The Original Pink-Eyed Dilution Mutation (*p*) Arose in Asiatic Mice: Implications for the *H4* Minor Histocompatibility Antigen, *Myod1* Regulation and the Origin of Inbred Strains

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ABSTRACT

Allelic variation of the mouse *pink-eyed dilution* (*p*) gene in common laboratory strains and wild mice was examined by Southern blot and by polymerase chain reaction. In these assays the original *p* mutation allele found in strains SJL/J, 129/J, B10.129(21m), P/J and FS/Ei most closely matches an Asian *Mus musculus* allele, confirming anecdotal accounts of the Asian origin of this mutation. In contrast, the wild-type allele found in other common laboratory strains was apparently derived from *Mus domesticus*. Analysis of chromosome 7 loci both proximal and distal to the *p* locus demonstrates that strains SJL/J, 129/J, B10.129(21M), P/J and FS/Ei contain DNA segments of varying length derived from *M. musculus*. Strains 129/J and B10.129(21M) contain the largest segment of *M. musculus*-derived DNA (about 5 cM), including the loci *Myod1*, *p*, three clustered $GABA_A$ receptor subunit loci (*Gabrg3*, *Gabra5* and *Gabrb3*), and *Snrpn*. The difference in the species origin of genes from this region of chromosome 7 may underlie the basis of the antigenicity of the minor histocompatibility antigen *H4*, defined by the strain B10.129(21M), and may account for the enhanced *Myod1* activity observed in SJL/J mice.

THE first mutation[1] identified at the mouse *pink-eyed dilution* (*p*) locus was the recessive allele designated *p* (reviewed by SILVERS 1979; GREEN 1989), referred to hereafter as the original *p* mutation. In addition to being one of the first mutations described in mice, this mutation and a mutation at the *albino* (*c*) locus were used to define the first linkage group in the mouse (HALDANE *et al.* 1915), now assigned to chromosome 7. The original *p* mutation also was used to define histocompatibility antigens and was found to be genetically inseparable from the minor histocompatibility locus, *H4* (SNELL and STEVENS 1961), which has been shown to be genetically complex (DAVIS and ROOPENIAN 1990; ROOPENIAN 1992; ROOPENIAN *et al.* 1993).

To date, more than 100 *p* alleles have been identified, some of which were *de novo* in origin and some of which were induced by X-rays or chemical mutagens (GREEN 1989; LYON *et al.* 1992; M. F. LYON, personal communication). In the homozygous state, each mutant *p* allele causes hypopigmentation ranging from a minor reduction in coat color to a dramatic reduction of both coat and eye color characteristic of the original *p* mutation. In addition to affecting pigmentation, several mutant alleles are associated with other abnormalities, including neurological disorders (p^{6H}, p^{25H}, p^{bs}, p^{cp}), cleft palate (p^{cp}), male sterility and female semi-fertility p^{6H}, p^{25H}, p^{bs}), genetic instability (p^{un}), and prenatal lethality (p^{81H}, p^{82H}, p^{87H}) (GREEN 1989; LYON *et al.* 1992; BRILLIANT 1992).

The original *p* mutation also was used as a visible marker in studies of the minor histocompatibility locus, *H4*. The original *p* mutation of strain 129/J was put on a C57BL/10 background (wild type at the *p* locus) to construct the congenic line B10.129(21M). The *H4* minor histocompatibility locus was initially defined by resistance of the B10.129(21M) strain to injected C57BL/10 leukemia cells after preimmunization with C57BL/10 thymus cells (SNELL and STEVEN 1961). Transplanted skin grafts (in both directions) also defined antigenic differences between C57BL/10 and B10.129(21M) (GRAFF *et al.* 1966), with faster rejection when the donor is B10.129(21M). However, the expression of the *H4* antigen is not limited to lymphocytes and skin cells, as *H4*-mediated graft rejection extends to liver (SCHULTZ *et al.* 1978) and heart (DETOLLA *et al.* 1977).

Recently, we (GARDNER *et al.* 1992) isolated and cloned a cDNA encoding the mouse *p* gene. When this cDNA was used as a hybridization probe for Southern blots containing genomic DNA from various common laboratory strains, two distinct classes of hybridization patterns were evident: those of mouse strains bearing the original *p* mutation and those of other laboratory mouse strains. Although the migration of most of the

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TABLE 1

Inbred mouse strains and species used in this study

Abbreviation	Strain (*p* alleles)	Origin
SJL	SJL/J *p/p*	Laboratory mouse[a]
129	129/J *p/p*	Laboratory mouse[a]
21M	B10.129(21M) *p/p*	Laboratory mouse[a]
P/J	P/J *p/p*	Laboratory mouse[a]
FS	FS/Ei *p/p*	Laboratory mouse[a]
B6	C57BL/6J +/+	Laboratory mouse[a]
B10	C57BL/10SnJ +/+	Laboratory mouse[a]
C3H	C3H/HeJ +/+	Laboratory mouse[a]
AKR	AKR/J +/+	Laboratory mouse[a]
DBA	DBA/2J +/+	Laboratory mouse[a]
SMJ	SM/J +/+	Laboratory mouse[a]
NZB	NZB/B1NJ +/+	Laboratory mouse[a]
BALB	BALB/cJ +/+	Laboratory mouse[a]
MOLF	MOLF/Ei +/+	*Mus m. molossinus*, Japan[b]
MOLD	MOLD/Rk +/+	*M. m. molossinus*, Japan[c]
MOL	MOL +/+	*M. m. molossinus*, Japan[d]
MOA	MOA +/+	*M. m. molossinus*, Japan[e]
MOM	MOM +/+	*M. m. molossinus*, Japan[e]
NEF	NEF +/+	*M. m. molossinus*, Japan[e]
CZ I	CZECH I +/+	*Mus m. musculus*, Czechoslovakia[d]
CZ II	CZECH II +/+	*M. m. musculus*, Czechoslovakia[d]
SKIVE	SKIVE/Ei +/+	*M. m. musculus*, Denmark[b]
CAST	CAST/Ei +/+	*Mus m. castaneus*, Thailand[b]
CAS	CAS +/+	*M. m. castaneus*, Thailand[d]
TIRANO	TIRANO/Ei +/+	*Mus domesticus*, Italy[b]
ZALENDE	ZALENDE/Ei +/+	*M. domesticus*, Switzerland[b]
WSB	WSB/Ei +/+	*M. domesticus*, Maryland, United States[d]
SPRET	SPRET/Ei +/+	*Mus spretus*, Spain[b]
SPRET-2	SPRET-2 +/+	*M. spretus*, Morocco[d]
HORT-H	HALBTURN +/+	*Mus hortulanus*, Austria[d]
HORT-P	PANCEVO +/+	*M. hortulanus*, Yugoslavia[d]

[a] From The Jackson Laboratory.
[b] From E. EICHER stocks; some were derived from stocks of M. POTTER or V. CHAPMAN.
[c] From T. RODERICK; MOLD/Rk shares a common ancestry with MOLF/Ei.
[d] From M. POTTER, N.I.H., Bethesda, Maryland.
[e] From T. TOMITA, Nagoya University, Japan.

hybridizing bands was different between each class, approximately the same number of fragments were detected. Thus the original *p* mutation did not appear to be associated with a deletion, duplication, inversion, insertion or translocation. At least two other loci near the *p* gene, *Saa* (TAYLOR and ROWE 1984) and *MyoD1* (KAY *et al.* 1993), also show curious polymorphisms specific to strains carrying the original *p* mutation.

While progress has been made in understanding the genetic complexity of the *p* locus (LYON *et al.* 1992), defining the *p* gene (GARDNER *et al.* 1992), understanding genetic reversion of the p^{un} allele (BRILLIANT *et al.* 1991; GONDO *et al.* 1993), and the molecular basis of the p^{cp} mutation (NAKATSU *et al.* 1993; CULIAT *et al.* 1993), until now, the basis for the variant hybridization pattern unique to the original *p* mutation has been unknown.

In this report we present data demonstrating that the original *p* mutation is of *Mus musculus* origin, whereas the *p* locus of other common laboratory mouse strains is of *Mus domesticus* origin, explaining the unique Southern blot pattern associated with laboratory strains bearing the original *p* mutation. We also present estimates of the size of the *M. musculus*-derived region of chromosome 7 that flanks the original *p* mutation carried in several inbred strains. These findings may help to explain the basis of the *H4* minor histocompatibility locus and alleleic variants of loci closely linked to the *p* locus, including *Saa* and *Myod1*.

MATERIALS AND METHODS

Mice and genomic DNA isolation: Genomic DNAs for Southern analysis from laboratory mice (maintained at The Jackson Laboratory, Bar Harbor, Maine, or The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia) were isolated as previously reported (BRILLIANT *et al.* 1991; TUCKER *et al.* 1989). DNA from inbred wild mice were from a previously described collection (TUCKER *et al.* 1989). Some of the founders for these laboratory stocks derived from wild isolates were originally obtained from M. POTTER (NIH), who also provided some tissues for DNA extraction. DNAs from other inbred wild mice were prepared from tissues obtained from T. TOMITA (Nagoya University) and T. RODERICK (The Jackson Laboratory). A list of the strains, species and subspecies used together with their ultimate geographical origin and the abbreviated designations used in figures and text are presented in Table 1. For species nomenclature, we follow the convention that *Mus domesticus* and *Mus musculus* are distinct species (TUCKER *et al.* 1989, 1992b; EICHER *et al.* 1989; SAGE *et al.* 1986), in contrast to others

(*e.g.*, NAGAMINE *et al.* 1992) who classify *Mus domesticus* as *Mus musculus domesticus*, a sub-species of *Mus musculus*. In more general agreement is that *Mus musculus* is composed of geographically distinct subspecies: *Mus m. musculus*, *Mus m. castaneus*, and *Mus m. molossinus* (the latter, found in Japan and Manchuria, is a hybrid of the first two subspecies) (YONEKAWA *et al.* 1986).

Gel electrophoresis, probe preparation, Southern hybridization: Agarose gel electrophoresis and Southern blotting to Gene Screen Plus membranes (NEN-Dupont, Boston) or to Hybond N+ (Amersham, Arlington Heights, Illinois) were as previously described (BRILLIANT *et al.* 1991). Fragments used for Southern hybridization included: a 1.35-kb *Dra*III fragment from pMC2701, a *p* cDNA clone (GARDNER *et al.* 1992); a 2.7-kb *Hin*dIII fragment from p393, a *Myod1* genomic DNA clone containing most of the gene-encoding sequences (gift of C. EMERSON); a 0.9-kb *Bam*HI-*Pst*I fragment from pBS908-1 encoding the RP2 gene of the *D7Rp2* locus (gift of G. WATSON) (BERGER *et al.* 1981); three cDNAs of $GABA_A$ receptor subunit genes: *Gabrg3* (gift of J. SIKELA) (WILSON-SHAW *et al.* 1991), a 3′ fragment of *Gabra5* (gift of A. TOBIN) (KHRESHCHATISKY *et al.* 1989), and a 0.7-kb *Eco*RI-*Xho*I fragment of *Gabrb3* (gift of J. WAGSTAFF and M. LALANDE) (WAGSTAFF *et al.* 1991); and an intron fragment of *Snrpn* (generated from primer sequences reported by LEFF *et al.* 1992). Probes were radiolabeled to a specific activity of $\sim 3 \times 10^9$ cpm/μg using the Multiprime Kit (Amersham) or Prime It Kit (Stratagene, La Jolla, California).

Polymerase chain raction (PCR), oligonucleotide primers and reaction conditions: Genomic DNAs were amplified using a Programmable Thermal Controller (MJ Research): 94°, 2′; 94°, 1′; 59°, 1′; 72°, 1′; repeat steps 2–4, 39 times; 72°, 5′; hold at 15° (end). Primer pair MHB13, 5′CACTATGCCTGCTTGGATGCTGC3′, and MHB14, 5′ATGGGACATCTGTGGTTACACTGG3′, were derived from a C57BL/6JEi p^{un}/p^{un} sequence (432 bp apart) surrounding the 5′ (left) boundary of a tandem duplication in the p^{un} allele. This sequence is identical to that found in wild-type C57BL/6JEi DNA and is intronic for the *p* gene (GONDO *et al.* 1993; Y. GONDO and M. H. BRILLIANT, unpublished).

Primer pair MHB46, 5′CCTCAGAGACAAGAGGCTAAGAG3′, and MHB47, 5′AGACACCAGGGAGTGCCTCTGC3′, were derived from C57BL/6JEi sequences (411 bp apart) within the putative first intron of the mouse *p* gene and flank a $(CA)_{23}$ repeat. To accentuate mobility differences, PCR products of MHB46 and MHB47 were digested with *Pst*I before gel electrophoresis to generate four *Pst*I fragments in C57BL/6JEi DNA: 11 bp, 42 bp, 114 bp [containing a $(CA)_{23}$ repeat], and 244 bp. All primers were obtained from The DNA Synthesis Facility, Fox Chase Cancer Center.

Reverse transcriptase-PCR (RT-PCR): Expression of *Gabrb3* and *p* (a control) was analyzed by RT-PCR in cultured melanocytes. Primers used for *Gabrb3* were: β3e4, 5′CGCCTACTCTGGGATCCCTCTCAACCTCACG3′, and β3e8, 5′TCGATCATTCTTGGCCTTGGCTGT3′. Primers used for *p* were: *p* e1, 5′CTTACACCAGGGTTGTGCTCCATCC3′, and *p* eX, 5′ACCGCACACAGCACCCAAGCTT3′. Primer sequences and RT-PCR conditions were as previously described (NAKATSU *et al.* 1993).

RESULTS

DNA from within the *p* gene in mice bearing the original *p* mutation is distinct from that of other common laboratory strains: Strains SJL/J, 129/J, B10.129(21M), P/J, and FS/Ei are homozygous for the original *p* mutation (RODERICK and GUIDI 1989). Southern blots of DNA from these strains (in comparison to other common laboratory strains) revealed multiple restriction fragment length variants when hybridized to cloned cDNA of the *p* locus (Figure 1A) (GARDNER *et al.* 1992). Because the gene encoding the *p* locus is organized in multiple exons distributed over a large genomic segment (of at least 100 kb, J. M. GARDNER, Y. NAKATSU and M. H. BRILLIANT, unpublished), it seemed unlikely that

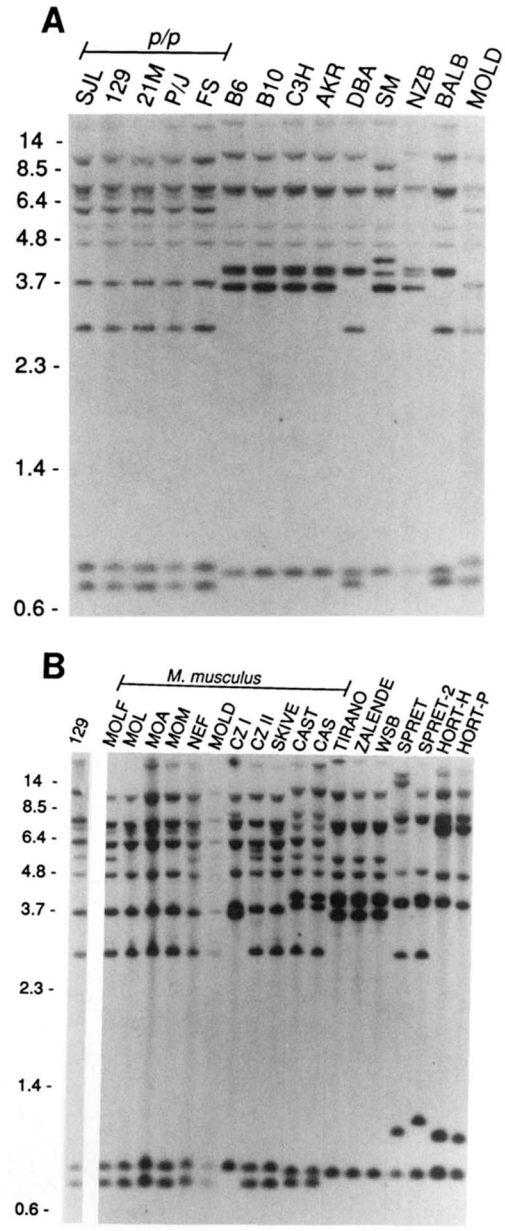

FIGURE 1.—Comparison of DNA from the *p* locus of various mouse strains and species by Southern blot. The full names of all strains and stocks (abbreviated in this figure), their *p* locus alleles and their origins are presented in Table 1. The size of marker fragments is indicated for each panel in kilobases. (**A**) The pattern of Southern hybridization of the *p* cDNA probe to *Sst*I-digested DNA from mice bearing the original *p* mutation (*p/p*) compared with the pattern observed in wild-type laboratory strains and *M. m. molossinus* (MOLD). (**B**) The pattern of Southern hybridization of the *p* cDNA probe to *Sst*I-digested DNA from mice bearing the original *p* mutation compared with wild mouse isolates.

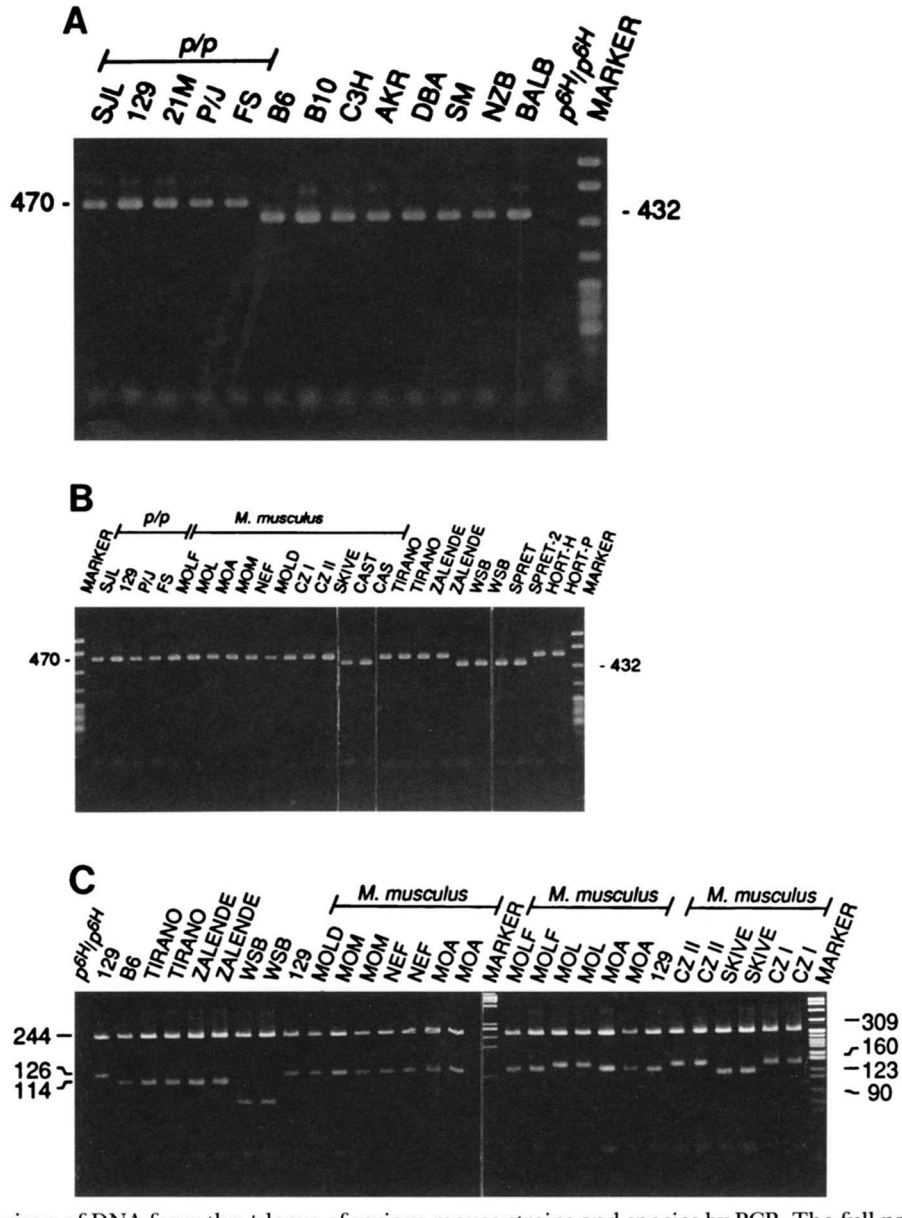

FIGURE 2.—Comparison of DNA from the *p* locus of various mouse strains and species by PCR. The full names of all strains and stocks (abbreviated in this figure), their *p* locus alleles and their origins are presented in Table 1. The size of marker fragments is indicated for each panel in base pairs. (**A**) The pattern of PCR amplification of genomic DNA from mice bearing the original *p* mutation (indicated by top bracket line) compared with those of common laboratory strains (wild-type for *p*) and with DNA from a mouse homozygous for the p^{6H} deletion. PCR primers (MHB13 and MHB14) were derived from the C57BL/6J sequence of an intron of the *p* gene associated with the p^{un} mutation (see MATERIALS AND METHODS). The products of these PCR reactions were resolved on 2% agarose gels (1% SeaKem GTG/1% NuSieve GTG; FMC Bioproducts, Rockland, Maine). (**B**) The pattern of PCR amplification of genomic DNA from mice bearing the original *p* mutation (indicated by top bracket line marked *p/p*) compared with those of wild mouse isolates (*M. musculus* samples and others species, as indicated). PCR primers MHB13 and MHB14 were used. The products of these PCR reactions were resolved on 2% agarose gels (1% SeaKem GTG/1% NuSieve GTG; FMC Bioproducts). (**C**) The pattern of PCR amplification of genomic DNA from strain 129/J bearing the original *p* mutation compared with those of common laboratory strains and wild mouse isolates (*M. musculus* samples and others species, as indicated). The PCR primers used in this assay were MHB46 and MHB 47, derived from the C57BL/6J sequence from within the first intron of the *p* gene (see MATERIALS AND METHODS). To accentuate the mobility differences, the resulting PCR products were digested with *Pst*I before gel electrophoresis. The sizes of certain product fragments (in bp) is indicated to the left. The digested products of these PCR reactions were resolved on 3.5% MetaPhor agarose gels (FMC Bioproducts).

such differences were the result of a simple genomic rearrangement (*i.e.*, deletion, duplication, inversion, insertion, or translocation). Rather, they were reminiscent of differences observed between species. To test this hypothesis, we employed the same Southern assay to survey the *p* alleles of several *Mus* species, especially Asian species, in light of the anecdotal accounts of an Asian origin of the original *p* mutation (KEELER 1931; TOKUDA

TABLE 2

Allele types of the *p* locus in wild mice

	Species[a]													
	M. m. molossinus						*M. m. musculus*			*M. m. castaneus*		*M. domesticus*		
Assay	MOLF	MOL	MOA	MOM	NEF	MOLD	CZI	CZII	SKIVE	CAST	CAS	TIRANO	WSB	ZALENDE
Southern	129	129[b]	129[b]	129[b]	129	129	129[b]	129[b]	129	?[c]	?[c]	B6	B6	B6
PCR (13/14)	129	129	129	129	129	129	129	129	129	B6	B6	129	129	129
PCR (46/47)	129	?[c]	129	129	129	129	?[c]	?[c]	?[c]	ND[d]	ND[d]	B6	?[c]	B6

[a] See Table 1 for abbreviations and origins of mouse strains and isolates.
[b] One or two RFLPs between the indicated sample's pattern on Southern blot and 129.
[c] Neither a 129 nor B6 allele.
[d] Not determined.

1935). The results of that survey are presented in Figure 1B and demonstrate that DNA from mice bearing the original *p* mutation gives a hybridization pattern distinct from inbred strains of pure *M. domesticus* (TIRANO, ZALENDE and WSB), *M. m. castaneus*, *M. spretus*, and *M. hortulanus*. However, the hybridization patterns obtained for laboratory strains bearing the original *p* mutation were identical to that of DNA from several isolates of *M. musculus*, a wild mouse ranging from Eastern Europe to Asia (including MOLF, NEF, MOLD, CZ II and SKIVE). The exceptional *M. musculus* isolates were MOL, MOA, MOM and CZ I, which appear to exhibit simple restriction fragment length variants. In contrast, the hybridization patterns of DNA from laboratory strains that do not bear the original *p* mutation matched the pattern observed for most isolates of *M. domesticus* (compare C57BL/6J, C57BL/10SnJ, C3H/HeJ and AKR/J in Figure 1A with TIRANO, ZALENDE and WSB in Figure 1B). Confirmation of these results was obtained by PCR analysis using primers from intronic sequences of the *p* gene (Figure 2, A, B and C). Primer pair MHB13 and MHB14 amplified a 470-bp product from the DNA of strains SJL/J, 129/J, B10.129(21M), P/J and FS/Ei in contrast to other common laboratory strains where a 432 bp product was amplified (Figure 2A). The 470-bp product also was amplified from the DNA of all *M. musculus* isolates as well as two of three *M. domesticus* isolates (TIRANO and ZALENDE). The 432-bp product was amplified in the third *M. domesticus* isolate (WSB), in two *M. m. castaneus* isolates (CAST-1 and CAST-2), and in two *M. spretus* isolates (SPRET-1 and SPRET-2) (Figure 2B).

A second pair of primers, MHB46 and MHB47, that flank an intronic $(CA)_n$ repeat, differentiated strains 129/J and C57BL/6J. A *PstI* digest of amplified DNA results in four fragments in C57BL/6J DNA. The largest two are visible in Figure 2C: a 244-bp fragment (common to all mouse strains and species studied) and a 114-bp fragment containing a $(CA)_{23}$ repeat in C57BL/6J, also present in two of three *M. domesticus* isolates (TIRANO and ZALENDE) but not in WSB, which exhibited a uniquely small (<90 bp) fragment. The other species exhibited various polymorphisms. Instead of a 114 bp fragment, strain 129/J produced a 126 bp fragment in common with *M. musculus* isolates MOLD, MOM, NEF, MOA and MOLF, but different from MOL, CZ II, SKIVE and CZ I. The above Southern and PCR results are summarized in Table 2 and, taken together, demonstrate that strains SJL/J, 129/J, B10.129(21M), P/J and FS/Ei are most similar to *M. musculus*, with identity to *M. m. molossinus* isolates MOLF, NEF and MOLD in all three assays.

DNA from the region flanking the *p* locus in mice bearing the original *p* mutation is distinct from that of other common laboratory strains: If the original *p* mutation was derived from *M. musculus*, a larger segment of *M. musculus*-derived genomic DNA flanking the *p* gene might be retained in mouse strains bearing the original *p* mutation. To test this possibility, we employed restriction fragment length polymorphism (RFLP) analysis to look for retention of *M. musculus* alleles for loci both proximal and distal to the *p* locus. To find RFLPs between mice bearing the original *p* mutation, other laboratory strains and wild mice, a panel of Southern blots with informative DNA digests was hybridized to probes for loci both proximal and distal to *p*. We also exploited previously reported RFLPs for *Myod1* (KAY *et al.* 1993) and *Snrpn* (LEFF *et al.* 1992).

The allelic variants for two loci proximal to the *p* locus, *D7Rp2* and *Myod1*, were examined (Figure 3, A and B). Strains SJL/J, 129/J, P/J and FS/Ei did not retain *M. musculus* alleles for the most proximal of these two loci, *D7Rp2*. However, we could not unambiguously determine the origin of the B10.129(21M) *D7Rp2* allele, as the allele found in C57BL/10SnJ, a progenitor of this congenic line, cannot be distinguished from the *M. musculus* allele. Because 129/J, the other progenitor of B10.129(21M), does not have a *M. musculus* allele of *D7Rp2*, the most likely origin of the B10.129(21M) allele was C57BL/10SnJ. Strains SJL/J, 129/J, B10.129(21M) and P/J retained a *M. musculus* allele for the other proximal allele examined, *Myod1*. In contrast, FS/Ei did not retain a *M. musculus Myod1* allele. These results suggest that the proximal limit of the *M. musculus* component in SJL/J, 129/J, B10.129(21M) and P/J lies between the *Myod1* and *D7Rp2* loci. Similarly,

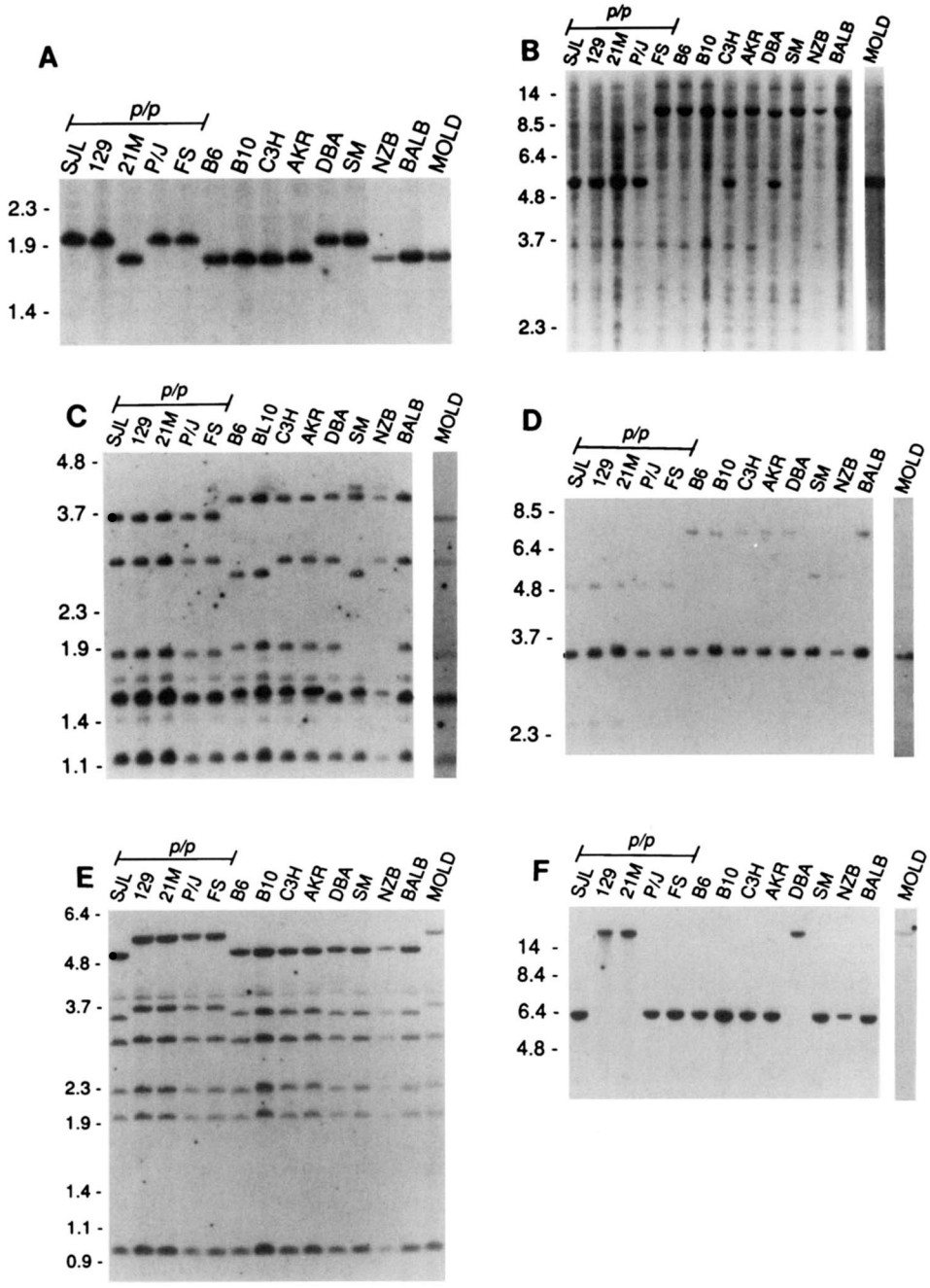

FIGURE 3.—Comparison of DNA at loci proximal and distal to the *p* locus in various mouse strains and species. Probes for the indicated loci (see MATERIALS AND METHODS) were hybridized to genomic DNA from the indicated mouse strains. The full names of all strains and stocks (abbreviated in this figure), their *p* locus alleles, and their origins are presented in Table 1. The migration of marker fragments (in kilobases) is indicated to the left of each panel. (**A**) *D7Rp2* to *Hin*dIII-digested DNA. (**B**) *Myod1* to *Sst*I-digested DNA. (**C**) *Gabrg3* to *Sty*I-digested DNA. (**D**) *Gabra5* to *Hin*dIII-digested DNA. (**E**) *Gabrb3* to *Pst*I-digested DNA. (**F**) *Snrpn* to *Taq*I-digested DNA.

the proximal limit for the *M. musculus* component in FS/Ei lies between the *p* and *Myod1* loci.

Allelic variants for loci distal to the *p* locus also were examined (Figure 3, C D, E and F). These included *Gabrg3*, *Gabra5* and *Gabrb3* (encoding the $GABA_A$ receptor subunits $\gamma 3$, $\alpha 5$ and $\beta 3$, respectively), previously found to be clustered 3′ (distal) to the mouse *p* gene (NAKATSU *et al.* 1993). The order of these loci relative to *p* has recently been determined (NAKATSU *et al.* 1993; Y. NAKATSU and M. H. BRILLIANT, in preparation), proximal to distal: *p*, *Gabrg3*, *Gabra5* and *Gabrb3*. In addition, we examined alleles of the *Snrpn* locus, previously mapped distal to *Gabrb3* (LEFF *et al.* 1992). Two strains, 129/J and B10.129(21M), retained *M. musculus* alleles for all of these loci, including *Snrpn*. P/J and FS/Ei retained *M. musculus* alleles for the three $GABA_A$ receptor loci, but not *Snrpn*. SJL/J retained *M. musculus* alleles for *Gabrg3* and *Gabra5*, but not *Gabrb3*. These results suggest that the distal limit of the *M. musculus* component in 129/J and B10.129(21M) extends beyond *Snrpn*. Similarly, the distal limit for the *M. musculus* segment in P/J and FS/Ei lies between the *Gabrb3* and *Snrpn* loci. In SJL/J, the distal limit for the *M. musculus* segment lies between the *Gabra5* and *Gabrb3* loci. A summary of the extent of the *M. musculus* segment centered around the *p* locus in strains

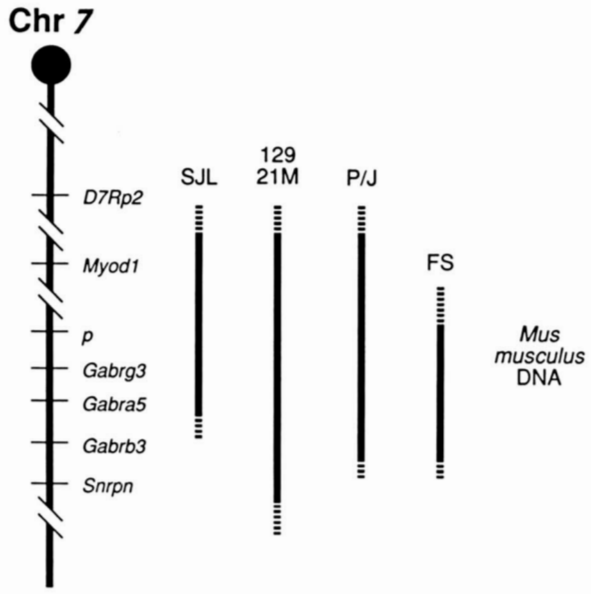

FIGURE 4.—Retention of *M. musculus* DNA in laboratory mice bearing the original *p* mutation. The relative order of the loci analyzed in this study is presented (HOLDENER *et al.* 1993; Y. NAKATSU and M. H. BRILLIANT, in preparation) in a diagram of mouse chromosome 7 (top to bottom, proximal to distal). At the right, that portion of chromosome 7 loci derived from *M. musculus* is indicated for the various strains.

SJL/J, 129/J, B10.129(21M), P/J and FS/Ei is presented in Figure 4.

DISCUSSION

We have found that the original *p* mutation was derived from *M. musculus* (most likely the Japanese/Manchurian subspecies, *M. m. molossinus*). In contrast, the wild-type and other mutant *p* alleles in laboratory strains were derived from *M. domesticus*. Thus, we have confirmed by molecular genetic analysis the anecdotal accounts (KEELER 1931; TOKUDA 1935) of the Asian origin of the original *p* mutation.

We also found that laboratory mouse strains bearing the original *p* mutation contain a *M. musculus*-derived segment of DNA flanking the *p* locus. These results are in agreement with an earlier observation by TAYLOR and ROWE (1984), who mapped the *Saa* locus (encoding serum amyloid A proteins) 3.8 cM proximal to *p* (very close to *Myod1*, HOLDENER *et al.* 1993). A *M. m. molossinus*-like hybridization pattern for *Saa* was noted in strains bearing the original *p* mutation: SJL/J, 129/J, B10.129(21M), and P/J, but not FS/Ei (TAYLOR and ROWE 1984), consistent with our data (Figure 4). These results impact on how we view the *H4* minor histocompatibility locus that is so tightly associated with the *p* locus. Moreover, other curious polymorphisms in this region can now be explained, including the one associated with a reported enhancement of *Myod1* gene activity (KAY *et al.* 1993).

The *H4* minor histocompatibility locus: The minor histocompatibility locus *H4* is tightly linked to the *p* locus as defined by the congenic strain B10.129(21M). As we have shown, the region around the *p* locus in strain B10.129(21M) was derived from *M. musculus*. A protein encoded by the DNA of one mouse species may be sufficiently different to elicit an immune response in another mouse species. Thus, a gene or genes within the segment of chromosome 7 derived from *M. musculus* in B10.129(21M) is likely to underlie the *H4* antigen. We have examined only a fraction of the genes expected to lie within this segment (approximately 5 cM of a total of 1, 800 cM; or 0.28% of the genome). Thus, the basis of the *H4* antigen need not be the *p* gene *per se*. Indeed, the *H4* antigen has been detected in graft rejection assays of organs, *e.g.*, liver (SCHULTZ *et al.* 1978) and heart (DETOLLA *et al.* 1977), where expression of the *p* gene is not observed (GARDNER *et al.* 1992; RINCHIK *et al.* 1993). Therefore, the basis of graft rejection of these organs must have a different etiology.

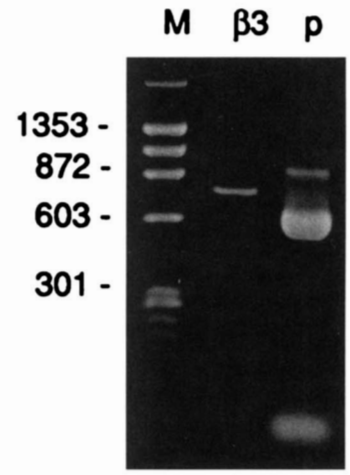

FIGURE 5.—Expression of transcripts in melanocytes by RT-PCR. The presence of transcripts of the *Gabrb3* gene (encoding the β3 subunit of the $GABA_A$ receptor), β3 lane, and the gene encoding the pink-eyed dilution gene product, p lane, were assayed by RT-PCR using previously published primers and protocols (see MATERIALS AND METHODS; NAKATSU *et al.* 1993). Marker fragment migration, M lane, is also shown.

In addition to the *p* gene, species-specific forms of at least six genes *Myod1*, *Gabrg3*, *Gabra5*, *Gabrb3* and *Snrpn* (this report) and *Saa* (TAYLOR and ROWE 1984) also are potentially expressed in these tissue grafts. It is possible that the product of these genes, or others yet to be defined within this genetic interval (individually or in combination), form the basis of the *H4* antigen. In support of a possible role in *H4* mediated skin graft rejection, we have detected *Gabrg3* gene expression in melanocytes by RT-PCR (Figure 5).

A further difference exists between the genomic DNA of strains with different *H4* alleles. RFLPs between C57BL/10JSn and B10.129(21M) or 129/J were detected by the 28RN probe, a genomic fragment adjacent to an intracisternal A particle (IAP) element originally used to identify the p^{un} mutation (BRILLIANT *et al.* 1991;

B. K. Lee, E. M. Eicher and M. H. Brilliant, unpublished). These RFLPs were revealed by a variety of restriction endonucleases and are consistent with the presence of a variant IAP element at this site in C57BL/10JSn DNA but not in B10.129(21M) or 129/J DNA. Thus, if produced, the unique proteins encoded by this element also are candidates for the *H4* antigen.

We note with interest the recent results of Roopenian *et al.* (1993) who identified loci encoding *H4* antigenic determinants both proximal and distal to the *p* locus. The genetic distance of this region is similar in size to the *M. musculus*-derived genomic segment that we describe here, spanning approximately 5 cM, centered around the *p* gene. The *H4* locus was found to contain at least two components, *H46* (mapping proximal to *p*) and *H47* (mapping distal to *p*). The *H46* antigen stimulates MHC class II-restricted $CD4^+$ helper T cells. The *H47* antigen stimulates MHC class I-restricted $CD8^+$ cytotoxic T cells. The strain distribution of the *H47* antigen (+ in SJL/J, − in 129/J and P/J) and its location distal to *p* (Roopenian *et al.* 1993), combined with our data (Figure 4) suggest that the gene encoding the *H47* antigen will be between *Gabra5* and *Snrpn* in a region that includes *Gabrb3*. Indeed, in light of its genomic location and expression in melanocytes (Figure 5), the protein encoded by *Gabrb3* is a candidate for the *H47* antigen.

A second minor histocompatibility locus associated with the *p* locus: In this report, we have postulated that the *H4* minor histocompatibility antigen, defined by graft rejection, is the result of antigenic differences in the proteins encoded by the *M. musculus* segment of the B10.129(21M) genome. However, there is an additional report of graft rejection associated with another allele of the *p* locus, p^{un}. Wettstein *et al.* (1988) reported that tissue grafts from the p^{un} allele were rejected by coisogenic wild-type (C57BL/6J) and p^{un} revertant strains. Previously, we have shown that the basis of the p^{un} mutation is a duplication of genomic sequences at the *p* locus (Brilliant *et al.* 1991). This duplication is ~70 kb in size and is completely and exactly removed in p^{un} revertant mice (Gondo *et al.* 1993). Moreover, this genomic duplication is internal to the *p* gene encoding sequences, such that p^{un} melanocytes produce a larger transcript that presumably contains reiterated exon sequences with a novel order, expressed at near normal abundance (Gardner *et al.* 1992). If translated, it would result in a novel protein that may be recognized as foreign when expressed in melanocytes, transplanted to coisogenic skin, and may account for the basis of the observed graft rejection. An alternative hypothesis is based on our observation that p^{un}/p^{un} melanocytes have an apparent growth disadvantage when compared with wild-type coisogenic melanocytes, *in vitro* (reduced doubling time) and *in vivo* (there are fewer melanocytes in both the choroid and retinal epithelium of p^{un} eyes) (D. Durham-Pierre and M. H. Brilliant, unpublished observation). Thus, it is possible that the observed slow graft "rejection" (without the complete graft destruction typically described for histocompatibility-mediated graft rejection) observed by Wettstein *et al.* (1988) is actually the result of infiltration of the graft by surrounding wild-type melanocytes that have a growth advantage over the resident mutant melanocytes. Short range infiltration by surrounding melanocytes into an area depleted of melanocytes (or into an area where melanocytes are at a growth disadvantage) has been previously documented (Silvers 1958).

Implications for *Myod1*: Kay *et al.* (1993) described a unique form of the *Myod1* locus in SJL/J mice and speculated that this form arose recently from one or more mutational events involving founder alleles. Our data suggests that the origin of the SJL/J *Myod1* allele is *M. musculus*, retained because it is linked to the *p* allele carried by SJL/J. The SJL/J form of *Myod1* is thought to be associated with higher efficiency muscle regeneration (Kay *et al.* 1993). If true, it may be an intrinsic property of the *M. musculus Myod1* gene, or it may reflect a unique interaction of the *M. musculus*-derived *Myod1* gene with other gene products encoded by non-*M. musculus* DNA in SJL/J mice. In light of our findings, it may be informative to assay muscle regeneration in *M. musculus* and in other laboratory strains containing the *M. musculus* allele of *Myod1*.

Origin of inbred strains: The wild-type *p* allele carried by most laboratory strains appears to be derived from *M. domesticus*. In contrast, the original *p* mutation carried in strains SJL/J, 129/J, B10.129(21M), P/J and FS/Ei was derived from *M. musculus*. Tucker *et al.* (1992a) and Nagamine *et al.* (1992) have found that the *Y* chromosome carried by most laboratory strains was derived from *M. musculus* (including 129/J, FS/Ei and P/J), with the exception of several strains (including SJL/J) with a *M. domesticus Y* chromosome. Many of the diverse alleles of a variety of genes found in different inbred mouse strains (defined by biochemistry or by RFLP) match the alleles found in *M. domesticus*, *M. musculus* and *M. m. castaneus* (Blank *et al.* 1986; Elliot 1989). It has been proposed that the majority of the genetic diversity observed among various inbred strains can be accounted for by the introgression of non-*M. domesticus* chromosomal segments and segregation of residual allogenicity during inbreeding (Blank *et al.* 1986). Our data confirm this notion in general and specifically confirm the speculation of Taylor and Rowe (1984) who proposed that the *Saa* locus in mice bearing the original *p* mutation was of *M. m. molossinus* origin.

Chinese and Japanese tradition considers a white mouse a sign of good luck. When found in the wild, such mice were often given as gifts to religious and political leaders. These mice were treasured and bred (Keeler 1931; Tokuda 1935). A keen insight into the genetics of several coat color loci, including *p*, *c* and *W*, is reflected

by very early accounts of these mutations (TOKUDA 1935). It is quite possible that one of the progenitors of SJL/J, 129/J, P/J and FS/Ei was selected and captured from the wild as a sign of good fortune so long ago.

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