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Genetics

A mutation in the β-globin gene detected in the progeny of a female mouse treated with ethylnitrosourea

(mutagenesis/hemoglobin/biochemical screening/genetic regulation)

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ABSTRACT **A mouse with a variant hemoglobin was discovered during electrophoretic screening of (C57BL/6J × DBA/2J) F_1 progeny of females treated with ethylnitrosourea. The variant trait was transmitted as a simple Mendelian alternate at the *Hbb* locus in all crosses except those involving the original carrier of the mutation. The proband mouse which received the mutation directly from the mutagen-treated parent was a germinal mosaic for the mutant and normal Hbb^s alleles. The mutant allele was designated Hbb^{s2}. The mutant haplotype specifies both an electrophoretically fast hemoglobin band and a hemoglobin band in the normal β^{single} hemoglobin position. Thus, the mutation has altered one of the tandemly duplicated genes at the Hbb^s locus. A comparison of the relative concentrations of the two hemoglobins in Hbb^{s2} mice demonstrates preferential expression of the mutant gene, possibly analogous to the enhanced expression of Hbb^{dmaj} in the Hbb^d haplotype. Analysis of the amino acid sequence of the variant β-globin revealed that the valine at position 60 was changed to glutamic acid. The simplest mutation mechanism for such an alteration is an A·T→T·A transversion.**

Considerable natural variation has been found at the loci encoding hemoglobins in mammals. The large number of alternate alleles and convenient access to the gene products have contributed to the extensive knowledge of the molecular structure and the expression of hemoglobin, as well as of the organization of hemoglobin genes (1–10).

The study of mouse hemoglobin mutations offers a chance to explore further the organization and function of this important region of the mammalian genome. Extensive natural variation has been described at the hemoglobin loci in inbred and wild mice (2–5). These spontaneous mutants have recently been supplemented with induced mutants. Experiments with x-rays and triethylenemelamine have yielded four α-thalassemias and one tandem gene duplication involving the β-globin locus (11, 12). Two more mutations induced by the mutagen ethylnitrosourea (EtNU) have been described, one mutation causing an electrophoretic mobility change in α-hemoglobin (13, 14) and the other causing altered mobility of the d-minor component of β-globin (15). Others are currently being analyzed (16).

This paper describes a new EtNU-induced β-globin mutant and discusses some of its properties.

MATERIALS AND METHODS

C57BL/6J and DBA/2J parental mice used for screening experiments were purchased from The Jackson Laboratory. EtNU was obtained from the National Toxicology Program's Chemical Repository. It was dissolved in a phosphate-citrate buffer (13) with the concentration adjusted to deliver 100 mg/kg in 0.3 ml by intraperitoneal injection. Controls were injected with the same volume of buffer. C57BL/6J females were treated and then mated with DBA/2J males over a period of 7 days to obtain progeny from meiotic and premeiotic oocytes exposed to EtNU. Kidney and blood samples were taken from the resulting F_1 progeny and subjected to electrophoretic analysis as described (13, 17).

The original F_1 mutant female was backcrossed to a DBA/2J male and to a son that carried the mutation. The resulting progeny and other descendants were mated in various combinations to allow genetic analysis. Hemoglobins of progeny were typed by starch gel electrophoresis (17), cellulose acetate electrophoresis (18), or both. Microdensitometry was performed immediately after isoelectric focusing of cystamine-treated hemoglobin samples (19).

Mutant mouse β-globin polypeptides were separated by chromatography on carboxymethylcellulose and analyzed as described (5, 20).

RESULTS

The original mutant animal was identified by the occurrence of an atypical band in the starch gel electrophoregram of its hemoglobin. To determine the β-globin haplotype, cystamine-treated blood from the variant animal was subjected to cellulose acetate electrophoresis. With this technique, a normal (B6 × D2) F_1 pattern consists of three bands. The most anodal band is specified by the Hbb^s haplotype of the C57BL/6J parent (Fig. 1). The other two bands are specified by the Hbb^d haplotype inherited from the DBA/2J parent: an intermediate band specified by the Hbb^{dmaj} gene and a cathodal light band specified by the Hbb^{dmin} gene. In addition to the three bands of hemoglobin expected from Hbb^s/Hbb^d mice, the mutant F_1 animal had a fourth band which migrated faster than the others.

The β-globin types of progeny of the backcross of the variant female to a normal DBA/2J male were classified by electrophoresis (Table 1). Transmission of a normal Hbb^d allele by the mutant animal was demonstrated by the recovery of 4 offspring among 11 of the backcross progeny with the two-banded pattern typical of Hbb^d/Hbb^d mice. Three other progeny from the same cross inherited the four-banded pattern of the original mutant female (Fig. 1), demonstrating that the mutant trait was heritable and, thus, caused by a germinal mutation. In addition, hemoglobin of some of the backcross progeny had the normal three-banded F_1 pattern, showing that the mutant female had transmitted gametes bearing the normal as well as the altered Hbb^s allele. Germinal mosaicism for the two Hbb^s alleles could explain such a transmission pattern.

When one of her mutant carrier sons was mated with her, 1 homozygous mutant animal was found among 14 progeny (Table 1). This animal, as well as homozygotes derived from

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Abbreviation: EtNU, ethylnitrosourea.

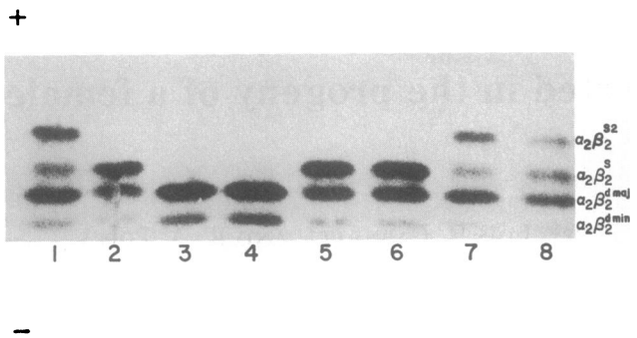

FIG. 1. Patterns of hemoglobin bands obtained by cellulose acetate electrophoresis of cystamine-treated blood samples. Hemoglobin from the original F_1 mutant is in lane 8. The samples in the other lanes are from the progeny in her first litter from a backcross to the DBA/2J male. Lanes 1 and 7: mutant F_1 pattern specified by genotype Hbb^{s2}/Hbb^{d}. Lanes 2, 5, and 6: normal F_1 pattern (Hbb^{d}/Hbb^{s}). Lanes 3 and 4: normal Hbb^{d}/Hbb^{d} pattern. The composition of the tetramers is shown at right.

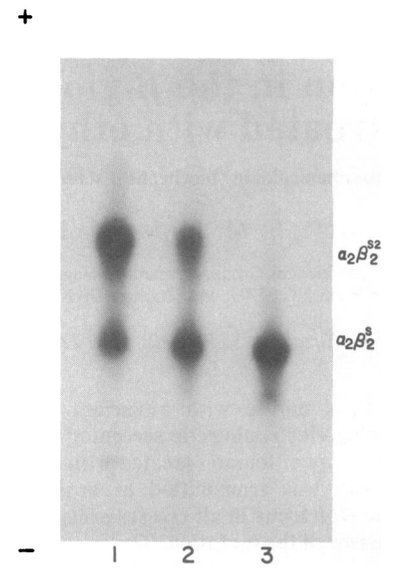

FIG. 2. Starch gel electrophoresis patterns of hemoglobin from an Hbb^{s2}/Hbb^{s2} homozygote (lane 1), an Hbb^{s2}/Hbb^{s} heterozygote (lane 2), and an Hbb^{s}/Hbb^{s} homozygote (lane 3).

other crosses, was fully viable and fertile. The mutant trait behaved as a Mendelian alternate at the *Hbb* locus in all subsequent matings (Table 2). In crosses of heterozygotes with each other, there was no significant deviation from the 1:2:1 ratio among the 47 progeny.

The mutant hemoglobin was always associated with an apparently normal β^{single} hemoglobin in mutant mice from all crosses (Fig. 2). The consistent presence of a two-banded pattern in the homozygote and the failure of these bands to assort independently provided additional evidence that the new mutation occurred in one of the tandemly duplicated genes in the Hbb^{s} haplotype of the treated female parent. The resulting new haplotype is provisionally designated Hbb^{s2}.

Upon either cellulose acetate or starch gel electrophoresis, the hemoglobin of Hbb^{s2}/Hbb^{s2} animals migrated as two bands (Fig. 2), corresponding to the products of the normal and mutant alleles. Relative concentrations of the normal and mutant hemoglobins were assessed by scanning densitometry of the hemoglobin patterns of animals of several genotypes (Table 3). In homozygous Hbb^{s2} mutants, the mutant hemoglobin comprised 74% of the total hemoglobin, whereas the normal β^{single} hemoglobin was only 26% of the total. In similar analysis of hemoglobin from DBA/2J mice, β^{dmajor} represents 73% of the total hemoglobin, and β^{dminor}, 27%. These values are consistent with those reported by Whitney (21).

The two types of β-globin polypeptides from Hbb^{s2} homozygotes were separated by carboxymethylcellulose chromatography (Fig. 3). The mutant polypeptide, $\beta^{single\text{-}2}$, was eluted earlier than the normal β^{single} polypeptide. The amino acid compositions of the tryptic peptides of both β-globins were identical with the exception of peptide βT-6. The expected Val-Lys dipeptide was present in β^{single}, but a unique Glu-Lys dipeptide was found in the mutant $\beta^{single\text{-}2}$, indicating that the mutation resulted in the substitution of glutamic acid for valine at position 60. No other changes in the mutant protein were detected by the methods employed.

DISCUSSION

Prior to this report it was not known whether both structural genes in the Hbb^{s} haplotype were expressed. Studies of hemoglobin composition in Hbb^{s} mice revealed only one β-globin polypeptide (20). Molecular studies on the DNA of Hbb^{s} mice demonstrated two structural genes with intact regulatory elements (8, 9). Furthermore, unpublished studies of *in vitro* transcription of β-globin mRNA from Hbb^{s}

Table 1. Crosses of the original F_1 hemoglobin mutant female

Cross	No. of litters	No. of progeny with *Hbb* type					Total no. of progeny
		s2/s2	*s2/d*	*d/d*	*s/d*	*s2/s*	
Backcross to DBA/2J Hbb^{s2}/Hbb^{d} (Hbb^{s}/Hbb^{d}) × Hbb^{d}/Hbb^{d}	3	0	3	4	4	0	11
Backcross of carrier son to original mutant Hbb^{s2}/Hbb^{d} (Hbb^{s}/Hbb^{d}) × Hbb^{s2}/Hbb^{d}	3	1	8	3	0	2	14

Table 2. Crosses involving mutant carriers other than the original F_1 mutant

Cross	No. of litters	No. of progeny with *Hbb* type				Total no. of progeny
		d/d	*s2/d* or *s2/s*	*s/d*	*s2/s2*	
Backcross of carriers to C57BL/6J Hbb^{d}/Hbb^{s2} × Hbb^{s}/Hbb^{s}	4	0	16	14	0	30
Crosses between heterozygotes Hbb^{d}/Hbb^{s2} × Hbb^{d}/Hbb^{s2}	8	11	26	0	10	47
Crosses between homozygotes Hbb^{s2}/Hbb^{s2} × Hbb^{s2}/Hbb^{s2}	2	0	0	0	9	9

Table 3. Relative concentrations of the hemoglobins

Genotype	No. of mice	% of total hemoglobin (mean ± SD)			
		$\beta^{single-2}$	β^{single}	β^{dmajor}	β^{dminor}
Hbb^{s2}/Hbb^{s2}	6	74 ± 6	26 ± 6		
Hbb^{s2}/Hbb^{s}	6	33 ± 2	67 ± 2		
Hbb^{s2}/Hbb^{d}	6	34 ± 5	15 ± 3	39 ± 7	12 ± 1
Hbb^{s}/Hbb^{d}	5		57 ± 4	33 ± 1	10 ± 2
Hbb^{d}/Hbb^{d}	5			73 ± 2	27 ± 2
Hbb^{s}/Hbb^{s}	6		100		

homozygous animals indicate that both genes are transcribed (B. Brown, M. Edgell, C. Hutchison, and S. Weaver, personal communication). Because the mutation described here makes the polypeptides distinguishable from each other, it is clear that the polypeptide products of both genes are present.

The relative difference in the concentration of the two hemoglobin types in Hbb^{s2}/Hbb^{s2} mice indicates that the expression of the two genes in the Hbb^{s2} haplotype in adults is not equivalent and may be comparable to that of the Hbb^{dmaj} and Hbb^{dmin} genes in the Hbb^{d} haplotype (21). Analysis of the β-globin DNA of the new mutant will determine whether the loci are in the same orientation as are the genes in the Hbb^{d} haplotype, and comparison of regulatory sequences in mice of different *Hbb* haplotypes might provide further insights into the regulation of expression at these loci.

The mutagenic process associated with the induction of the new mutation resulted in germinal mosaicism for the normal and mutant Hbb^{s} alleles in the F_1 mutant female. Germinal mosaicism may have resulted because fixation of the mutation occurred after fertilization. The resultant embryo would have been comprised of both normal and mutant cells. Mosaic mutants are induced in *Drosophila* in postmeiotic germ cells (22). However, similar mosaicism has not been reported in studies of mutants induced in the late germ-cell stages of mice by irradiation (23) and chemicals (24). The few reported mosaic mutants detected in the mouse visible-specific-locus test are most likely of spontaneous origin (25).

The study of altered gene products specified by mutant genes such as the one reported here can provide information about the *in vivo* mechanisms of mutagen action. Extrapolating from the amino acid substitution, the simplest possible mutational mechanism for the induction of Hbb^{s2} is a T·A→A·T transversion in a GTG triplet specifying valine at position 60 (26). Studies of normal Hbb^{s} DNA have shown that a GTG triplet is at position 60 (S. Weaver, personal communication). However, it is possible that the EtNU treatment could have caused other alterations in the Hbb^{s} haplotype undetectable by analysis of the protein. Determination of the nucleotide sequence of the mutant Hbb^{s2} gene will show whether other changes have occurred.

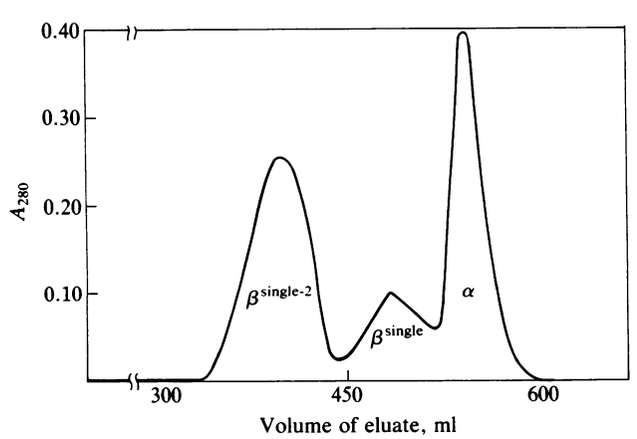

FIG. 3. Carboxymethylcellulose chromatography of globin chains from Hbb^{s2} homozygotes.

As the molecular bases of more mutations induced *in vivo* become known, it will be interesting to see whether other EtNU-induced mutations show the same base-change specificity. An α-globin mutation induced in spermatogonia by EtNU was shown to be associated with an A·T→T·A transversion (14).

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