within the BALB/c 590-bp *Hin*dIII/*Bgl* II or 1000-bp *Bgl* II/*Hin*dIII fragments. This A+T-rich segment extends for 119 bp before sequence homology with the BALB/c chromosome resumes, within the *bh0* IVS2 at a point 138 bp from its 5′ end. From this point to the *Acc* I site there are 10 differences in 210 nucleotides between the C57BL/10 and BALB/c chromosomes. It is not immediately obvious why, to the left of the deletion (in intergenic DNA), the BALB/c and C57BL/10 sequences are >99% homologous (ignoring the $(C\text{-}A)_n$ length difference), whereas to the right of the deletion (within IVS2 of *bh0*), the allelic IVS2 sequences are only 95% homologous.

DISCUSSION

Characteristics of the Deletion. The number of bases removed by the deletion is larger than would be predicted on the basis of restriction fragment lengths due to the unexpected presence of the 119-bp A+T-rich segment in the Hbb^s chromosome. This A+T-rich DNA is located between the regions of BALB/c and C57BL/10 sequence homology. Whether the segment was inserted into the C57BL/10 chromosome or deleted from the BALB/c cluster is unclear. An analogous situation has been found in the case of a spontaneous deletion of the *b1* gene in the DBA strain (26). This deletion results in a β-thalassemia in the homozygous condition. In addition to the deletion of 3709 bp, 68 bp of A+T rich DNA have been inserted (D. Kuebbing, personal communication).

Thirty-one base pairs to the left of the break in homology, the repeating dinucleotide $(C\text{-}A)_n$ is found in both the C57BL/10 and BALB/c chromosome. This perfect $(C\text{-}A)_n$ tract is imbedded in a longer run of alternating purines and pyrimidines. The function, if any, of such DNA is not known. However, Hamada (27) reported that tracts of $(T\text{-}G)_n$ occur at a high frequency in human, mouse, *Drosophila*, calf, and salmon DNAs. In addition, regions of alternating $(T\text{-}G)_n$ have been implicated as having a role in recombination events (28–31). Whether or not the $(R\text{-}Y)_n$ tract to the left of the *bh0* deleted segment had anything to do with the *bh0* deletion process is unclear. However, the absence of similar sequences in BALB/c to the left and right of the deleted segment suggests that some mechanism other than homologous recombination was involved.

Is the *bh0* Gene Transcribed? Our data demonstrate that the deletion in the Hbb^s complex includes the first two exons of the *bh0* gene, 5′ flanking DNA, transcriptional regulatory region, and part of IVS2 including its 5′ splice site. These results are inconsistent with earlier reports of *bh0* transcripts in both MEL cells carrying the Hbb^s haplotype as well as in C57BL embryos (8). The RNA used in these studies was prepared from a dimethyl sulfoxide-induced MEL cell line,

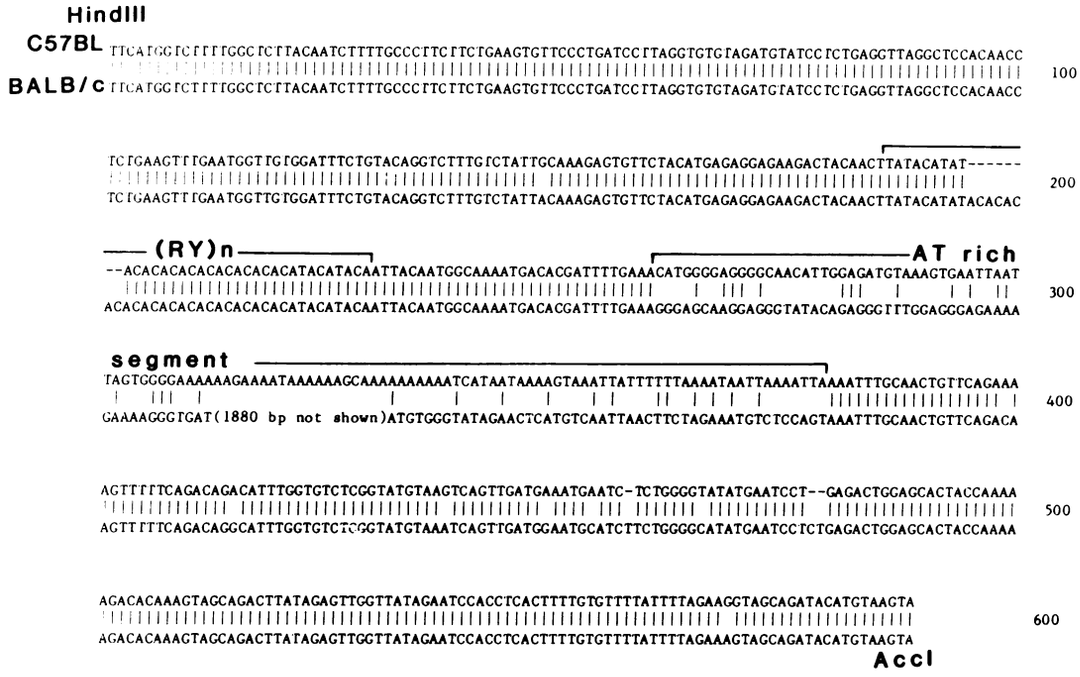

FIG. 5. Sequence of the DNA surrounding the deletion. Gaps introduced into the sequence in order to maximize homology are indicated as dashes.

GM979, and from C57BL/Cas embryos at day 10 of gestation. The investigators performed nuclease S1 protection experiments using a probe derived from a BALB/c (*Hbb*^{d}) *bh0* gene. The probe extended from a point within the *bh0* IVS*2* to a point 37 bp upstream of the polyadenylylation site. A low-abundance RNA species, whose protected size of 134 bp corresponds to the size expected for the *bh0* third exon, was observed.

Several explanations for the existence of this apparent bh0 transcript are possible: transcription originated at a cryptic promoter; RNA was transcribed from the related *y* or *bhl* genes; RNA was transcribed from another, hypothetical, related gene; and the *bh0* gene in the *Hbb*^{s} haplotype carried by the C57BL/Cas strain and GM979 cell line is not deleted. The first three possibilities are discussed below. We have no information with which to address the fourth possibility.

The *Hbb*^{s} and *Hbb*^{d} *bh0* genes are over 99% homologous throughout the third coding block and for at least 250 bp downstream from the termination codon (data not shown). For this reason, the *Hbb*^{d} probe should protect an Hbb^s transcript originating at a hypothetical cryptic promoter. As the size of the observed protected fragment corresponds to the size of a transcript spliced at the 5′ end of the third coding block, we believe that if RNA were transcribed from a cryptic promoter, then the illegitimate transcript must include a suitable donor splice site.

The third exons of the *Hbb*^{s} *y* and *bhl* genes are quite different from the *Hbb*^{d} *bh0* gene in the region downstream of the termination codon. The C57BL/10 *bhl* 3′ untranslated region is less than 65% homologous to the *bh0* 3′ untranslated region (data not shown), and no products protected from S1 nuclease should be observed. Although we do not know the sequence of the 3′ untranslated region of the C57BL/10 *y* gene, evidence suggests that only shorter fragments would be protected. Nuclease S1 mapping experiments performed with the same RNAs (from GM979 cells and C57BL/Cas embryos) but with an *Hbb*^{d} 3′-labeled probe extending from within the BALB/c bh0 third coding block to beyond the polyadenylylation site yielded bands of the *bh0* expected size as well as shorter fragments (B. A. Brown, personal communication). These shorter bands correspond to the sizes predicted for an *Hbb*^{d} y transcript protected by the bh0 probe. As the cell line and embryos from which the RNA was obtained carried the *Hbb*^{s} haplotype, we take this as indirect evidence that *Hbb*^{s} and *Hbb*^{d} *y* genes are identical in this region and, therefore, that the paradoxical bh0 transcript is not the product of the *Hbb*^{s} *y* gene.

We also addressed the hypothesis that there was another *bh0*-like third exon elsewhere in the genome. A C57BL/10 315-bp *Eco*RI fragment, H (see Fig. 1), containing the majority of the *Hbb*^{s} *bh0* third exon was used to probe *Bam*HI and *Eco*RI digests of genomic DNA (data not shown). In both C57BL/10 and BALB/c DNA, strongly hybridizing fragments correspond to the *bh0* third exon. The fragments containing the third exons of *y* and *bh1* hybridize weakly. Therefore, no other *bh0*-like third coding block sequences are dispersed in the mouse genome.

***bh0* May Be a Recently Disabled Pseudogene.** The deletion of an apparently intact β-globin gene might be expected to have detrimental consequences. In humans, deletions ranging from 615 bp to over 105 kb in the β-globin gene complex result in forms of β-thalassemia and hereditary persistence of fetal hemoglobin (32). In mice, a naturally occurring 3709-bp deletion that removes the entire adult *b1* gene also results in a thalassemia when homozygous (26). In contrast, we know of no pathology or morbidity related to hematological deficiencies in inbred mice carrying the *Hbb*^{s} haplotype with the deleted *bh0* gene.

The hemoglobin of *Hbb*^{d} mice contains no detectable bh0 polypeptide. All identified globin chains have been assigned to a structural gene locus (5, 7, 8). However, the intact *bh0* gene has no obvious sequence changes within the 5′ flanking DNA that would affect its transcription. In fact, the gene can be transcribed in a cell-free system (B. A. Brown, personal communication). The only potential irregularity observed in the *bh0* gene is an insertion of over 200 bp within the first intervening sequence (IVS*1*) (12). The insertion is located 26 bp to the left of the putative lariat branch point (33). With the insertion, the length of the IVS*1* becomes nearly three times the average for vertebrate β-globin genes. Whether the insertion effects the message stability or splicing is not known.

We suggest that the *bh0* gene in the Hbb^d haplotype is to all intents and purposes a pseudogene. If there is a bh0 protein product, it is below the detectable limit. The fact that the gene can be deleted demonstrates unambiguously that it is dispensable. Unlike other mouse β-globin pseudogenes (9, 10), the amino acid sequence encoded by the Hbb^d *bh0* gene is not obviously defective. We believe that the *bh0* gene may have been disabled recently, possibly due to the insertion of 200 bp within its IVS*1*. The partial deletion of the *bh0* gene in the Hbb^s haplotype demonstrates the continuing fluidity of gene clusters and one fate of a gene whose role is no longer essential for development.

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1. Fantoni, A., Bank, A. & Marks, P. A. (1967) *Science* **157,** 1327–1328.
2. Craig, M. L. & Russell, E. S. (1964) *Dev. Biol.* **10,** 191–201.
3. Russell, E. S. & McFarland, E. C. (1974) *Ann. N.Y. Acad. Sci.* **241,** 25–38.
4. Fantoni, A., De la Chapelle, A. & Marks, P. A. (1969) *J. Biol. Chem.* **244,** 675–681.
5. Jahn, C. L., Hutchison, C. A., III, Phillips, S. J., Weaver, S., Haigwood, N. L., Voliva, C. F. & Edgell, M. H. (1980) *Cell* **21,** 159–168.
6. Leder, P., Hansen, J. N., Konkel, D. A., Leder, A., Nishioka, Y. & Talkington, C. (1983) *Science* **209,** 1336–1342.
7. Hansen, J. N., Konkel, D. A. & Leder, P. (1982) *J. Biol. Chem.* **257,** 1048–1052.
8. Farace, M. G., Brown, B. A., Raschella, G., Alexander, J., Gambari, R., Fantoni, A., Hardies, S. C., Hutchison, C. A., III, & Edgell, M. H. (1984) *J. Biol. Chem.* **259,** 7123–7128.
9. Phillips, S. J., Hardies, S. C., Jahn, C. L., Edgell, M. H. & Hutchison, C. A., III (1984) *J. Biol. Chem.* **259,** 7947–7954.
10. Hutchison, C. A., III, Hardies, S. C., Padgett, R. W., Weaver, S. & Edgell, M. H. (1984) *J. Biol. Chem.* **259,** 12881–12889.
11. Konkel, D. A., Maizel, J. V., Jr., & Leder, P. (1979) *Cell* **18,** 865–873.
12. Hill, A., Hardies, S. C., Phillips, S. J., Davis, M. G., Hutchison, C. A., III, & Edgell, M. H. (1984) *J. Biol. Chem.* **259,** 3739–3747.
13. Hutton, J. J., Bishop, J., Schweet, R. & Russell, E. S. (1962) *Proc. Natl. Acad. Sci. USA* **48,** 1505–1513.
14. Stern, R. H., Russell, E. S. & Taylor, B. A. (1976) *Biochem. Genet.* **14,** 373–381.
15. Voliva, C. F., Martin, S. L., Hutchison, C. A., III, & Edgell, M. H. (1984) *J. Mol. Biol.* **178,** 795–813.
16. Shyman, S. & Weaver, S. (1985) *Nucleic Acids Res.* **13,** 5085–5093.
17. Burton, F. H., Loeb, D. D., Chao, S. F., Hutchison, C. A., III, & Edgell, M. H. (1985) *Nucleic Acids Res.* **13,** 5071–5084.
18. Brown, B. A., Padgett, R. W., Hardies, S. C., Hutchison, C. A., III, & Edgell, M. H. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2753–2757.
19. Weaver, S., Comer, M. B., Jahn, C. L., Hutchison, C. A., III, & Edgell, M. H. (1981) *Cell* **24,** 403–411.
20. Dale, R. M. K., McClure, B. A. & Houchins, J. P. (1985) *Plasmid* **13,** 31–40.
21. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
22. Maniatis, T., Jeffrey, A. & Kleid, D. G. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1184–1188.
23. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3683–3688.
24. Blin, N. & Stafford, D. W. (1976) *Nucleic Acids Res.* **3,** 2303–2308.
25. Rougeon, F. & Mach, B. (1977) *Gene* **1,** 229–239.
26. Skow, L. C., Burkhart, B. A., Johnson, F. M., Popp, R. A., Popp, D. M., Goldberg, S. Z., Anderson, W. F., Barnett, L. B. & Lewis, S. E. (1983) *Cell* **34,** 1043–1052.
27. Hamada, H., Petrino, M. G. & Kakunago, T. (1982) *Proc. Natl. Acad. Sci.* USA **79,** 6465–6469.
28. Slightom, J. L., Blechl, A. E. & Smithies, O. (1980) *Cell* **21,** 627–638.
29. Stringer, J. R. (1982) *Nature (London)* **296,** 363–366.
30. Flanagan, J. G., Lefranc, M.-P. & Rabbitts, T. H. (1984) *Cell* **36,** 681–688.
31. Treco, D., Thomas, B. & Arnheim, N. (1985) *Mol. Cell. Biol.* **4,** 2029–2038.
32. Collins, F. S. & Weissman, S. M. (1984) *Prog. Nucleic Acid Res. Mol. Biol.* **31,** 315–465.
33. Reed, R. & Maniatis, T. (1985) *Cell* **41,** 95–105.