Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 6968–6972, August 1992
Genetics

Genetic and molecular analysis of recessive alleles at the pink-eyed dilution (*p*) locus of the mouse

(complementation analysis/cleft palate/developmental mutations/deletion mapping)

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Contributed by Mary F. Lyon, May 5, 1992

ABSTRACT **Recessive mutant alleles at the pink-eyed dilution (*p*) locus on mouse chromosome 7 reduce pigmentation of both the coat and eyes. Here we describe the properties and complementation interactions of 10 *p* alleles, including 6 not previously reported. Several alleles that cause additional phenotypes affecting development, reproduction, and behavior were shown to be deletions by using DNA probes derived from the *p* region. An alignment of functional and marker-defined units is proposed, giving a linear complementation map that orders at least four functional loci. The characterization of a nested set of deletions around *p* will facilitate detailed molecular analyses of the genes and developmental functions associated with this part of the mouse genome.**

The pink-eyed dilution (*p*) locus is defined by one of the earliest known coat-color mutations of the mouse. While the wild-type allele (+) generates intense pigmentation in both the coat and eyes, recessive alleles at this locus reduce pigmentation, primarily in eumelanin [reviewed by Silvers (1)]. The *p* and albino (*c*) loci on mouse chromosome 7 constituted the first linkage group identified in mammals (2), and a wealth of mutations at each locus has been recovered in mutagenesis experiments. Radiation-induced deletions surrounding the *c* locus have been particularly useful in constructing detailed functional maps of a 6- to 11-centimorgan region on chromosome 7 (3–5).

At least 12 alleles at the *p* locus have been described (1, 6). In addition to pigmentation, some alleles affect reproduction, development, and behavior. Because all *p* mutations associated with pleiotropic effects were radiation-induced, it seems likely that pleiotropism is due to multilocus deletions that alter a gene required for normal pigmentation (*p*) in addition to one or more adjacent genes that control distinct functions (7). Indeed, the p-6 Harwell (p^{6H}) allele is deleted for one unique sequence derived from within the pink-eyed unstable (p^{un}) duplication (8).

In this report we present studies on the properties of and complementation interactions between four previously reported and six newly induced *p* alleles, including molecular analysis with DNA probes derived from the *p* region. Together these analyses characterize a nested set of deletions around the *p* locus that will facilitate high-resolution functional and physical mapping in this interesting region of the mouse genome.

MATERIALS AND METHODS

***p* Mutations.** In all, 20 mutant *p* alleles were studied (Table 1). Ten of these were generated in the course of mutagenesis experiments with various types and doses of radiation at the Medical Research Council Radiobiology Unit, Harwell, England. This set of 10 alleles included 4 previously described—p^{d}, p^{bs} (formerly designated p^{24H}), p^{cp} (formerly p^{11H}), and p^{25H}—and 6 not previously reported—p^{81H}, p^{82H}, p^{84H}, p^{86H}, p^{87H}, and p^{88H}. These mutations were tested for complementation in as many pairwise combinations as possible. The remaining 10 mutations analyzed included 3 spontaneously arising alleles (*p*, p^{J}, and p^{un}), 6 independent p^{un+} revertants (designated p^{un+1J} through p^{un+6J}), and the radiation-induced p^{6H} allele. These 10 alleles were included only in DNA analyses.

Viability and Fertility Tests. For tests of viability, $p^{?}/p\ c^{ch}$ mice were crossed to $+c^{ch}/+c^{ch}$ (homozygous chinchilla) mice. (The symbol $p^{?}$ denotes any pink-eyed dilution allele other than *p*.) If intercrossing their non-chinchilla offspring (putatively $p^{?}/+/+c^{ch}$) produced no phenotypically pink-eyed young (>20 offspring collected), $p^{?}$ was presumed to cause embryonic lethality in homozygotes.

For tests of fertility, mice in question were mated to p^{d}/p^{d} homozygotes. Males were caged with two p^{d}/p^{d} females for 1 month and were judged sterile if neither female became pregnant. Females were caged singly with p^{d}/p^{d} males for 2 months. Those that bred were allowed to have at least two litters and the rearing of young was evaluated.

Probes. DNA fragments from within and around an ≈75-kilobase-pair (kbp) duplication associated with the p^{un} mutation (8) have been cloned. Three such fragments were used here as hybridization probes in Southern blot analysis: 28RN, a 390-bp *Rsa* I–*Nde* I DNA fragment from within the p^{un} duplication (8); 300L, a 1.7-kbp *Eco*RI–*Hin*dIII fragment isolated from λU300; and 700R, a 1.4-kbp *Eco*RI insert from p700R, originally isolated from λU700. The 300L and 700R probes flank the p^{un} duplication (Y.G., J.M.G., Y.N., and M.H.B., unpublished work).

Southern Blot Analysis. Inbred mice [including strains BDP/J, C57BL/6J, C57BL/10J, C3H/HeJ, FS/Ei, I/LnJ, P/J, SJL/J, B10.129(21M)/Sn, and 129/J] and mice carrying *p* alleles were obtained from the Foundation Stocks or research colonies at The Jackson Laboratory. DNA samples from mice carrying p^{81H}, p^{82H}, p^{84H}, p^{86H}, p^{87H}, or p^{88H} were obtained from the Medical Research Council. At least two mice from each strain or *p*-locus genotype were analyzed. Five to 10 μg of genomic DNA was restriction endonuclease-digested according to the supplier's recommendations (GIBCO/BRL), and the resulting DNA fragments were separated by electrophoresis through 0.8% agarose gels. DNA was transferred to nylon membranes (Zeta-Probe, Bio-Rad) with 0.4 M NaOH (12). Filters were prehybridized for 2–4 hr at 65°C in 0.5 M sodium phosphate, pH 7.2/7% SDS/1 mM

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Table 1. Origin and effects of *p* alleles studied

Allele					Fertility			
Symbol	Name	Origin	Pigment	Viability	♂	♀	Size and gait	Ref(s).
p	Pink-eyed dilution	Spontaneous	Light eyes and coat	Viable	F	F	Normal	6
p^J	Pink-eyed dilution-J	Spontaneous	Similar to p/p	Viable	F	F	Normal	*
p^{un}	Pink-eyed unstable	Spontaneous	Similar to p/p, reverts to wild type	Viable	F	F	Normal	8
p^{un+1J} to p^{un+6J}	Pink-eyed unstable revertant-1J to -6J	Spontaneous	Wild type	Viable	F	F	Normal	8, †
p^d	Dark pink-eye	x-rays‡	Intermediate	Viable	F	F	Normal	9, §
p^{bs}	p-black-eyed sterile	Neutrons	Dark eyes, coat between p/p and p^d/p^d	Viable (reduced)	S	P	Small, jerky	9, §
p^{cp}	p-cleft palate	Neutrons	Similar to p/p	Leaky, neonatal lethal	ND	P	Small, jerky	§, ¶
p^{6H}	p-6 Harwell	x-rays‖	Similar to p/p	Viable	S	P	Small, jerky	9, 11
p^{25H}	p-25 Harwell	Neutrons	Similar to p/p	Viable (reduced)	S	P	Small, jerky	9, 11, §
p^{81H}	p-81 Harwell	x-rays**	Similar to p/p††	Prenatal lethal	—	—	—	§
p^{82H}	p-82 Harwell	x-rays**	Similar to p/p††	Prenatal lethal	—	—	—	§
p^{84H}	p-84 Harwell	x-rays**	Similar to p/p	Viable	F	F	Normal	§
p^{86H}	p-86 Harwell	x-rays**	Similar to p/p	Viable	F	F	Normal	§
p^{87H}‡‡	p-87 Harwell	x-rays**	Similar to p/p††	Prenatal lethal	—	—	—	§
p^{88H}	p-88 Harwell	x-rays**	Similar to p/p	Viable	F	F	Normal	§

All mutations are recessive; phenotypes are for homozygous mice, except where noted. F, fertile; S, sterile; P, poor; ND, not determined; —, homozygotes were inviable and so not tested.

*The Jackson Laboratory, unpublished.

†All six alleles were derived independently (E.M.E., unpublished).

‡3 Gy to fetus.

§This report.

¶Note that Phillips (9) reported p^{cp}/p^{cp} males as fertile and with a normal gait; Johnson and Hunt (10) reported p^{cp}/p^{cp} males as sterile.

‖6 Gy to male parent.

**3 + 3 Gy, 24-hr interval, to male parent.

††Refers to genotype $p/p^?$ since $p^?/p^?$ is lethal.

‡‡Associated with a translocation T(7D3;11B1), with breakpoints on chromosome 7 not near the *p* locus.

EDTA and were hybridized for 18 hr at 65°C with denatured ^{32}P-labeled probe (1–5 × 10^6 cpm/ml) in fresh prehybridization solution (13). Probes were labeled with [α-^{32}P]dCTP by the random-priming method (14). Filters were washed at 65°C in 15 mM NaCl/1.5 mM sodium citrate, pH 7/0.1% SDS and exposed to Kodak XAR-5 film for 1–7 days at −80°C with intensifying screens. Filters were stripped for reuse by washing twice at 95°C in 15 mM NaCl/1.5 mM sodium citrate, pH 7/0.1% SDS.

RESULTS

Pigmentation. All except two of the *p* alleles studied resembled the original *p* allele in their effects on pigmentation. The alleles p^{6H}, p^{25H}, p^{84H}, p^{86H}, p^{88H}, and p^J produced the typical p/p color when homozygous or in combination with *p*. The lethal alleles p^{81H}, p^{82H}, and p^{87H} (see below) generated typical p/p color in compounds with *p*, p^{25H}, or p^{cp}. Surviving p^{cp}/p^{cp} homozygotes (see below) also displayed typical p/p coat and eye color. The two exceptional alleles were p^{bs} and p^d. In p^{bs}/p^{bs} mice the eye color was indistinguishable from wild type both at birth and at weaning, and the coat color was slightly darker than that of p/p but lighter than that of p^d/p^d mice. The eyes of p^d/p^d mice were lightly pigmented at birth (though darker than those of p/p mice) and darkened by weaning. The color of the coat was considerably darker than that of p/p and p^{bs}/p^{bs} mice, somewhat resembling that of brown (b/b) mice. The compounds of p^d with *p* alleles that generate typical p/p color all resulted in intermediate effects: eyes were lighter at birth than those of p^d/p^d mice (but not as light as those of p/p mice) and darkened by weaning; ears and tail were lighter than those of p^d/p^d mice but coat color in other regions was indistinguishable. Similarly, compounds with p^{bs} showed intermediate effects: p^{cp}/p^{bs} and p^{25H}/p^{bs} mice had pigmented eyes at birth and coats of a color intermediate between p/p and p^{bs}/p^{bs} mice, whereas the coats of p^{bs}/p^d mice resembled those of p^d/p^d mice but the eyes were slightly darker.

Viability, Growth, and Behavior. Mendelian segregation of *p* alleles was observed in most intercross and testcross combinations, but some exceptions were noted, as follows. Among the new alleles, p^{81H}, p^{82H}, and p^{87H} were presumed to cause prenatal lethality in homozygotes, as intercrossing heterozygotes produced no pink-eyed progeny. By contrast, mice homozygous for p^{84H}, p^{86H}, or p^{88H} were fully viable. The recessive lethality controlled by p^{81H}, p^{82H}, and p^{87H} was complemented by all other alleles tested. However, these three alleles failed to complement each other (Table 2, experiment A). As reported previously, mice homozygous for p^{cp} usually died at birth due to cleft palate. However, the defect is "leaky" in that occasional p^{cp}/p^{cp} homozygotes may survive to adulthood. This (leaky) neonatal lethal effect of p^{cp} was complemented by p^{81H}, p^{82H}, and p^{87H} as well as p^{bs}, p^d, and p^{25H} (summarized in Fig. 1). Notably, significantly fewer p^{bs}/p^{bs} and p^{25H}/p^{25H} homozygotes than expected were recovered from intercrosses (Table 2, experiment B), indicating a reduced viability for mice of these genotypes.

Mice homozygous for the alleles p^{bs}, p^{6H}, p^{25H}, and p^{cp} often were smaller than control ($p^?/+$ and $+/+$) littermates. The heterozygotes p^{bs}/p^{25H}, p^{25H}/p^{81H}, and p^{25H}/p^{82H} also were small, but p^{cp} complemented this effect, as compounds

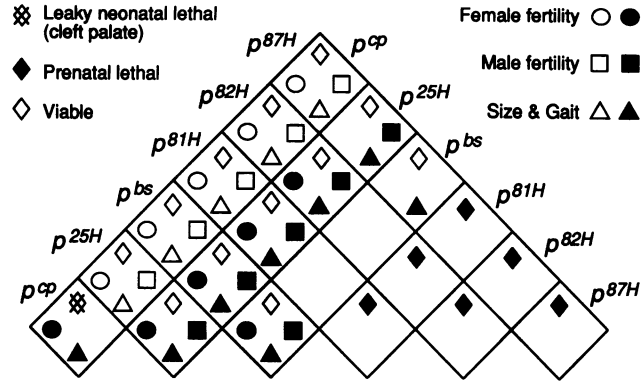

FIG. 1. Effects and complementation reactions of various *p* alleles. Open symbols denote the normal and filled symbols the abnormal phenotype.

of p^{cp} with p^{bs}, p^{25H}, p^{81H}, or p^{82H} were of normal size (Fig. 1).

Mice homozygous for the alleles p^{bs}, p^{6H}, and p^{25H} displayed a mildly jerky gait with some tremor, confirming the observations of Phillips (9, 11) regarding p^{bs}, p^{6H}, and p^{25H}, and of Hunt and Johnson (15) regarding p^{25H}. These alleles did not complement each other, as p^{bs}/p^{25H} animals also had an abnormal gait. By contrast, p^{bs} and p^{25H} were complemented by both p^{cp} and p^{d}. The alleles p^{81H} and p^{82H} also failed to complement p^{25H}; p^{25H}/p^{81H} and p^{25H}/p^{82H} mice had a jerky gait, whereas p^{81H}/p^{cp} and p^{82H}/p^{cp} mice displayed a normal gait. Surprisingly (in view of the normal size and behavior of p^{cp}/p^{bs} and p^{cp}/p^{25H} animals), six p^{cp}/p^{cp} mice that survived to adulthood showed jerky behavior (see also ref. 16). (Abnormal gait has not been noted in previous descriptions of p^{cp} homozygotes; see ref. 9). These results are summarized in Fig. 1.

Male Fertility. The results of fertility testing (Table 3) confirmed the findings of others (9–11, 15, 17) that p^{bs}/p^{bs} and p^{25H}/p^{25H} males are sterile and that p^{bs} and p^{25H} do not complement each other, as p^{bs}/p^{25H} males also were sterile. On the other hand, p^{cp} complemented p^{bs} and p^{25H} in that p^{cp}/p^{bs} and p^{cp}/p^{25H} males were fertile. As expected, heterozygotes of p^{d} with all other alleles tested were fertile. Of the new alleles tested, homozygous p^{84H}/p^{84H}, p^{86H}/p^{86H}, and p^{88H}/p^{88H} males all proved fertile. By contrast, p^{81H}, p^{82H}, and p^{87H} each failed to complement p^{25H}: p^{25H}/p^{81H}, p^{25H}/p^{82H}, and p^{25H}/p^{87H} males were sterile, whereas p^{cp}/p^{81H}, p^{cp}/p^{82H}, and p^{cp}/p^{87H} males were fertile (see Fig. 1). No p^{cp}/p^{cp} males were tested for fertility.

Table 2. Segregation of *p* alleles in crosses involving lethal and semilethal *p* alleles

Cross	No. of offspring			
	Total	p^x/p^{y*} Obs	Exp	*P*
Experiment A				
$p^{81H}/p^{d} \times p^{82H}/p^{d}$	32	0	8	<0.003
$p^{81H}/p^{d} \times p^{87H}/p^{d}$	32	0	8	<0.003
$p^{82H}/p^{d} \times p^{87H}/p^{d}$	32	0	8	<0.003
Experiment B				
$+/p^{bs} \times +/p^{bs}$	702	110	176	<0.0001
$+/p^{25H} \times +/p^{25H}$	531	96	133	<0.0003

Obs, observed; Exp, expected. An expected value is the number of progeny predicted if the pink-eyed class of mice indicated is fully viable. *P* values were calculated by the χ^2 method.
*In experiment A, "p^x/p^y" refers to light pink-eyed progeny, p^{81H}/p^{82H}, p^{81H}/p^{87H}, or p^{82H}/p^{87H}; in experiment B, "p^x/p^y" indicates p^{bs}/p^{bs} or p^{25H}/p^{25H}.

Table 3. Results of fertility tests

Genotype	No. of mice tested				
	Male		Female		
	F	S	F	P	S
p^{d}/p^{d}	8	0	14	1	0
p^{bs}/p^{bs}	0	5	0	2	6
p^{cp}/p^{cp}	—	—	0	3*	0
p^{25H}/p^{25H}	0	5	0	4	3
p^{d}/p^{bs}	8	0	8	0	0
p^{d}/p^{cp}	4	0	2	0	0
p^{d}/p^{25H}	3	0	5	0	0
p^{bs}/p^{cp}	7	0	3	0	0
p^{bs}/p^{25H}	0	3	0	4	0
p^{cp}/p^{25H}	5	0	5	0	0
p^{81H}/p^{d}	5	0	1	0	0
p^{81H}/p^{cp}	7	0	3	0	0
p^{81H}/p^{25H}	0	2	1	1	0
p^{82H}/p^{d}	4	0	3	0	0
p^{82H}/p^{cp}	4	0	4	0	0
p^{82H}/p^{25H}	0	5	0	4	0
p^{87H}/p^{cp}	3	0	2	0	0
p^{87H}/p^{25H}	0	4	0	3	0
p^{84H}/p^{84H}	3	0	3	0	0
p^{86H}/p^{86H}	4	0	5	0	0
p^{88H}/p^{88H}	7	0	4	0	0

F, fertile; S, sterile; P, produced but failed to rear litters.
*These females were crossed with known $+/p^{cp}$ males: one produced three offspring, none pink-eyed; the other two produced some pink-eyed young, and all those examined (four from each female) exhibited cleft palate.

Female Fertility. Previous authors have reported that females homozygous for p^{bs}, p^{6H}, or p^{25H} are poor breeders (9), semi-sterile (17), or sterile with hypoplastic ovaries (10). In the present work some p^{bs}/p^{bs} and p^{25H}/p^{25H} females indeed were sterile (Table 3). Others, however, produced litters but failed to rear them (the young apparently dying without being suckled), suggesting that the defect in these females involves lactation, maternal behavior, or both. Thus, various aspects of reproduction in females are affected by these alleles. No p^{bs}/p^{bs} or p^{25H}/p^{25H} females were normally fertile.

There was no complementation between p^{bs} and p^{25H}: all four p^{bs}/p^{25H} females tested produced litters but failed to rear them. By contrast, all p^{bs}/p^{cp} and p^{cp}/p^{25H} females tested were fertile and reared litters normally. Notably, the three p^{cp}/p^{cp} females tested failed to rear litters and were of low fecundity in that they produced a few, small litters (Table 3; Fig. 1).

Among the new alleles tested, homozygous p^{84H}/p^{84H}, p^{86H}/p^{86H}, and p^{88H}/p^{88H} females were fertile and reared litters. Compounds of p^{81H} or p^{82H} with p^{cp} were fertile, but p^{81H} and p^{82H} failed to complement the sterility of p^{25H} (Table 3; Fig. 1).

DNA Analysis. Three cloned DNA sequences derived from the *p* region (see *Materials and Methods*) were used to probe Southern blots of endonuclease-digested DNA from mice with various *p*-allele genotypes (Fig. 2). Each probe revealed a single hybridizing fragment in DNA from p^{J}, p^{d}, p^{bs}, p^{25H}, p^{84H}, p^{86H}, or p^{88H} homozygotes; mice homozygous for any of six independent p^{un+} revertant alleles (p^{un+1J} through p^{un+6J}); or wild-type mice from the inbred strains C57BL/6J, C57BL/10J, and C3H/HeJ. While a differently sized fragment was revealed with each probe, all of these mutant *p* alleles were associated with a fragment apparently equal in size to that of wild type. By contrast, DNA from the seven inbred *p*-bearing strains tested [BDP/J, FS/Ei, I/LnJ, P/J, SJL/J, B10.129(21M)/Sn, and 129/J] displayed a uniquely sized (*p*-specific) hybridizing fragment with each probe.

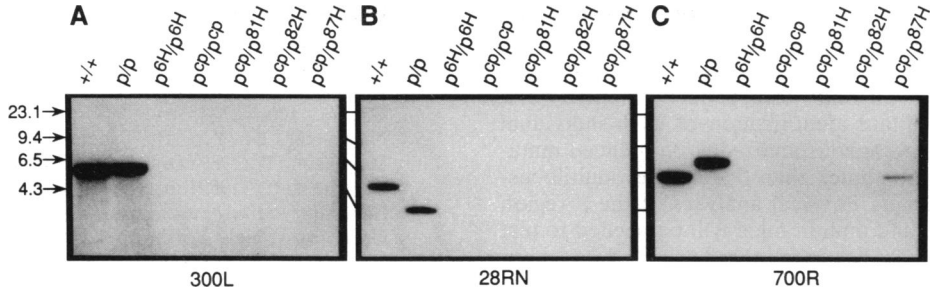

FIG. 2. Southern blot analysis of *Pst* I-digested DNA from mice with various *p*-locus genotypes. Probes are indicated below the autoradiograms. Relative positions and sizes (kbp) of *Hin*dIII-digested λ phage DNA fragments are shown at left. DNA in the last three lanes of each blot was under-loaded compared with the first four lanes, based on ethidium bromide staining and rehybridization with a probe for the tyrosine hydroxylase gene, also on mouse chromosome 7 (18) (data not shown).

DNA from p^{6H}/p^{6H} mice, previously shown to be deleted for the 28RN sequence (8), failed to hybridize with 300L and 700R as well. Similarly, DNA from p^{cp}/p^{cp} homozygotes and compounds of p^{cp} with the recessive lethal alleles p^{81H} and p^{82H} failed to hybridize with all three probes. DNA from compounds of p^{87H} and p^{cp} failed to hybridize with 28RN or 300L but did hybridize with 700R.

DISCUSSION

The results reported here parallel the conclusions drawn by others (7, 9–11, 17) regarding complexity at the *p* locus. In addition, the description of three new *p* alleles that confer recessive prenatal lethality, as well as evidence for reduced viability generated by the p^{bs} and p^{25H} alleles, extends the scope of developmental functions associated with *p* mutations. Finally, the identification of deleted sequences associated with a subset of the *p* alleles studied may provide insight into the basis of their pleiotropic nature.

The most unusual findings from complementation analysis concern the allele p^{cp}. This allele clearly and unambiguously complemented the effects of the alleles p^{bs} and p^{25H} not only on male and female fertility, but also on body size and gait. Furthermore, while the prenatal lethal alleles p^{81H}, p^{82H}, and p^{87H} failed to complement the male sterility, small size, and jerky gait generated by p^{25H}, each produced fertile, normal compounds with p^{cp}. Nevertheless, all six surviving p^{cp}/p^{cp} adults showed small size, jerky gait, and (for the three females tested) impaired fertility. These results suggest that the genetic lesions in p^{bs}, p^{6H}, p^{25H}, p^{81H}, p^{82H}, and p^{87H} that lead to similar end effects (impaired growth, locomotion, and fertility) in homozygotes and noncomplementing compounds are distinct from the genetic lesion of p^{cp}, such that functional gene products can be obtained in trans heterozygotes. Thus far, the features of jerky gait, small size, and impaired fertility generated by p^{bs}, p^{6H}, p^{25H}, p^{81H}, p^{82H}, and p^{87H} have not been separated and hence could be due to a single gene. The identification and study of additional *p* alleles may help to resolve these functional units.

It is of interest to consider the number of genes underlying the various phenotypic effects controlled by alleles at the *p* locus. The irradiation-induced alleles p^{84H}, p^{86H}, and p^{88H} produce an effect like that of the original *p* allele on pigmentation but cause no other abnormal effects. As radiation produces predominantly null alleles, the phenotype of these alleles—like that of *p* itself—may represent the null effect at the *p* locus. All of the alleles with pleiotropic effects were identified among the progeny of irradiated mice (see Table 1). As radiation is known to produce a range of DNA lesions, including intralocus and multilocus deletions, the complex developmental aspects of many *p* mutations are most likely due to deletions or other changes in neighboring genes (7). In fact, five *p* mutations that cause complex effects—namely, p^{6H}, p^{81H}, p^{82H}, p^{87H}, and p^{cp}—were shown here to involve physical deletions. Furthermore, because p^{cp} fully complements the developmental, reproductive, and behavioral deficiencies controlled by p^{bs}, p^{6H}, p^{25H}, p^{81H}, p^{82H}, and p^{87H}, the gene involved in pigmentation (*p*, the only noncomplementing functional unit) would seem to be uninvolved in these additional functions.

If we assume that each *p* allele with pleiotropic effects is the result of a single multilocus deletion, it is possible to construct a hypothetical complementation map of functional and marker-defined units that accounts for all analyzed *p*-locus mutations (Fig. 3). This map predicts the location of at least four loci that affect development, reproduction, or behavior, and may be refined with the analysis of additional mutations. Indeed, the effects of additional genes in the intervals defined by the p^{81H}, p^{82H}, p^{87H}, and p^{cp} deletions

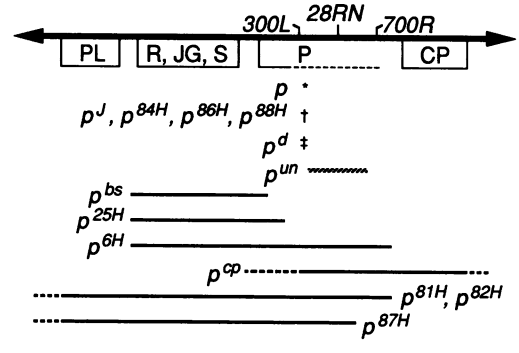

FIG. 3. Hypothetical complementation map of the *p* region. No correlation with genetic or physical distance is implied. Orientation of the map with respect to the centromere is not known. Relative positions of three probe sequences used are shown above the map. Boxes below the map represent functional units, defined by complementation analysis of *p* mutations. The phenotypes associated with homozygous deletion of each functional unit are symbolized: PL, prenatal lethality; R, JG, S, runting, jerky gait, and sterility; P, defective pigmentation; and CP, cleft-palate syndrome, including runting, jerky gait, and sterility features distinct from R, JG, S (see text). Horizontal lines below the map represent the presumed extent of lesions. Hatched bar represents the duplicated region associated with the p^{un} mutation (ref. 8 and Y.G., J.M.G., Y.N., and M.H.B., unpublished work). Other symbols: ⋆, the original *p* mutation, an allele that is associated with a distinct restriction map with all three probes; †, four presumed null mutations that affect pigmentation only and are associated with wild-type fragment sizes in Southern blot analysis; and ‡, the p^{d} mutation, a hypomorphic allele that affects only pigmentation and displays wild-type-sized hybridizing fragments in Southern blot analysis. While p^{bs} and p^{25H} also display wild-type fragment sizes with the probes tested here, the lesion associated with p^{25H} is shown extending further into the P functional unit based on its more extreme pigmentation defect. It cannot be determined from the data reported here whether any of the DNA probe sequences used lies within P or between P and CP.

could be masked by the lethal phenotypes they control. Efficient germ-line point mutagenesis (reviewed by Rinchik, ref. 19) together with single-generation screening strategies that depend on the availability of genetically marked deletions (20) might facilitate identification of such individual genes. Of course, spontaneous and radiation-induced mutations often are more complex than this simple, multilocus-deletion model assumes. Physical analyses of the *p* region from both wild-type and mutant mice will be needed to test this assumption.

Heritable deletion mutations have been useful for constructing detailed functional maps of several chromosomal regions (3–5, 21–23). Likewise, the characterization of a set of overlapping deletion mutations around *p* will allow strategies to be designed for making a detailed molecular analysis of the genes and developmental functions associated with this part of the mouse genome. The *p* region is of particular interest in view of its homology with a part of human chromosome 15q11–q13 (24, 25), a region associated with the Prader–Willi and Angelman syndromes, and with profound imprinting effects (26–29). If the genes mediating the pleiotropic effects of certain *p* mutations have homologues in a conserved linkage group on human chromosome 15q11–q13 (see ref. 16), they also may be involved in some of the pleiotropic effects of the Prader–Willi and Angelman syndromes. For example, the gene encoding the type A γ-aminobutyrate receptor β3 subunit has been mapped to the Angelman/Prader–Willi region in man and the *p* region in mouse (30). It is possible that mutations in this gene are responsible for some of the common neurological features of the Prader–Willi, Angelman, and *p* syndromes.

Note Added in Proof. Gardner *et al.* (31) have cloned a gene encoded by the *p* locus. This gene apparently mediates the pigmentation phenotype of *p*-locus mutations, as aberrant expression of this gene was found in six homozygous mutants characterized by severe hypopigmentation [*p* (the original mutant allele), p^{un}, p^{J}, p^{6H}, p^{25H}, and p^{cp}]. By contrast, transcripts of this gene were detected in revertants of p^{un} and in homozygous mutants characterized by intermediate pigmentation (p^{bs}, p^{d}, and p^{9J}).

We thank Edward P. Evans for data on the p^{87H}-associated translocation. We are grateful to Susan Deveau and Sue Morse for technical assistance and Suzanne Ruddy for some DNA extractions. We thank Muriel T. Davisson, Joseph Nadeau, Randy Strich, and Linda Washburn for reviewing the manuscript. Work carried out at The Jackson Laboratory was supported in part by Grant GM20919 (to E.M.E.) and Training Grant CA09217 (to T.R.K.) from the National Institutes of Health. Work carried out at The Institute for Cancer Research was supported by National Institutes of Health Grants GM43840, GM22167, CA06927, and RR05539; the Pew Charitable Trusts; and by an appropriation from the Commonwealth of Pennsylvania. Y.N. is the recipient of a Uehara Fellowship.

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