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Genetics

# Molecular basis of dark-eyed albinism in the mouse

(*c* locus/mouse mutation/pigmentation/transgenic mice/tyrosinase)

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**ABSTRACT** **Dark-eyed albino ($c^{44H}$) is a recessive allele at the mouse albino (*c*) locus, which encodes tyrosinase (monophenol,L-dopa:oxygen oxidoreductase, EC 1.14.18.1), the key enzyme in melanin synthesis. Similar to type IB oculocutaneous albinism in humans, overall production of pigment is greatly reduced in dark-eyed albino mice and obvious only in the eyes. We have studied the molecular basis of the $c^{44H}$ mutation and show that expression of the tyrosinase gene is not affected. After sequencing tyrosinase cDNA isolated from $c^{44H}/c^{44H}$ homozygotes, we uncovered a single base alteration from wild type leading to a serine-to-isoleucine exchange. The importance of this mutation was demonstrated by generating transgenic mice containing a mutated tyrosinase minigene. This showed that the single base change was sufficient to severely depress pigment production in transgenic mice. We therefore conclude that the point mutation is responsible and sufficient to generate the dark-eyed albino phenotype.**

A large number of genes and mutations affecting coat color in mice have been identified and characterized over the past years (1, 2). Some mutations have their origin in the mouse fancy stock, but most of them have arisen spontaneously or were induced following mutagenesis by radiation or chemicals. These mutations act at various levels in the melanogenic process (3). A family of three related genes, the tyrosinase-related family, is important for synthesis of melanin. Genes coding for tyrosinase-related protein 1 (TRP-1) and tyrosinase-related protein 2 (TRP-2) were shown to map to the *b* locus and slaty locus, respectively, and to influence color or quality of pigment produced (3, 4). The enzyme tyrosinase (monophenol,L-dopa:oxygen oxidoreductase, EC 1.14.18.1) is encoded by the albino (*c*) locus in the mouse and is indispensable for production of pigment (5).

Many mutations at this locus lead to viable or lethal albino phenotypes caused by deletions of part of or the whole tyrosinase gene, thus possibly encompassing other genes (6, 7). Apart from these deletions only a few specific mutations have been identified. The original albino/*c* allele is due to a point mutation in exon 1 of tyrosinase, thus changing a cysteine residue to serine (8–10). Both a temperature-sensitive tyrosinase enzyme found in the himalayan mutation ($c^h$) and a tyrosinase enzyme showing slightly reduced activity (chinchilla, $c^{ch}$) seem to be caused by point mutations as suggested by sequencing of the coding region of the tyrosinase gene (11, 12).

The dark-eyed albino mutation ($c^{44H}$) has been induced in spermatogonial stem cells by combined hydroxyurea and x-ray treatment. It represents an allele of the *c* locus (13). Adult homozygous mice have dark eyes and traces of pigment in hairs of the anogenital region but lack pigment of the body fur (13, 14). The phenotype is very similar to that characteristic for type IB ("yellow") oculocutaneous albinism (OCA) in humans. Such patients usually lack detectable melanin pigment at birth but typically accumulate some pigment in eyes and hair during childhood and adult life. More recently, human tyrosinase gene mutations associated with type IB OCA have been identified and shown to be due to point mutations (15, 16). No corresponding mouse mutation has been characterized.

Two major alternatives exist to explain the reduced pigmentation as a consequence of a mutation at the *c* locus. This could be due to either a mutation in the 5′ region affecting transcription or a mutation in the coding region changing activity or stability of the protein. We have demonstrated that the defect resides within the coding region of the gene. By isolating and sequencing the cDNA from the $c^{44H}$ mutation, we identified a point mutation leading to an amino acid exchange in the protein. The importance of this mutation was then corroborated by introducing it back into transgenic mice.

## MATERIALS AND METHODS

**DNA and RNA Analyses.** NMRI/Han mice were obtained from IFFA Credo (L'Arbresle, France) and $c^{44H}/c^{44H}$ mice were kindly provided by Bruce Cattanach (Medical Research Council Radiobiology Unit, Chilton, U.K.). They were maintained and bred at the animal facility of the institute. Genomic DNA was isolated from tail biopsies of mice essentially as described (17). Southern blot analysis was performed by hybridization to $^{32}$P-labeled DNA probe, pTy1.1, generated by random priming. pTy1.1 was constructed by cloning PCR-amplified sequences of exons 1 and 2 of the mouse tyrosinase gene (primers 1 and 5; bases −20 to +1117) in a TA Cloning vector (Invitrogen).

For PCR analyses on DNA or cDNA, we used *Taq* polymerase (Perkin–Elmer or Appligene, Strasbourg, France). Amplifications were done in 100 μl consisting of 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 20 pmol of each primer, and 2.5 units of *Taq* polymerase. Either 30 or 40 cycles were run, each consisting of 2 min at 94°C, 1 min at 55°C, and 2 min at 72°C. Following the last cycle, elongation was allowed to proceed for 10 min. We used the following primers (sequences in 5′–3′ direction): 1 (CTTAGCCAAAACATGTGATA, bases −20 to −1), 2 (CAGGCAGAGGTTCCTGCCAG, +231 to +250), 3 (GTGGGGATGACATAGACTGA, +540 to +521), 4 (GATCATTTGTAGCAGATCAG, +901 to +920), 5 (TTCCAGTGTGTTTCTAAAGC, +1117 to +1098), 6 (GGGATCGTTGGCCGATCCCTGTACTTGGG, +1234 to +1206), 7 (GAGCCTTACTTGGAACAAGCC, +1475 to +1495), and 8 (CTTAGAAACTAGGACTTAGG, +1835 to +1816).

RNA was isolated from mouse eyes and mouse skin by the guanidinium isothiocyanate procedure including centrifugation through CsCl (17, 18). First-strand cDNA was generated from 2.5 μg of total RNA by using random hexamers (first-strand cDNA synthesis kit, Pharmacia). To determine RNA

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Abbreviation: OCA, oculocutaneous albinism.

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levels, PCR was performed on 1/10th of the cDNA from $c^{44H}/c$ mice with primers 2 and 3. In a separate set of experiments, the PCR mixture was diluted 1:200, and a final PCR cycle was performed in fresh buffer to avoid heterodimeric DNA (19). For every RNA preparation, one sample was amplified without adding reverse transcriptase, thus controlling for the presence of genomic DNA and contaminants. PCR products were digested with *Dde* I, separated in a high-percentage (2.5% or 4%) NuSieve GTG agarose (FMC) gel, and blotted to charged membranes (GeneScreenPlus, DuPont, or Nytran Plus, Schleicher & Schuell). Membranes were hybridized to a random-primed $^{32}$P-labeled DNA probe isolated from pTy0.13 [+406 to +540; subcloned in pBluescript (Stratagene) by using primer 3 and the *Apa* I site at +406]. Quantification was performed with a PhosphorImager (Molecular Dynamics). For Northern blots, 20 μg of total RNA was run in a formaldehyde-containing agarose gel, blotted to nylon membrane (Hybond, Amersham), and hybridized to pTy1.1 ($^{32}$P-labeled by random priming) (17, 18). Photographs of the membrane showed that equal amounts of RNA were loaded.

**Subcloning and Sequencing of $c^{44H}$ Coding Region.** PCRs were performed on genomic DNA and cDNA isolated from $c^{44H}/c^{44H}$ homozygotes. PCR products were subcloned into a TA Cloning vector (Invitrogen). Tyrosinase gene-specific or SP6/T7 promoter-specific primers were used for nucleotide sequence determination with Sequenase (United States Biochemical). For any region of the cDNA, products of at least two independent PCRs were cloned and analyzed by sequencing.

**Transgenic Mice.** A *Bgl* II–*Sph* I fragment (+124 to +1168) of the tyrosinase minigene TyBS (9) was replaced by a *Bgl* II–*Sph* I fragment containing the mutation at +519 (isolated from pTy1.1). By sequencing the insert and the cloning sites of the mutated minigene TyBSmut519, no deviation to TyBS was found (except for the mutation at +519). Vector sequences were removed from both minigenes (TyBS and TyBSmut519) by cleavage with *Kpn*I and *Eco*RI. The fragments were isolated and purified from agarose gels by using glass powder (Geneclean, Bio 101), dissolved in 10 mM Tris, pH 7.4/0.1 mM EDTA, and injected into fertilized eggs derived from albino NMRI/Han mice (12, 17). For TyBS, 153 fertilized eggs were transferred to pseudopregnant females, which gave rise to 33 newborns. For TyBSmut519, 220 fertilized eggs were transferred and 52 mice were born. Transgenic animals were identified by pigment production (in skin or eye) and/or Southern blot analysis. Eyes were isolated from 4-month-old transgenic nonpigmented animals (and nontransgenic albino mice) and analyzed for traces of pigment production under a stereomicroscope.

## RESULTS

**The Dark-Eyed Albino Mutation ($c^{44H}$).** Mice homozygous for $c^{44H}$ are viable and fully fertile in both sexes (13). A homozygous male ($c^{44H}/c^{44H}$) was bred to albino ($c/c$) NMRI/Han females to generate heterozygous offspring. Eye pigmentation in $c^{44H}/c$ heterozygous mice is only clearly seen at an age of 3–4 months, while skin and hair are still unpigmented (Fig. 1). These matings thus confirmed that $c^{44H}$ represents an allele of the *c* locus (13, 14).

The albino mutation creates a new *Dde* I site in exon 1 of the tyrosinase gene (10). Using two primers, nos. 2 and 3 which are located 5′ and 3′ of the *Dde* I polymorphism, we amplified genomic DNA from NMRI/Han and $c^{44H}$ homozygotes. After digestion with *Dde* I, the fragments expected for albino (130 bp and 35 bp) were detected when DNA from NMRI/Han mice was amplified (see Fig. 3). As in DNA from wild-type ($C/C$) mice, the *Dde* I site was not present in $c^{44H}$, thus yielding a fragment of 165 bp (not shown). With respect

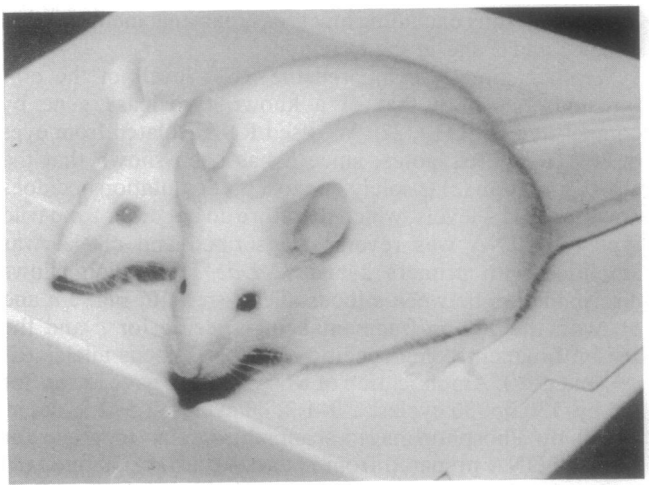

FIG. 1. Phenotype of dark-eyed albino mice. A dark-eyed albino mouse ($c^{44H}/c$, at right) and an albino mouse ($c/c$, at left) are both unpigmented in hair and skin. Eyes of the dark-eyed albino mouse are lightly pigmented.

to the albino mutation the $c^{44H}$ tyrosinase gene does not differ from wild type. This polymorphism therefore allowed us to discern DNA from heterozygous newborns ($c^{44H}/c$) and homozygous newborns.

**The Mutation Does Not Affect Expression of the Tyrosinase Gene.** Previous results suggested that the *c*-locus mutation chinchilla-mottled ($c^m$) was caused by a rearrangement in the tyrosinase gene upstream sequence which affected the level of transcription of the gene (20). We have therefore analyzed genomic DNA from mutant mice ($c^{44H}/c$) for alterations in restriction patterns compared with that of the wild-type tyrosinase gene (C57BL/6J; *C*; data not shown) and the chinchilla tyrosinase gene ($c^{ch}/c$) to depict possible rearrangements in the upstream region of the mutant (Fig. 2). DNA was digested with various restriction enzymes and Southern blots were hybridized to an exon 1/exon 2-specific probe. Cleavage with *Hin*dIII (4.8 kb and 8.0 kb) and *Eco*RI (4.7 kb and 12.0 kb) yielded the expected fragments (21). With another enzyme (*Pvu*II), no difference was found as well. Since these digestions do not allow analysis of the more 5′ region (*Eco*RI cuts at −2.5 kb; *Hin*dIII at −80 bp), we cleaved DNA with *Apa* I, which cuts in exon 1 and about 19 kb upstream (20). These analyses revealed no changes in restriction pattern among the four alleles analyzed (*C*, $c^{ch}$,

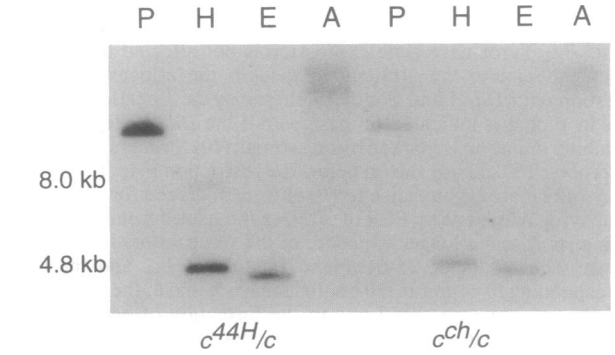


FIG. 2. The upstream region of the mutant tyrosinase gene is not altered. Southern blot analysis of DNA from dark-eyed albino mice ($c^{44H}/c$) and chinchilla mice ($c^{ch}/c$) shows that the three alleles are not rearranged (P, *Pvu*II; H, *Hin*dIII; E, *Eco*RI; A, *Apa*I). Ten micrograms ($c^{44H}/c$) or 5 μg ($c^{ch}/c$) of DNA was loaded per lane. The *Eco*RI fragment encompassing exon 2 (12.0 kb) is present on the original autoradiograph. The fragment lengths due to cleavage with *Hin*dIII are indicated.

$c^{44H}$, and $c$) thus excluding any gross rearrangements around exons 1 and 2 and in the 5′ region.

We then directly compared the RNA levels of the $c^{44H}$ tyrosinase gene to that of a known tyrosinase gene by competitive PCR (19, 22). We used RNA isolated from eyes of $c^{44H}/c$ heterozygotes, since it has been shown that the albino mutation ($c$) is solely due to a point mutation and does not affect RNA levels, which therefore do not differ from wild type ($C$). RNA was reverse transcribed and cDNA was amplified with primers 2 and 3. *Dde* I digestion allows discrimination between $c$-locus alleles (ref. 10; Fig. 3 *a* and *b*), with the 130-bp fragment being specific for $c$ and the 165-bp fragment being specific for other $c$-locus alleles ($C$, $c^{ch}$, and $c^{44H}$). Amplification of RNA samples gave ratios (for 165 bp/130 bp: 30 cycles, 1.0–1.3; 40 cycles, 1.5–2.3, determined by PhosphorImager scanning) similar to those for genomic DNA prepared from heterozygous $c^{44H}/c$ mice (30 cycles, 1.0–1.3; 40 cycles, 1.6–1.7). We diluted the final PCR mixture (40 cycles) 1:200 and performed one additional PCR cycle (19), which then resulted in a 1:1 ratio in genomic DNA and cDNA. This suggested that, in heterozygous ($c^{44H}/c$) animals, both $c$-locus alleles were equally expressed. Additionally, we performed a Northern blot analysis, which showed no difference in tyrosinase RNA present in skin of homozygous albino ($c/c$), homozygous dark-eyed ($c^{44H}/c^{44H}$), or heterozygous ($c^{44H}/c$) mice (Fig. 3*c*). We therefore conclude that RNA levels generated from $c^{44H}$ and $c$ are not different and that the mutation does not act on the level of transcription.

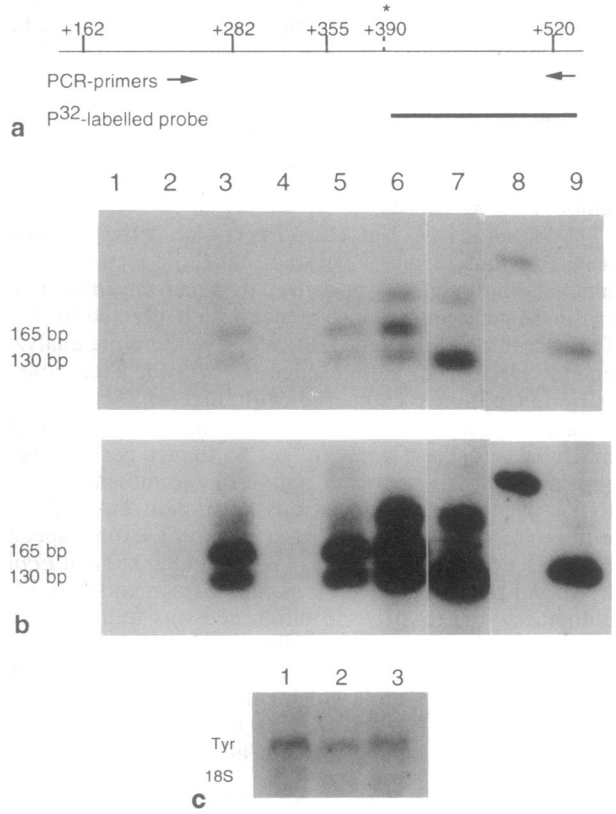


FIG. 3. Tyrosinase gene expression is not affected by $c^{44H}$. (*a*) Scheme depicting the strategy to discern the $c$-locus alleles by digestion with *Dde* I and Southern blot analysis. Location of *Dde* I sites in exon 1 is indicated (in base pairs); the *Dde* I site present in the albino mutation is marked by an asterisk (10). Primers 2 and 3 and the probe pTY0.13 are shown below the restriction map. (*b*) Eyes of 4-day-old heterozygous mice ($c^{44H}/c$) were analyzed for expression of the tyrosinase gene by PCR of cDNA synthesized from total RNA. Both a short and a longer exposure of the same autoradiograph are shown. The $c^{44H}$-specific fragment runs at 165 bp, and the band corresponding to $c$ runs at 130 bp. In lanes 3, 5, and 6, the upper band (165 bp) is overrepresented due to the formation of heterodimeric DNA (19), which is not cut by *Dde* I. Samples from amplified plasmid and genomic DNAs were run on the same gel. Lane 1, control PCR (40 cycles) was performed on total RNA (mouse 1) without reverse transcriptase; lanes 2 and 3, 30 and 40 cycles, mouse 1, $c^{44H}/c$; lanes 4 and 5, 30 and 40 cycles, mouse 2, $c^{44H}/c$; lane 6, genomic DNA of mouse 1 $c^{44H}/c$; lane 7, genomic DNA of $c/c$; lane 8, plasmid TyBS ($C$), uncleaved; lane 9, plasmid TyBS ($C$). (*c*) Northern blot analysis of total RNA (20 μg) isolated from skin of 3-day-old mice. Lane 1, albino ($c/c$); lane 2, dark-eyed heterozygous, ($c^{44H}/c$); lane 3, dark-eyed homozygous ($c^{44H}/c^{44H}$).

**The $c^{44H}$ Tyrosinase Gene Contains a Point Mutation.** According to the results presented above, we considered it likely that the mutation was due to an aberrant tyrosinase gene. To discover this mutation we sequenced the tyrosinase cDNA from homozygous $c^{44H}/c^{44H}$ mice. We used PCR to amplify cDNA and genomic DNA from dark-eyed albino mice with primers flanking the coding sequence of the mouse tyrosinase gene. PCR products were subcloned in plasmid vectors and sequenced. When compared with the wild-type tyrosinase gene, one single base change was found in several independent experiments (Fig. 4*a*): a G → T substitution at base +519 which causes a change from serine to isoleucine at amino acid 128. This event destroys an *Alu* I restriction enzyme site (AGCT to ATCT) in exon 1 of the tyrosinase gene. *Alu* I thus cleaves once (+225) when exon 1 of the tyrosinase gene is derived from $c^{44H}$ mice, and twice (+225 and +518) when it is derived from wild-type ($C$) or albino ($c$) mice. This polymorphism allowed us to confirm the presence of the mutant in several independent mice by restriction enzyme digestions of PCR-amplified products (Fig. 4*b*). We therefore conclude that the $c^{44H}$ tyrosinase gene contains a point mutation at position +519.

**The Mutation Is Sufficient to Generate the Dark-Eyed Albino Phenotype.** Having identified a point mutation in the gene we asked whether it represented the molecular basis for the decreased pigmentation. Preliminary experiments using *in vitro* translation showed that the mutant gene was able to produce a full-length tyrosinase protein (unpublished data). We therefore tested the function of the mutated tyrosinase gene *in vivo* by using transgenic mice. Such experiments have shown that tyrosinase minigenes introduced into mice do

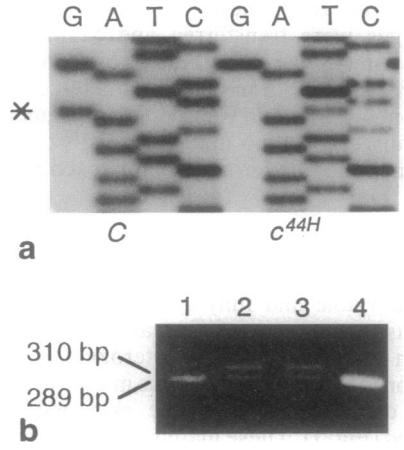


FIG. 4. The $c^{44H}$ tyrosinase gene contains a point mutation. (*a*) Section of sequencing gel showing the sequence difference between wild-type ($C/C$) and homozygous dark-eyed albino ($c^{44H}/c^{44H}$). (*b*) The $c^{44H}$ mutation removes a *Alu* I restriction site (AGCT) from the tyrosinase gene. Ethidium bromide-stained gel of *Alu* I-digested PCR-amplified DNA from NMRI/Han mice ($c/c$; lane 1), heterozygous dark-eyed albino mice ($c^{44H}/c$; lanes 2 and 3), and a wild-type tyrosinase minigene (TyBS; $C$; lane 4). Amplification with primers 2 and 3 results in fragment lengths of 310 bp in $c^{44H}$ and 289 bp and 21 bp (not shown) in albino and wild type.

rescue the albino phenotype. The resulting transgenic mice are pigmented to a varying degree in skin, fur, and eyes (9, 12, 23, 24). We used the construct TyBS (kindly provided by Paul Overbeek, Howard Hughes Medical Institute, Houston), which is composed of 2.25 kb of tyrosinase 5′ sequence coupled to tyrosinase cDNA. Expression from this construct was reported for a high percentage (about 80–90%) of transgenic mice (9, 25). Using TyBS, we obtained 4 pigmented mice among 5 transgenics (Fig. 5). When we injected a mutated version of the minigene (TyBSmut519; Fig. 5), none of the 52 newborn mice was pigmented. Southern blot analysis revealed that 7 of them were transgenic with copy numbers from about 1 to 20 (Fig. 5). Even after 4 months none of the founders showed signs of pigmentation in eyes. However, looking at isolated eyes under a dissecting microscope revealed pigmentation in animals from two lines (Fig. 6). This demonstrated that the mutant transgene was functional and could lead to pigment production. This weak pigmentation in 2 out of 7 transgenic lines in comparison with the wild-type transgene (4 out of 5 lines expressed the transgene in eye and/or skin) corresponds to the reduced pigmentation of dark-eyed albino mice. We therefore conclude that the characterized point mutation is both responsible for and sufficient to generate the dark-eyed albino phenotype.

## DISCUSSION

In humans, absent or reduced activity of tyrosinase is known as the genetic disorder of type I OCA (15, 16, 26). Whereas

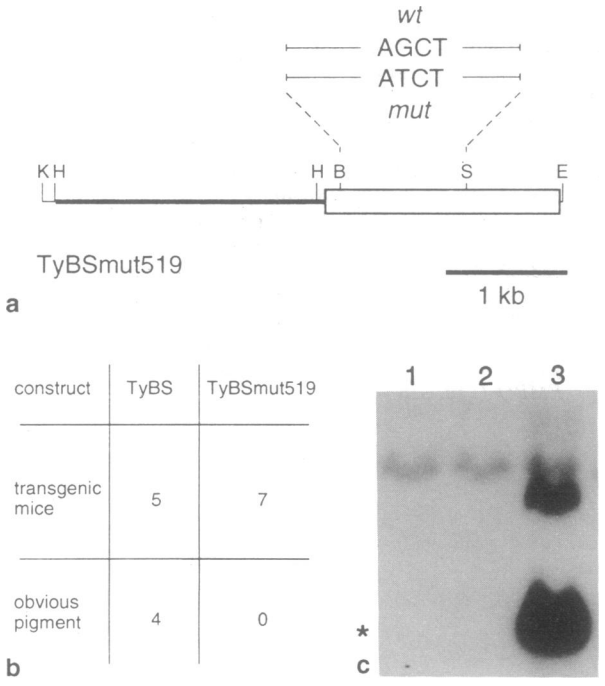


FIG. 5. Transgenic mice generated with a mutated tyrosinase minigene. (*a*) The tyrosinase minigene TyBSmut519 used for microinjection. The region exchanged from TyBS (wild type, *wt*) is indicated. Coding sequence of TyBSmut519 is shown as an open box, and upstream sequence as a thick line. K, *Kpn* I; H, *Hin*dIII; B, *Bgl* II; S, *Sph* I; E, *Eco*RI. (*b*) Table comparing obvious pigmentation in mice generated with TyBS and TyBSmut519. (*c*) Southern blot of *Hin*dIII-digested DNA from transgenic (line 310, lane 3) and nontransgenic (lanes 1 and 2) mice. The transgene-specific band of 2 kb, predicted by head-to-tail integration of the transgene, is indicated by a star. The additional transgene-specific upper band (4 kb) is presumably due to inverted orientation of the transgene. In DNA from nontransgenic mice (first two lanes), only the endogenous fragments corresponding to exon 1 (4.8 kb) and exon 2 (8.0 kb; faint band at top; see Fig. 2) are detected.

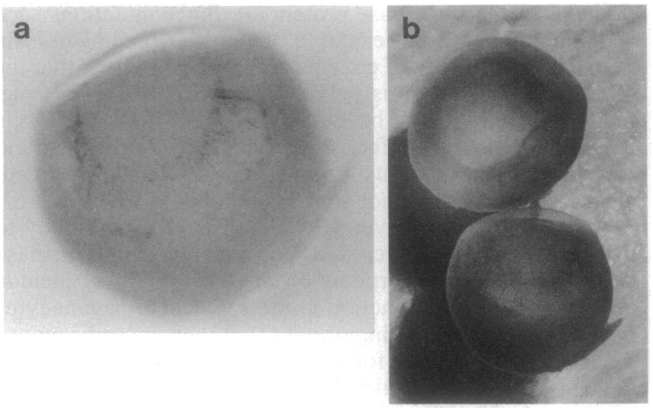


FIG. 6. Pigment production in eyes of a mouse transgenic for TyBSmut519. (*a*) Eye of a 4-month-old transgenic mouse (line 310). Pigmentation is visible in the iris and the ciliary epithelium. (*b*) Eyes of a nontransgenic mouse (above) and a transgenic mouse (below, line 310). No pigment is present in the eye of the nontransgenic albino mouse.

type IA OCA is tyrosinase-negative, type IB OCA is characterized by greatly reduced tyrosinase activity and little or no pigment at birth. DNA analyses of patients with type I OCA have demonstrated that mutations in the tyrosinase gene constitute the basis of these disorders, and a series of different allelic mutations of the human tyrosinase gene have been identified in patients with type I OCA (15, 16, 26). In mouse, the situation is different. Besides radiation-induced deletions of the *c* locus (e.g., $c^{14\ CoS}$; refs. 7, 17, and 27) only a few specific mutations have been analyzed (albino, chinchilla, and himalayan). These are all caused by point mutations in the coding region (3, 28). Results indicate that the *c*-locus mutation chinchilla-mottled is caused by a rearrangement in the tyrosinase 5′ flanking sequence and thus affects levels of transcription (20).

Dark-eyed albino is characterized by marked reduction but not an absence of melanin synthesis (refs. 13 and 14; Fig. 1). Low amounts of pigment are present both in neural crest-derived melanocytes and in the pigment epithelium of the retina. This excludes a mutation in a putative cis element responsible for specific expression in neural crest-derived melanocytes. Our results show that in the $c^{44H}$ tyrosinase gene, neither the 5′ region is rearranged nor the expression reduced (Figs. 2 and 3). Sequence analysis depicted a point mutation in exon 1. Experimental proof for the importance of this mutation came from reintroducing it back into mice. The mutated tyrosinase transgene did not lead to obvious pigmentation in albino mice, and decreased pigmentation could be detected in eyes of only two lines (Fig. 6). The lower pigmentation in transgenic TyBSmut519 versus dark-eyed albino mutant mice fits to the results obtained with different tyrosinase minigenes (9, 12, 23, 25, 28, 29), and it has been shown recently that wild-type pigmentation in tyrosinase-transgenic mice seems to require much more upstream sequence (30).

Mutations leading to decreased tyrosinase expression in humans (type IB OCA) are rare compared with those leading to complete loss of pigment (type IA OCA). The three identified mutations are in exon 2 and exon 4 of the human tyrosinase gene, and the exon 2 mutation seems to be the most frequent cause of type IB OCA in the general Caucasian population (15, 16, 26). The mutation that we have characterized is a mutation in tyrosinase exon 1 which alters but does not abolish pigment production. All other characterized mutations in exon 1 of the tyrosinase gene abrogate expression and infer a complete albino phenotype. The $c^{44H}$ mutation at codon 128 cannot be allocated to one of the important

domains of the tyrosinase protein (5). Since mutations such as albino affecting, for example, the copper-binding domain in exon 1, cause lack of enzyme activity, we suggest that the effect of the mutation is only subtle, since, otherwise, pigment production would be totally abolished. How such single amino acid changes affect the function of the protein and thereby influence production and deposit of pigment is not clear and requires further studies.

A mouse model for type IB OCA offers the possibility to analyze the effect of decreased but not abolished tyrosinase activity and pigment production *in vivo*. This may include analyses on hearing function in the ear, where melanocytes have been shown to be important (31, 32). Transgenic mouse models for melanoma formation have been reported with development of melanotic tumors being related to production of pigment (33, 34). The dark-eyed albino mouse enables analyses of how melanosis or melanoma formation are influenced by reduced pigment production.

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1. Silvers, W. K. (1979) *The Coat Colors of Mice: A Model for Mammalian Gene Action and Interaction* (Springer, New York).
2. Lyon, M. E. & Searle, A. G. (1989) *Genetic Strains and Variants of the Laboratory Mouse* (Oxford Univ. Press, Oxford), 2nd Ed.
3. Jackson, I. J. (1991) *BioEssays* **13**, 439–446.
4. Jackson, I. J., Chambers, D. M., Tsukamoto, K., Copeland, N. G., Gilbert, D. J., Jenkins, N. A. & Hearing, V. J. (1992) *EMBO J.* **11**, 527–535.
5. Hearing, V. J. & Tsukamoto, K. (1991) *FASEB J.* **5**, 2902–2909.
6. Russell, L. B., Russell, W. L. & Kelly, E. M. (1979) *Genetics* **91**, 127–139.
7. Rinchik, E. M., Stoye, J. P., Frankel, W. N., Coffin, J., Kwon, B. S. & Russell, L. B. (1993) *Mutat. Res.* **286**, 199–207.
8. Shibahara, S., Okinaga, S., Tomita, Y., Takeda, A., Yamamoto, H., Sato, M. & Takeuchi, T. (1990) *Eur. J. Biochem.* **189**, 455–461.
9. Yokoyama, T., Silversides, D. W., Waymire, K. G., Kwon, B. S., Takeuchi, T. & Overbeek, P. A. (1990) *Nucleic Acids Res.* **18**, 7293–7298.
10. Jackson, I. J. & Bennett, D. C. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7010–7014.
11. Kwon, B. S., Halaban, R. & Chintamaneni, C. (1989) *Biochem. Biophys. Res. Commun.* **161**, 252–260.
12. Beermann, F., Ruppert, S., Hummler, E., Bosch, F. X., Müller, G., Rüther, U. & Schütz, G. (1990) *EMBO J.* **9**, 2819–2826.
13. Cattanach, B. M. & Rasberry, C. (1988) *Mouse News Lett.* **81**, 64 (abstr.).
14. Jones, J., Peters, J., Ball, S., Cattanach, B. S. & Kwon, B. S. (1988) *Mouse News Lett.* **82**, 123 (abstr.).
15. Giebel, L. B. & Spritz, R. A. (1992) *Pigment Cell Res. Suppl.* **2**, 101–106.
16. Tomita, Y. (1993) *J. Invest. Dermatol.* **100**, 186S–190S.
17. Beermann, F., Hummler, E., Schmid, E. & Schütz, G. (1993) *Mech. Dev.* **42**, 59–65.
18. Davis, L. G., Dibner, M. D. & Battey, J. F. (1986) *Basic Methods in Molecular Biology* (Elsevier, New York).
19. Becker-André, M. & Hahlbrook, K. (1989) *Nucleic Acids Res.* **17**, 9437–9446.
20. Porter, S., Larue, L. & Mintz, B. (1991) *Dev. Genet.* **12**, 393–402.
21. Ruppert, S., Müller, G., Kwon, B. & Schütz, G. (1988) *EMBO J.* **7**, 2715–2722.
22. Foley, K. P., Leonard, M. W. & Engel, J. D. (1993) *Trends Genet.* **9**, 380–385.
23. Tanaka, S., Yamamoto, H., Takeuchi, S. & Takeuchi, T. (1990) *Development* **108**, 223–227.
24. Mintz, B. & Bradl, M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9643–9647.
25. Overbeek, P. A., Aguilar-Cordova, E., Hanten, G., Schaffner, D. L., Patel, P., Lebowitz, R. M. & Lieberman, M. W. (1991) *Transgenic Res.* **1**, 31–37.
26. Oetting, W. S. & King, R. A. (1993) *Hum. Mutat.* **2**, 1–6.
27. Russell, L. B., Montgomery, C. S. & Raymer, G. D. (1982) *Genetics* **100**, 427–453.
28. Beermann, F., Ganss, R. & Schütz, G. (1994) *Adv. Dev. Biochem.* **3**, in press.
29. Klüppel, M., Beermann, F., Ruppert, S., Schmid, E., Hummler, E. & Schütz, G. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3777–3781.
30. Schedl, A., Montoliu, L., Kelsey, G. & Schütz, G. (1993) *Nature (London)* **362**, 258–261.
31. Steel, K. & Barkway, C. (1989) *Development* **107**, 453–463.
32. Conlee, J. W., Parks, T. N. & Creel, D. J. (1986) *Brain Res.* **363**, 28–37.
33. Bradl, M. L., Klein-Szanto, A., Porter, S. & Mintz, B. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 164–168.
34. Iwamoto, T., Takahashi, M., Ito, M., Hamatani, K., Ohbayashi, M., Wajjwalku, W., Isobe, K. & Nakashima, I. (1991) *EMBO J.* **10**, 3167–3175.