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15. We thank P. Sager and J. McGowan for discussions and for the provision of AZT; I. Chen and members of his laboratory for providing the HIV isolates JR-FL and JR-CSF and for advice on PCR methods; M. Brown for technical assistance; E. Yee and L. Osborne for providing SCID-hu mice for infection studies with HIV; and F. Hamilton and K. K. Smith for review of the manuscript.

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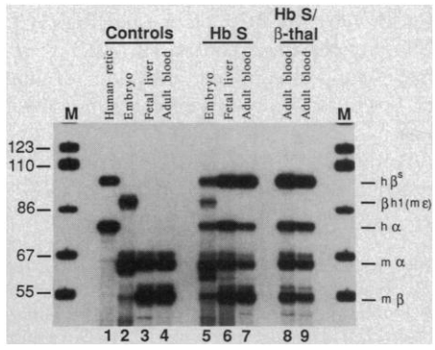

Fig. 2. Primer extension analysis of total RNA from Hb S and Hb S/β-thal mice. Authentic human β^s- and human α-globin primer extension products are 98 and 76 bp, respectively; correct mouse βh1- (ε), α-, and β-globin products are 87, 65, and 53 bp, respectively (*15*). Lanes 1 to 4 are total RNA samples from normal controls, lanes 5 to 7 are from Hb S mice, and lanes 8 to 9 are from Hb S/β-thal mice. Lane 1, human reticulocytes; lanes 2 and 5, 11-day mouse embryo (10 μg); lanes 3 and 6, 16-day mouse fetal liver (1.0 μg); and lanes 4, 7, 8, and 9, adult mouse reticulocytes (0.25 μg). Hb S mice contain five copies of HS I–V α and seven copies of HS I–V β^s. Hb S/β-thal animals were derived by mating Hb S mice with the β-thalassemic mouse line C57BL/6.*Hbb*$^{d\text{-}3thJ}$ (*7*).

Human Sickle Hemoglobin in Transgenic Mice

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DNA molecules that contain the human α- and β^s-globin genes inserted downstream of erythroid-specific, deoxyribonuclease I super-hypersensitive sites were coinjected into fertilized mouse eggs and a transgenic mouse line was established that synthesizes human sickle hemoglobin (Hb S). These animals were bred to β-thalassemic mice to reduce endogenous mouse globin levels. When erythrocytes from these mice were deoxygenated, greater than 90 percent of the cells displayed the same characteristic sickled shapes as erythrocytes from humans with sickle cell disease. Compared to controls the mice have decreased hematocrits, elevated reticulocyte counts, lower hemoglobin concentrations, and splenomegaly, which are all indications of the anemia associated with human sickle cell disease.

SICKLE CELL ANEMIA WAS THE FIRST genetic disease to be understood at the molecular level (*1*). An A to T transversion in the sixth codon of the human β-globin gene causes an amino acid change from a polar glutamic acid residue to a nonpolar valine in the β-globin polypeptide. This change decreases the solubility of deoxygenated Hb S. At low oxygen tensions, Hb S molecules polymerize to form extensive networks of intracellular fibers (*2*). These fibers distort the erythrocytes into a variety of sickled shapes. The sickled cells are rigid and nondeformable and can occlude the microvasculature, which causes local hypoxia and tissue damage (*3*).

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High concentrations of functional human hemoglobin can be synthesized in transgenic mice when human α- and β-globin genes are inserted immediately downstream of the erythroid-specific, deoxyribonuclease (DNase) I super-hypersensitive sites normally located 50-kb upstream of the human β-globin gene (*4*). These results suggested that high concentrations of sickle hemoglobin could be produced in transgenic mice if human α- and β^s-globin genes were inserted downstream of the super-hypersensitive (HS) sites and injected into fertilized eggs. Therefore, cosmid clones that contained all five upstream sites (HS I–V) and either the human α- or β^s-globin gene were constructed (Fig. 1) (*5*). The 26-kb fragments containing HS I–V α and HS I–V β^s were purified from vector sequences and coinjected into fertilized eggs (*6*). Injected eggs were implanted into the uteri of foster mothers; tail DNA of the progeny was analyzed for intact copies of the transgenes. Three transgenic animals had head-to-tail tandem arrays of both transgenes. Hemolysates from these animals were analyzed on isoelectric focusing (IEF) gels (*4*). All three mice synthesized Hb S; the highest expressor was mated to control animals to establish a line. These transgenic mice were then bred with a strain of β-thalassemic (β-thal) mice to reduce endogenous mouse β-globin levels (*7*). Hb S/β-thal animals derived from this mating were het-

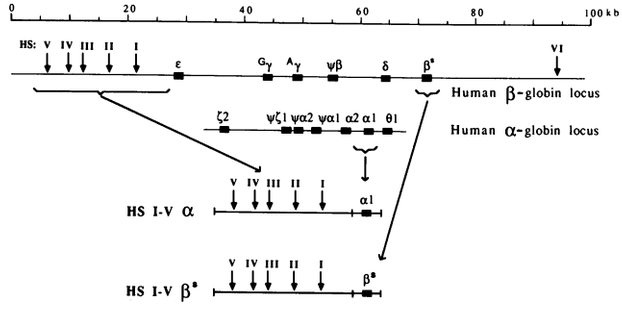

Fig. 1. HS I–V α and HS I–V β^s constructs. One hundred kilobases of the human β-globin locus and 35 kilobases of the human α-globin locus are illustrated. Cosmids containing HS I–V α1 and HS I–V β^s were constructed as described (*5*). The 26-kb inserts were purified from vector sequences, mixed at a 1:1 molar ratio (final DNA concentration was 2 ng/μl) and coinjected into fertilized mouse eggs (*6*).

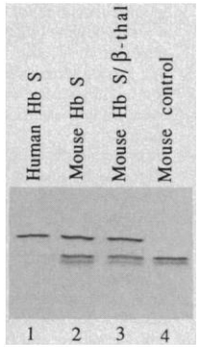

Fig. 3. Isoelectric focusing gel of Hb S and Hb S/β-thal hemolysates. Hemolysates were prepared and separated on a nondenaturing IEF gel (*4*). Lane 1 (human Hb S) is from a homozygous sickle cell patient and lane 4 is a mouse control. Lanes 2 and 3 are Hb S and Hb S/β-thal hemolysates, respectively. The Hb S/β-thal animals are heterozygous for the transgenes and heterozygous for the β-thal mutation. The multiple endogenous mouse hemoglobin bands are primarily due to different α-globin haplotype combinations in F2 animals from C57BL6/SJL crosses (*16*; also see *11*).

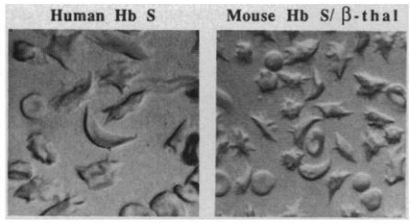

Fig. 4. Deoxygenated erythrocytes from a human sickle cell patient and a Hb S/β-thal transgenic mouse. Cells were deoxygenated with nitrogen gas and visualized by light microscopy (*12*). Erythrocytes from the homozygous sickle cell patient are shown in the first panel (human Hb S), and the second panel illustrates cells from a Hb S/β-thal transgenic mouse. The cells in both panels were photographed at the same magnification.

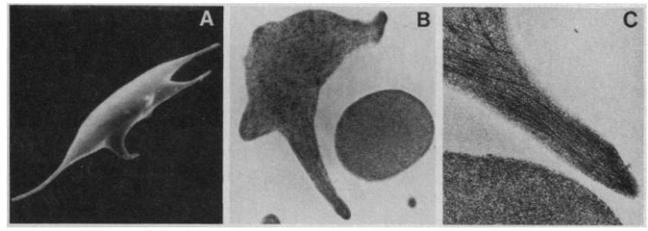

Fig. 5. Scanning and transmission electron micrographs of deoxygenated erythrocytes from Hb S/β-thal mice. Cells were deoxygenated with nitrogen gas and prepared for microscopy (*13*). (**A**) Scanning electron micrograph of a Hb S/β-thal erythrocyte; (**B** and **C**) transmission electron micrographs of Hb S/β-thal erythrocytes.

Table 1. Mouse hematological values.

	Control (n = 8)	β-thal* (n = 5)	HbS (n = 8)	Hb S/β-thal* (n = 6)
RBC (10^6/mm^3)	10.6 ± 0.7	12.0 ± 0.5	11.3 ± 0.7	9.3 ± 1.1
Hb (g/dl)	15.4 ± 0.4	17.2 ± 0.4	15.6 ± 1.4	13.5 ± 1.1
†HCT (%)	49.9 ± 2.2	53.2 ± 1.8	50.9 ± 1.6	45.0 ± 2.1
Reticulocytes (%)	2.4 ± 0.2	1.0 ± 0.7	2.7 ± 1.4	7.4 ± 3.7

*β-thal heterozygote. †HCT, hematocrit.

erozygous for the transgenes and heterozygous for the β-thal mutation.

Total RNA from erythroid tissue of progeny at different developmental stages was analyzed for correctly initiated globin mRNA by primer extension (*4, 8, 9*) (Fig. 2). Lane 1 is human reticulocyte RNA and lanes 2 to 4 are mouse control RNA from an 11-day embryo, 16-day fetal liver, and adult reticulocytes, respectively. Correctly initiated human (α and β) and mouse (α, βh1, and β) globin mRNAs were detected at the appropriate developmental stages. The switch from mouse embryonic (βh1) to adult β between 11 and 16 days of development is clear (lanes 2 and 3), whereas the switch from embryonic (χ) to adult α occurs before day 11 and is not illustrated here. Lanes 5 to 7 are RNA from an 11-day embryo, 16-day fetal liver, and adult reticulocytes of Hb S mice. Correctly initiated human α- and β^s-globin mRNA were present at all developmental stages. Lanes 8 and 9 are primer extensions from adult reticulocyte RNA of two Hb S/β-thal animals. The ratio of human β- to mouse β-globin mRNA increased in the Hb S/β-thal animals (compare lane 7 to 8 and 9). The amount of β^s-globin mRNA in Hb S and Hb S/β-thal animals was three and five times that of endogenous mouse β-globin mRNA, respectively (*10*). There were equivalent amounts of human α- and endogenous mouse α-globin mRNA.

To examine globin protein levels, we analyzed hemolysates from Hb S and Hb S/β-thal mice by isoelectric focusing (Fig. 3). Lanes 1 and 4 are hemolysates from a homozygous sickle cell patient and a mouse control. Lanes 2 and 3 are hemolysates from Hb S and Hb S/β-thal mice. Hb S synthesized in these transgenic mice had the same isoelectric point as native Hb S and quantitation of the bands by densitometry suggested that Hb S constituted 50% of total hemoglobin in both Hb S and Hb S/β-thal animals. Although we expected that the ratio of Hb S to normal mouse Hb would increase in Hb S/β-thal mice, they appeared to be equivalent (*11*). However, a functional difference between these two lines was observed.

Erythrocytes from Hb S and Hb S/β-thal mice and a patient with sickle cell disease were deoxygenated and examined by light microscopy (*12*) (Fig. 4). Human sickle cells assumed a variety of sickle shapes when Hb S polymerized at low oxygen tensions (*3*). Only a minor fraction (<1%) of cells from the Hb S mice displayed sickled shapes; however, the majority (>90%) of cells from the Hb S/β-thal animals were sickled. Except for their smaller size, sickled erythrocytes from the transgenic mice were indistinguishable from those of the patient with sickle cell disease.

Deoxygenated erythrocytes from Hb S/β-thal mice were examined by scanning and transmission electron microscopy (EM) (*13*). The erythrocytes had projections or spicules that are characteristic of sickled cells (Fig. 5A) (*3*). These structures result from the formation of Hb S fibers (Fig. 5, B and C).

Several hematological parameters for mouse controls, Hb S mice, and Hb S/β-thal animals were examined (Table 1). Red blood cell (RBC) counts, Hb concentrations (Hb), and hematocrits (HCT) were somewhat decreased and reticulocyte counts were increased in Hb S/β-thal mice compared to Hb S animals and controls. All of these values indicate that the Hb S/β-thal mice are mildly anemic. In addition, the spleens of Hb S/β-thal animals were two to four times larger than control spleens. Splenomegaly is common in children with sickle cell disease and can be life threatening (*3*).

Although the Hb S/β-thal mice are mildly anemic, they are otherwise healthy and appear to mimic the condition of sickle cell trait in humans (*14*). Individuals with sickle trait can suffer tissue damage from occlusion of the microvasculature under hypoxic conditions (*3*). We are now exposing Hb S/β-thal animals to oxygen tensions and pressures that simulate high altitudes to determine whether these animals will develop the tissue and organ damage that is characteristic of sickle cell disease. If the tissue damage associated with chronic vaso-occlusive episodes occurs in these mice, they will represent an excellent model of sickle cell disease. Although sickle cell disease was the first genetic disorder to be understood at a molecular level, no adequate treatment or cure is available. Hopefully, new drug and gene therapies can be designed and tested in these animals and, therefore, provide new strategies for treating this debilitating disease.

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5. Cosmids that contained HS I–V α or Hs I–V β^s were constructed as described for HS I–V β (*9*). The human α1-globin gene is within a 3.8-kb Bgl II–Eco RI fragment and the β^s-globin gene is within a 4.1-kb Hpa I–Xba I fragment.
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10. Globin mRNA levels were quantitated by solution hybridization as described by T. M. Townes *et al.*, *EMBO J.* **4**, 1715 (1985).
11. Accurate quantitation of Hb S in mice of the "single" haplotype is difficult because of heterodimer formation (*4*) in IEF gels; the top band, $h\alpha_2h\beta^s_2$, in Fig. 2 is contaminated with $h\alpha_2m\beta_2$ and the bottom band, $m\alpha_2m\beta_2$ is contaminated with $m\alpha_2h\beta^s_2$. The ratio of $h\alpha_2h\beta^s_2$ to $m\alpha_2m\beta_2$ may actually be increased in the Hb S/β-thal mice but the change may be obscured by an increase in $m\alpha_2h\beta^s_2$ in the bottom band. The β^s polypeptides are presumably in excess because there is five times more human β^s-globin mRNA than mouse β-globin mRNA in the Hb S/β-thal animals (see Fig. 3). However, direct measurement of β^s-globin levels is difficult because human β^s and mouse β^{single} polypeptides migrate at the same position on denaturing gels.
12. Cells were suspended in saline buffered with 10 m*M* sodium phosphate (*p*H 7.4) and slowly (3 to 5 ml/min) deoxygenated with nitrogen gas [K. Horiuchi *et al.*, *Blood* **71**, 46 (1988); T. Asakura and J. Mayberry, *J. Lab. Clin. Med.* **104**, 987 (1984)].
13. For scanning electron microscopy deoxygenated RBCs were fixed in 2% glutaraldehyde in cacodylate buffer, *p*H 7.2, for 5 days. The fixed cells were washed with cacodylate buffer and collected on a filter. The filter was floated in 2% osmium tetroxide for 10 min and dehydrated in a graded series of ethanol and dried with a Deuton Vacuum apparatus. The specimen was splattered with platinum palladium and examined with a Philips scanning electron microscope. For transmission electron microscopy, deoxygenated RBCs were fixed overnight in 2% glutaraldehyde in cacodylate buffer, *p*H 7.2, washed with cacodylate buffer and treated with 2% osmium tetroxide for 1 hour. The samples were dehydrated in a graded series of ethanol and embedded in Epon resin. Thin sections were cut with an LKB Ultrotome III and examined with a Philips 300 electron microscope.
14. In principle the ratio of Hb S to mouse Hb can be increased by making the mice homozygous for the transgenes and homozygous for the β-thal mutation. One advantage of this transgenic line is that different ratios of Hb S to mouse Hb can be attained by the appropriate genetic crosses. However, it is possible that progeny with higher Hb S to mouse Hb ratios will not be viable because higher Hb S levels in early development may not be tolerated.
15. The human α- and β^s-globin oligonucleotides used in the primer extension analysis were as described (*4*). The mouse βh1 primer 5′-ATAGCTGCCTTC-TCCTCAGCT-3′ corresponds to sequences from +67 to +87 of the mouse βh1 gene.
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Tumorigenicity in Human Melanoma Cell Lines Controlled by Introduction of Human Chromosome 6

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Chromosome banding analysis of human malignant melanoma has documented the nonrandom alteration of chromosome 6. To determine the relevance of chromosome 6 abnormalities in melanoma, a normal chromosome 6 was directly introduced into melanoma cell lines. The resulting (+6) microcell hybrids were significantly altered in their phenotypic properties in culture and lost their ability to form tumors in nude mice. The loss of the chromosome 6 from melanoma microcell hybrids resulted in the reversion to tumorigenicity of these cells in mice. The introduction of the selectable marker (psv$_2$neo) alone into melanoma cell lines had no effect on tumorigenicity. These results support the idea that one or more genes on chromosome 6 may control the malignant expression of human melanoma.

MALIGNANT TRANSFORMATION IS envisioned as a multistep process (*1*) with recent studies highlighting the importance of tumor suppressor genes in the development of human cancers (*2*). Although evidence supporting the existence of tumor suppressor genes (whose inactivation would release a cell from normal growth control) is accumulating (*3*), to date, only two tumor suppressor genes (*RB* and p53) have been cloned (*4*). Evidence supporting the presence of tumor suppressor genes in human malignancies has primarily come from two lines of investigation: (i) the study of allelic loss [by restriction fragment length polymorphism (RFLP) analysis] and (ii) the study of somatic cell fusion experiments. The former experimental approach has documented allelic loss of specific chromosome regions in several cancers including colon cancer, lung cancer, Wilms' tumor, retinoblastoma, and neuroblastoma (*5, 6*). In melanoma, RFLP allelic loss of several chromosome regions has been described for melanoma cell lines (*7*). In addition to reports of allelic loss, direct support for the presence of suppressor genes in cancer has resulted from the use of interspecific (*8*) and, more recently, intraspecific human somatic cell hybrids (*9*). Somatic cell hybridization involves the introduction of a complete genome from a normal cell into a cancer cell via cell fusion. In contrast, by microcell hybridization, the effect of introducing a single human chromosome into a recipient cell can be studied (*10*).

Our reason for selecting human chromosome 6 for microcell hybridization relates to its frequent involvement in structural alterations in melanoma (*11*). The most frequent alteration of chromosome 6 in melanoma is simple deletion of the long arm (6q−) (*11*), although a nonreciprocal translocation site involving chromosome 6 (leading to loss of sequences on distal 6q) has been described (*12*). Almost 40% of melanomas exhibit loss of sequences on 6q (*11, 12*). We report here the introduction of a chromosome 6 derived from a normal human diploid fibroblast into melanoma cell lines. Our results suggest that one or more genes on chromosome 6 have a role in tumorigenic expression of human melanoma.

A human-mouse microcell hybrid was generated that contained a single copy of chromosome 6 (derived from a normal human diploid fibroblast) as its only human component (*13*). Retention of chromosome 6 resulted from the insertion of the neomycin antibiotic resistance gene (psv$_2$neo) into

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