Molecular Analysis of 36 Mutations at the Mouse *pink-eyed dilution* (*p*) Locus

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ABSTRACT

Thirty-six radiation- or chemically induced homozygous-lethal mutations at the *p* locus in mouse chromosome 7 have been analyzed at 17 loci defined by molecular probes to determine the types of lesions, numbers of *p*-region markers deleted or rearranged, regions of overlap of deletion mutations, and genetic distances between loci. A linear deletion map of the [*Myod1, Ldh3*]–[*Snrpn, Znf127*] region has been constructed from the molecular analyses of the *p*-locus deletions. The utility of these deletions as tools for the isolation and characterization of the genes specifying the neurological, reproductive, and developmental phenotypes genetically mapped to this region will grow as more detailed molecular analyses continue.

THE pink-eyed dilution (*p*) locus is one of seven loci used as markers for experimental mutagenesis in the mouse specific locus test, which was designed primarily to assess the average rates of mutation induction at selected loci in the mammalian genome (RUSSELL 1951). Over 100 *p* mutations have been recovered in the progeny of irradiated or chemically treated animals over many years at the Oak Ridge National Laboratory and elsewhere, and a few have appeared spontaneously in control (untreated) groups. Many of the Oak Ridge mutations have been preserved in breeding stocks (RUSSELL *et al.* 1995, accompanying report), and subsets of them have already been successfully exploited in the genetic and molecular analyses of the *p* gene itself (RINCHIK *et al.* 1993a) and of the region of mouse chromosome 7 immediately surrounding the *p* locus (CULIAT *et al.* 1993, 1994; NICHOLLS *et al.* 1993; STUBBS *et al.* 1994). Several other *p* mutations, recovered primarily at the MRC Radiobiology Unit, Harwell, England, have been used in similar types of analyses (GARDNER *et al.* 1992; LYON *et al.* 1992; NAKATSU *et al.* 1993).

The accompanying report (RUSSELL *et al.* 1995) summarizes the origin, method of induction, and initial genetic analyses of 45 Oak Ridge *p*-locus mutations. Most of these were radiation-induced, and they include 38 *p*, five p^x ("dark pink-eyed"), and two p^m (mottled) mutations. Of the 38 *p* mutations in this accompanying report, 33 are prenatally lethal when homozygous (p^{pl}), four are neonatally lethal (p^{nl}), and one is juvenile-lethal (p^{jl}). The p^x mutations include two that are prenatally lethal (p^{xpl}) and three that are juvenile lethal (p^{xjl}); both p^m mutations are also juvenile lethal (p^{mjl}) when homozygous. These mutations have been placed into complementation groups according to the results of a large series of pairwise *trans* complementation crosses. These genetic analyses have also provided evidence that most of these mutations are probably deletions and have assigned several biological functions to specific intervals of a deletion map (RUSSELL *et al.* 1995).

The present report expands the resolution of the genetically derived deletion map of the region covered by the Oak Ridge *p* deletions by adding data from 17 loci defined by molecular probes. Thirty-five of the 36 homozygous-lethal *p* mutations included in this report are the same ones described and analyzed by RUSSELL *et al.* (1995); however, 10 mutations ($p^{12R250M}$, p^{103G}, p^{48PB}, p^{17FATw}, p^{12DTR}, p^{39DSD}, p^{18CoS}, p^{15ThP}, p^{39K} and p^{1OZb}) included in the RUSSELL analysis have been omitted from this report, and one homozygous-lethal *p* mutation, $p^{17MNURf}$, is included in this report but not in the RUSSELL report. Omissions include the p^x mutations $p^{12R250M}$, p^{103G}, p^{48PB}, p^{17FATw} and p^{12DTR}, and the p^m mutations p^{39DSD} and p^{18CoS}; these seven mutations are being analyzed in an ongoing study, and those results will be reported separately. The prenatally lethal *p* mutations p^{15ThP}, p^{39K}, and p^{1OZb} have not yet been tested with the available molecular probes. For 20 of the 36 mutations analyzed in this report, data reporting the deletion of several selected loci (including *p* as well as other tightly linked loci) have previously been reported (SCRABLE *et al.* 1990; CULIAT *et al.* 1993, 1994; NICHOLLS *et al.* 1993; RINCHIK *et al.* 1993a). A more comprehensive molecular analysis of the entire set of deletions, as reported here, should facilitate their use as tools in the molecular genetic analysis of this region of mouse chromosome 7.

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MATERIALS AND METHODS

Seventeen different molecular probes mapping near *p* were used to test for deletions or rearrangements in the DNA from 36 mutations. For those mutations that cause early lethality in homozygotes, *M. spretus*/*p** F_1 interspecific hybrid animals were used to differentiate by restriction fragment length variant (RFLV) analysis the mutant chromosome from the wild-type *M. spretus* chromosome (Table 1). Alternatively, when possible, we tested for deletion of loci by hybridizing labeled probes to Southern blots of DNA from deletion homozygotes or from compound heterozygotes carrying overlapping *p*-locus deletions as previously described (CULIAT *et al.* 1993, 1994).

Mice: Induction and recovery of *p*-locus mutations are described in the accompanying report (RUSSELL *et al.* 1995). One mutation ($p^{17MNURf}$), included in this study but not in the companion study, was induced in spermatogonial stem cells by methylnitrosourea at a dose of 75 mg/kg. A 129/Rl × *M. spretus* interspecific backcross (IB) typed for a number of chromosome-7 loci was generated by crossing *M. spretus* males (+ +/+ +) to 129/Rl − $p\ c^h/p\ c^h$ females. F_1 females ($p\ c^h$/+ +) were crossed back to 129/Rl − $p\ c^h/p\ c^h$ males (JOHNSON *et al.* 1989). The resulting N2 progeny were scored for the *p* and c^h phenotypes, and DNA was saved from all animals.

F_1 interspecific hybrids (*M. musculus* × *M. spretus*) heterozygous for lethal *p* mutations were generated by crossing *M. spretus* males ($+^{Spt}/+^{Spt}$) to female carriers of lethal *p* mutations (+/*p**, where *p** represents any lethal *p* mutation). F_1 females, which could be either $+/+^{Spt}$ or $p^*/+^{Spt}$) were then test mated to *M. musculus p*/*p* males to identify those females that carried *p**. DNAs were saved from the $+^{Spt}/p^*$ interspecific hybrid females, as well as from the *M. spretus* sires and the *M. musculus* +/*p** dams.

For neo- or postnatally lethal mutations, homozygous *p**/*p** DNAs were generated by intercrossing +/*p** or p^{7R75M}/p^* within a given stock. (p^{7R75M}, a fully viable p^x-type mutation that yields darker progeny when homozygous than when heterozygous with most *p* alleles, was used only as a marker of the nonlethal chromosome and was not among the *p* alleles analyzed in the present study.) Mice homozygous for p^{nl} or p^{jl} mutations were identified by their unpigmented eyes (RUSSELL *et al.* 1995). The derivation of compound heterozygotes that carry complementing mutations is described elsewhere (CULIAT *et al.* 1993; RUSSELL *et al.* 1995).

Molecular probes and Southern blotting: Seventeen molecular probes were employed in Southern blot analysis to assay for deletions or other types of rearrangements among *p*-region mutations and to provide data for the construction of a map of the overlap among deletions. The probes, the loci they recognize, the primer sequences for those probes derived by PCR, the restriction enzymes that gave useful RFLVs, and the references for each are listed in Table 1; the relative positions of these probes to each other are shown in Figure 1. The preparation of genomic DNAs, Southern blotting, and labeling of probes were all done as described (JOHNSON *et al.* 1989).

RESULTS

For loci mapping within the *p*-region deletion complex, deletion mapping shows the proximal-to-distal order as [*Ldh3–Myod1*], *ru2, Gas2, D7H15F37S1, p, Gabrg3, D7Cwr15, Gabra5, Gabrb3, Hpve6a.* Both *Znf127* and *Snrpn* are outside any *p*-region deletion tested. These data are described briefly below, beginning with the *p* gene itself and then proceeding in proximal-to-distal order, and are summarized in Table 2. We have also determined genetic distances between selected *p*-region loci by interspecific backcross mapping, as summarized in Table 3.

The *p* gene: Both the mouse *p* gene and its human homologue (*P*) have recently been identified (GARDNER *et al.* 1992; RINCHIK *et al.* 1993a). The locus appears to encode an integral membrane transporter protein with 12 transmembrane domains predicted from the amino acid sequence (RINCHIK *et al.* 1993a; LEE *et al.* 1994, 1995; ROSEMBLATT *et al.* 1994) and may be involved in transport of tyrosine. Mutations in the mouse *p* or human *P* gene are associated with melanin reduction in the skin, hair, and eyes (GARDNER *et al.* 1992; RINCHIK *et al.* 1993a; LEE *et al.* 1994, 1995; SPRITZ 1994).

In many cases, it was possible to test by Southern-blot analysis for the deletion of genomic restriction fragments recognized by DN10, a human *P* cDNA (RINCHIK *et al.* 1993a), in DNA derived from p^{nl} or p^{jl} homozygotes or from compound heterozygotes carrying a p^{pl} opposite another *p* mutation that complemented for early embryonic lethality (one example being of the general genotype $p^{pl}/p^{7FR60Lb}$). Twenty-nine of 33 mutations tested (three not tested) showed deletion of all fragments recognized by DN10. One p^{pl}, p^{2DFiOD}, is not deleted for any *p*-locus probes tested but has been shown to be a large paracentric inversion (RUSSELL *et al.* 1995). Finally, three mutations, $p^{4THO\text{-}II}$ (NICHOLLS *et al.* 1993), $p^{12R30Lb}$ and p^{9DTW}, partially delete the *p* locus, as indicated by the presence of only some of the *Eco*RI fragments detected by the DN10 human cDNA; for example, an *Eco*RI digest of C3H/Rl or 101/Rl wild-type genomic DNA exhibits eight hybridizing fragments; $p^{4THO\text{-}II}$ deletes seven of those eight fragments, while p^{9DTW} deletes one (data not shown).

Loci proximal to *p*: *Human 11p15 and 19q loci:* As had been previously reported, the human *11p15* loci *Ldh3, Ldh1, Saa1, Kcnc1, Tph* and *Myod1* map proximal to *p* within a 500-kb segment of DNA that is deleted in $p^{46DFiOD}$ but not in any other of three Oak Ridge *p* mutations tested (STUBBS *et al.* 1994). The gene order within this cluster has been shown by physical mapping to be *Ldh3, Ldh1, Saa1, Tph, Kcnc1, Myod1* (STUBBS *et al.* 1994), but the orientation of the entire cluster relative to *p* has not yet been determined. We tested whether any other *p* mutations deleted *M. musculus* RFLVs detected by *Ldh3* or *Myod1* probes in *M. spretus*/*p** F_1 DNAs. As shown in Table 2, none of the 32 additional deletions tested deletes *Myod1* and none of 31 tested (five not tested) deletes *Ldh3;* thus, none tested but $p^{46DFiOD}$ extends to this cluster of human *11p15* loci. Some deletions that do not include *ru2* (RUSSELL *et al.* 1995, see below) or *Myod1, e.g.*, $p^{15DThWb}$ and $p^{2FBCfFob}$, were not tested for deletion of some of the other members of the [*Ldh3–Myod1*] cluster.

TABLE 1

Probes used for *p*-region deletion mapping

Probe	Locus	Source	Enzyme/RFLV[a]	Reference
Ldhc	*Ldh3*	520-bp *Kpn*I-*Eco*RI fragment, mouse intron 1	*Eco*RI M = 6.3 S = 4.4	ZHOU *et al.* (1994)
pUCLD	*Ldh1*	1.7-kb *Eco*R1-*Hind*III fragment of pUCLD-14; mouse genomic	*Bam*HI M = 7.3 S = 14.1	MENDEL (1987)
pSA30	*Saa1*	1.5-kb mouse genomic *Pst*I fragment	*Msp*I M = 10 S = 6	MORROW *et al.* (1991) MENDEL (1987)
pG4Th	*Tph*	ATCC probe #63150	*Bam*HI M = 16.2, 3.0 S = 14.8, 3.3	STOLL *et al.* (1990)
KV4c	*Kcnc1*	Rat cDNA	*Bam*HI[b] M = 15.7 S = 21.9	HAAS *et al.* (1993)
pMYOD1	*Myod1*	Human cDNA	*Eco*RI M = 16.0 S = 13.0	DAVIS *et al.* (1987)
201/225 (PCR)[c]	*Gas2*	Mouse brain RNA, 246-bp product 5′AAATGATGTGCACTGCCCTGA 5′GAACTAAGCTGTAGAGA	*Taq*I M = 8.0, 6.6 S = 4.2	COLOMBO *et al.* (1992)
MN7	*D7H15F37S1*	Microdissection (human)	*Taq*I M = 2.5 S = 2.7	BUITING *et al.* (1990)
DN10	*D7H15S12* (=*p*)	Human cDNA	*Eco*RI-8 fragments[d]	RINCHIK *et al.* (1993a)
304/305 (PCR)	*Gabrg3*	Mouse brain RNA, 306-bp product 5′TCAAGCTGTCGAAAGCCAAC 5′GTCCAGCTCAGAGACATCAA	*Pvu*II M = 7.5 S = 1.8	CULIAT *et al.* (1994)
TM15	*D7Cwr15*	Microdissection (mouse)	*Taq*I M = 4.2 S = 2.9	JOHNSON (1990)
300/301 (PCR)	*Gabra5*	Rat brain RNA, 183-bp product 5′GTTGGTGACACCAGGAATTCAGC 5′CTCAGAAGTCTTCTCCTCAGA	*Bam*HI M = 3.9 S = 17.0	CULIAT *et al.* (1993)
218/219 (PCR)	*Gabrb3*	Rat genomic DNA, 258-bp product 5′GTTGGTGACACCAGGAATTCAGC 5′GTACAGCCAGTAAACTAAGTTG	*Pst*I M = 3.6 S = 1.8	NICHOLLS *et al.* (1993)
E6-AP (PCR)	*Hpve6a*	Human cell line DNA, 377-bp product 5′CACAAATCACAATGAAGA 5′GAACTAAGCTGTAGAGA	*Taq*I[e] M = 16.9	NAKAO *et al.* (1994)
pSNRPN	*Snrpn*	Human cDNA exons 2–8	*Taq*I[b] M = 5.6 S = 15.8	LEFF *et al.* (1992)
34-1-111	*Znf127,* formerly *D15S91h*	Mouse brain cDNA	*Eco*RI M = 2.7 S = 4.6	NICHOLLS *et al.* (1993)
pIGF-1-R.8	*Igf1r*	0.7-kb *Eco*RI fragment of human cDNA	*Bam*HI M = 5.5 S = 3.5	ULLRICH *et al.* (1986)

[a] M, *M. musculus* RFLV; S, *M. spretus* RFLV. Fragment sizes given in kb.
[b] Nonpolymorphic fragments of other sizes are not listed.
[c] Some probes were generated by PCR with the DNA/RNA source and primers indicated.
[d] Deletion of fragments tested using homozygous or compound heterozygous DNAs rather than DNAs from interspecific hybrids.
[e] Only the one polymorphic *M. musculus* fragment is listed.

TABLE 2

Deletion mapping of *p*-region loci

Allele[a]	Locus																	Phenotype[b]
	Ldh3	*Ldh1*	*Saa1*	*Tph*	*Kcnc1*	*Myod1*	*Gas2*	*D7H15F37S1*	*p*	*Gabrg3*	*D7Cwr15*	*Gabra5*	*Gabrb3*	*Hpve6a*	*Snrpn*	*Znf127*	*Igf1r*	
46DFiOD	−	−	−	−	−	−	−	−	−	−	−	+	+	N	+	+	+	PL
15DThWb	N	+	+	N	N	+	−	−	−	−	−	+	+	N	+	+	+	PL
47DTD	+	+	+	N	N	+	−	−	−	−	−	−	−	+	+	+	+	PL
8R250M	+	+	+	N	N	+	−	−	−	−	−	−	−	+	+	+	+	PL
3R30M	+	+	+	+	+	+	−	−	−	−	+	N	N	N	+	+	+	PL
2MNURf	+	+	+	N	N	+	−	−	−	+	+	+	+	N	N	+	+	PL
17MNURf	+	+	+	N	N	+	−	−	−	+	+	+	+	N	N	+	+	PL
8OK	+	+	+	N	N	+	−	−	−	−	+	+	+	N	N	+	+	PL
2FBCfFob	N	+	N	N	N	+	−	−	N	−	+	+	+	N	N	+	N	PL
24Zb	+	+	+	N	N	+	−	−	−	−	−	+	+	+	+	+	+	PL
23DFiOD	+	+	+	N	N	+	+	−	−	−	−	−	−	+	+	+	+	PL
2HATh	+	+	+	N	N	+	+	−	−	−	−	−	−	+	+	+	+	PL
30PUb	+	+	+	N	N	+	+	−	−	−	−	−	−	−	+	+	+	PL
132G	+	+	+	N	N	+	+	−	−	−	−	−	−	+	+	+	+	PL
116G	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	NL
8FDFoD[c]	+	N	N	N	N	+	+	−	−	−	−	−	+	+	+	+	+	PL
83FBFo	+	+	+	N	N	+	+	−	−	−	−	−	+	N	+	+	+	PL
1DTTMb	+	N	N	N	N	+	+	−	N	−	−	−	−	+	N	+	+	PL
41DTD	+	+	+	N	N	+	+	−	−	−	+	+	+	N	+	+	+	PL
4R250H	+	+	+	N	N	+	+	−	−	−	+	+	+	N	+	+	+	PL
3DTR	+	+	+	N	N	+	+	−	−	+	+	+	+	N	+	+	+	PL
12R30Lb	+	+	N	N	N	+	+	−	P	+	+	+	+	N	+	+	+	PL
3FR60Lg	+	+	+	N	N	+	+	−	−	+	+	+	+	N	+	+	+	PL
58HATh	+	+	+	N	N	+	+	−	−	+	+	+	+	N	N	+	+	PL
1MNURf	+	+	+	N	N	+	+	−	−	+	+	+	+	N	N	+	+	PL
25DVT	+	+	+	N	N	+	+	−	−	+	+	+	+	N	+	+	+	PL
226THO-I[c]	+	+	+	N	N	+	+	−	−	−	−	−	+	+	+	+	N	PL
7FR60Lb	+	N	N	+	+	+	+	−	−	−	−	−	−	+	+	+	N	NL
55PB	+	+	+	N	N	+	+	−	−	−	−	−	−	+	+	+	N	PL
45DTD	+	+	+	N	N	+	+	−	−	−	−	−	−	+	+	+	+	PL
4THO-II	+	+	+	N	N	+	+	+	P	−	−	−	−	+	+	+	+	NL
3RD300H	+	N	N	N	N	+	−	−	−	+	+	N	+	+	N	N	N	PL
19DVT	N	+	+	N	N	+	+	−	N	−	−	−	−	+	N	+	N	PL
2DFiOD	+	+	+	+	+	+	N	+	+	+	+	N	+	N	N	+	+	PL
26FATw	+	+	+	N	N	+	+	+	−	−	−	+	+	N	N	N	N	PL
9DTW	+	+	+	N	N	+	+	+	P	+	+	N	N	N	N	N	N	JL

+, not deleted; −, deleted; P, partial deletion (one or some, but not all hybridizing bands absent); N, not tested. Deletion of the probe tested is not necessarily equivalent to deletion of any or all of a coding unit.

[a] Each allele is an independent radiation- or chemically induced *p* mutation. For example, 46DFiOD represents $p^{46DFiOD}$.

[b] PL, prenatal lethal; NL, neonatal lethal; JL, juvenile lethal. Lethality phenotypes were determined by complementation analyses (RUSSELL *et al.* 1995, acompanying report).

[c] Deletion endpoint within the *Gabrb3* gene (CULIAT *et al.* 1993).

TABLE 3

Recombination distances between loci within the *p*-region deletion complex

Interval	% Recombination	95% confidence limits (cM)	Reference
Myod1-p	5.5 cM (10/182)[a]	2.9–9.7	NICHOLLS *et al.* (1993)
Myod1/Ldh3–Gas2	3.3 cM (6/184)	1.4–7.0	This study
Gas2–p	2.7 cM (5/184)	1.1–6.1	This study
p-D7Cwr15	0 (0/184)	0–1.8	This study
D7Cwr15–Gabrb3	1.1 cM (2/184)	0.19–3.6	This study
Gabrb3–Snrpn/Znf127[b]	0 (0/184)	0–1.8	This study
Snrpn–Znf127	0 (0/184)	0–1.8	This study
Znf127[b]*–Igf1r*	6.0 cM (11/182)	2.9–10.5	NICHOLLS *et al.* (1993)
p–Znf127[b]	1.1 cM (2/182)	0.2–3.8	NICHOLLS *et al.* (1993)

[a] cM = centimorgans. Numbers in parentheses represent total number of recombinants/total progeny tested.
[b] *Znf127* is *D15S9h-1* in NICHOLLS *et al.* (1993).

Previous data (MENDEL 1987) had shown that *Lhb* is not deleted in $p^{46DFiOD}$, so the proximal breakpoint of $p^{46DFiOD}$ lies between *Lhb* and the [*Ldh3–Myod1*] cluster. Therefore, no other *p* deletions were tested with *Lhb*.

ru2: The deletion of the coat-color locus *ru2* by $p^{46DFiOD}$, but not by any other Oak Ridge *p* deletion, has been shown by genetic means (RUSSELL *et al.* 1995). The placement of *ru2* relative to the [*Ldh3–Myod1*] cluster can be inferred from data taken from mapping of the breakpoints of Is(In7;X)1Ct, a radiation-induced mutation that resulted in the insertion of a portion of central chromosome 7 into an *X* chromosome (CATTANACH 1961, 1966). The chromosome-7-proximal breakpoint of Is(In7;X)1Ct lies between *ru2* and the [*Ldh3–Myod1*] cluster, as evidenced by the fact that *Saa1* and *Ldh1* are on the 7^{Df} derivative chromsome (TAYLOR and ROWE 1984; JOHNSON *et al.* 1989, D. K. JOHNSON, unpublished results), whereas *ru2*, like *p*, is on the X^7 derivative chromosome (EICHER 1970; RUSSELL 1983; JOHNSON *et al.* 1989; D. K. JOHNSON, unpublished results). Therefore, *ru2* must lie between *p* and the [*Ldh3–Myod1*] cluster.

Results from the hybridization of the probes for *p* and *Myod1* to 182 Oak Ridge IB segregants indicated a genetic distance for this interval of 5.5 ± 1.7 cM (NICHOLLS *et al.* 1993). Hybridization of the probes for *Ldh1* and *Ldh3* to DNAs from these same IB mice gave no evidence for recombination within the 500-kb [*Ldh3–Myod1*] cluster (HANDEL *et al.* 1992; STUBBS *et al.* 1994).

Gas2 locus: The Growth Arrest-2 (*Gas2*) gene, for which a human cognate gene is unknown, was cloned from mRNA derived from growth-arrested National Institutes of Health 3T3 cells (SCHNEIDER *et al.* 1988) and mapped to mouse chromosome 7 using recombinant inbred DNAs (COLOMBO *et al.* 1992). Analysis of *M. spretus/p** F_1 DNAs demonstrated that the *Gas2* locus is deleted in 11 *p* mutations of 35 tested (Table 2). The locus maps 2.2 cM proximal to *p* in the Oak Ridge IB (95% confidence limits: 0.7 and 5.2 cM, for four recombinants in 184 segregants). The *Ldh3–Gas2* (or *Myod1–Gas2*) distance is 2.7 cM [95% confidence limits: 1.1 and 6.1 cM, for five recombinants in 184 segregants (Table 3)].

D7H15F37S1 locus: This locus, defined by an anonymous human DNA microclone (MN7) that recognizes a large (14–15 kb) transcript in mouse brain RNA (BUITING *et al.* 1992), was previously reported to map proximal to *p* and to be deleted in three Oak Ridge National Laboratory (ORNL) *p* mutations ($p^{46DFiOD}$, p^{3R30M} and $p^{7FR60Lb}$) and one Harwell mutation (*p6H*) (NICHOLLS *et al.* 1993). In the present study, *D7H15F37S1* was found to be deleted in 28 of 33 additional mutations tested. The locus is deleted in all of the mutations that delete *Gas2* and in others that do not delete *Gas2*, thereby placing it within the *Gas2–p* interval, consistent with its not being deleted in $p^{4THO-II}$. *D7H15F37S1* has not been tested on the IB.

Loci distal to *p*: *$GABA_A$ receptor subunit genes:* Three genes that encode subunits of the type-A γ-aminobutyric acid ($GABA_A$) receptor are clustered distal to *p* (CULIAT *et al.* 1993; NAKATSU *et al.* 1993) in the order *Myod1, p, Gabrg3, Gabra5, Gabrb3* (CULIAT *et al.* 1994). Deletion-mapping data for these three genes were previously reported by CULIAT *et al.* (1993, 1994) for 19 of the ORNL *p* mutations. Table 2 incorporates results from the testing of 13 additional mutations for deletion of *Gabra5* (four not tested), 15 additional mutations for *Gabrb3* (two not tested), and 17 for *Gabrg3*.

D7Cwr15 locus: This locus, defined by an anonymous DNA microdissection clone (JOHNSON *et al.* 1989), is deleted in 20 of 36 mutations tested and was not separated from *p* in 184 backcross segregants (upper 95% confidence limit, 1.78 cM). *D7Cwr15* can be placed distal to *p* because $p^{4THO-II}$, which breaks within the *p* gene and deletes all three $GABA_A$ subunit genes (CULIAT *et al.* 1994), also deletes *D7Cwr15*. The pattern of deletion of *D7Cwr15* (Table 2) indicates that it maps between the *Gabrg3* and *Gabra5* genes; thus, p^{3R30M}, $p^{2FBC/Fob}$ and p^{8OK}, all of which delete proximal loci *Gas2*,

D7H15F37S1 and *p*, delete *Gabrg3* but not *D7Cwr15*. In the IB two in 184 segregants were recombinant between *D7Cwr15* and *Gabrb3*, the most distal of the three $GABA_A$ subunit loci (1.1 cM; 95% confidence interval 0.19–3.63 cM).

Other human 15q11-q13-region loci: The *p–Znf127* (*D15S9h-1*) region is highly homologous in gene order to the human chromosome *15q11-q13* region that also contains the critical regions for the Prader-Willi and Angelman syndromes (NICHOLLS *et al.* 1993). None of the 33 mutations tested (a set that included all 13 mutations that delete *Gabrb3*) extends far enough distally to delete *Znf127*. Similarly, none of 24 mutations tested (which also included the deletions of *Gabrb3*) deletes *Snrpn*, a locus that maps in humans within the Prader-Willi critical region (LEFF *et al.* 1992). Thus, these two loci cannot be ordered relative to *p* with the Oak Ridge *p* deletions. In the 184 IB segregants, no *Gabrb3–Snrpn*, *Gabrb3–Znf127*, or *Snrpn–Znf127* recombinants were detected.

The human *HPVE6A* locus, which encodes a protein that interacts with the E6 oncogene of human papilloma virus and the p53 tumor suppressor protein, maps to human *15q11-q13* between *GABRB3* and *SNRPN* (NAKAO *et al.* 1994). Among 13 deletions that include *Gabrb3*, and thus extend distally from *p*, a *M. musculus*-specific RFLV at the *Hpve6a* locus was found to be deleted in only p^{30PUb}, a deletion that also extends proximally from *p* to include *l(7)1Rl* (RUSSELL *et al.* 1995).

Construction of a deletion map of the [*Myod1-Ldh3*]–[*Snrpn-Znf127*] interval: Figure 1 presents a deletion map of the [*Myod1-Ldh3*]–[*Snrpn/Znf127*] interval that is based on the data presented in Tables 2 and 3. $p^{46DFiOD}$, the deletion that extends the most proximally from *p*, removes a cluster of human *11p15* genes (*Ldh3, Ldh1, Saa1, Kcnc1, Tph,* and *Myod1*) as well as the phenotypically defined locus *ru2* (STUBBS *et al.* 1994; RUSSELL *et al.* 1995). Thus, $p^{46DFiOD}$ extends proximally ≥5.5 ± 1.7 cM (NICHOLLS *et al.* 1993) from *p*. The deletion extending the most distally, p^{30PUb}, deletes *Hpve6a* but not *Snrpn* or *Znf127*. *Znf127* was reported to map 1.1 ± 0.8 cM from *p* in the Oak Ridge IB (NICHOLLS *et al.* 1993). *Snrpn* did not recombine with *Znf127* in the sample of 184 backcross segregants, and the most distally extending deletion, p^{30PUb}, includes neither locus; thus *Snrpn* and *Znf127* cannot be ordered by either IB or deletion analyses. However, recent evidence from human mapping studies suggests that *Snrpn* may lie between *Gabrb3* and *Znf127* (WAGSTAFF *et al.* 1991; OZCELIK *et al.* 1992; MUTIRANGURA *et al.* 1993; NAKAO *et al.* 1994).

Interestingly, one mutation, p^{26FATw}, which does not delete the proximal loci *D7H15F37S1* and *Gas2*, is prenatally lethal. Evidence from p^{116G} and $p^{7FR60Lb}$ localizes *D7H15F37S1* distal to *l(7)1Rl*. If that is, in fact, the case, p^{26FATw} must be designated a "skipper," which either deletes noncontiguous distal loci along with *l(7)1Rl* or is a more complex rearrangement that involves a prenatal-lethal function outside the *p*-region deletion complex. Experiments are planned to study the time-of-death of p^{26FATw} homozygotes.

DISCUSSION

We have constructed a linear deletion map of the [*Myod1-Ldh3*]–*Snrpn/Znf127* region of mouse chromosome 7 that places the deduced breakpoints of 35 agent-induced homozygous-lethal deletions into specific intervals of an initial molecular map (Figure 1). The one mutation not deleted for any probes tested, p^{2DFioD}, has been shown cytogenetically to be a paracentric inversion (RUSSELL *et al.* 1995) and is not included in Figure 1.

Differential survival of compound heterozygotes that carry *p* mutations affecting the region just proximal to *p*, in the vicinity of the *D7H15F37S1* locus, has allowed the assignment of two functions: mild juvenile-lethal (61% surviving through weaning or beyond and 32% to 237 days) and severe juvenile lethal (28% surviving through weaning or beyond and 4% to 237 days) (RUSSELL *et al.* 1995). One or both of these phenotypes may be identical to the neurological, fitness, and male-sterility syndrome exhibited by mice homozygous for the p^{6H} deletion. This phenotype, previously designated "R, JG, S" (LYON *et al.* 1992) and mapped more finely by Nicholls *et al.* (1993) to the region immediately proximal to *p* in the vicinity of the *D7H15F37S1* locus, is likely to be due to loss of function of one highly pleiotropic gene (RINCHIK *et al.* 1995).

We have shown by deletion mapping that the *Growth arrest2* (*Gas2*) gene, for which a human cognate gene is currently not available, maps between the [*Myod1–Ldh3*] cluster and the *D7H15F37S1* locus, some 2 cM proximal to *p*. *l(7)1Rl* lies proximal to *p* (RUSSELL *et al.* 1995) and is deleted in all 11 mutations that delete *Gas2*. Twenty-two mutations that delete *D7H15F37S1* but not *Gas2* fail to complement *l(7)Rl1* (RUSSELL *et al.* 1995, accompanying report). Evidence that *l(7)1Rl* is distal to *D7H15F37S1* is provided by p^{116G} and $p^{7FR60Lb}$, both of which delete both *D7H15F37S1* and *p* but not *l(7)1Rl*. Thus, *l(7)1Rl* must map between *Gas2* and *D7H15F37S1*. No *in vivo* function has yet been assigned to the *Gas2* gene. Unfortunately, the *Gas2, l(7)1Rl, p* gene order means that the *Gas2* null phenotype cannot be studied using the *p* deletions, since all mice homozygous for deletions that include *Gas2* also delete *l(7)1Rl* and thus die around the time of implantation.

In this same context, it is interesting that embryos homozygous for p^{2MNURf} elicit an implantation reaction but then die shortly thereafter (RUSSELL *et al.* 1995). Because p^{2MNURf} deletes the *Gas2* locus, it can be concluded that there are no loci between *Gas2* and *p* that

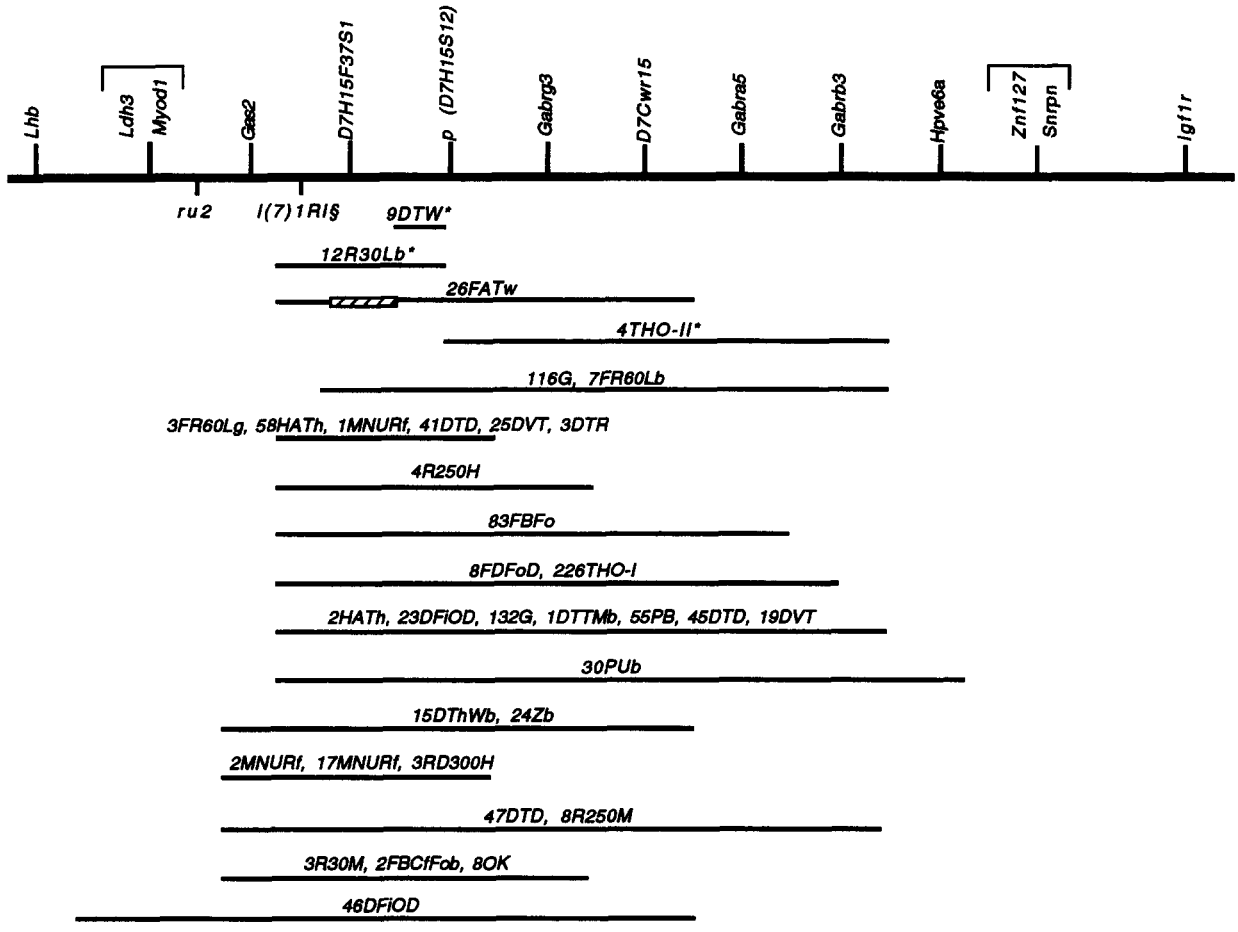

FIGURE 1.—A deletion map of the *p* region. The centromere is to the left, and loci above the chromosome include all those listed in Table 1. The *Ldh3* and *Myod1* bracketed pair represent a 500-kb gene cluster that includes *Ldh3, Ldh1, Saa1, Tph, Kcnc1,* and *Myod1* (STUBBS *et al.* 1994). The *Znf127* and *Snrpn* bracketed pair indicates that the gene order has not been determined relative to *p* in mouse. The two loci below the chromosome, *l(7)1Rll* (RUSSELL *et al.* 1995) and *ru2* are phenotype-defined and have not yet been accessed molecularly. The solid lines beneath the chromosome represent the presumed extent of each deletion (or deletions in cases where endpoints cannot be discriminated with existing data). The hatched box within the p^{26FATw} deletion line indicates the possibility that this mutation is a "skipper," *i.e.*, deletion of noncontiguous loci or other rearrangement. * indicate deletions that have removed one or more, but not all, hybridizing fragments at one of the end markers. §*l(7)1Rl* is not a locus but is a functional unit (RUSSELL *et al.* 1995) that maps to the interval whose proximal boundary is defined by the proximal breakpoints of 10 deletions (*e.g.*, $p^{15DThWb}$ and p^{3R30M}) and whose distal boundary is defined by the proximal breakpoints of 20 deletions (*e.g.*, $p^{3FR60Lg}$ and p^{2HATh}).

are required for preimplantation development. Because it is known that $p^{4THO\text{-}II}/p^{4THO\text{-}II}$ homozgotes die at birth from cleft palate (CULIAT *et al.* 1993, 1994), it follows that no loci important for preimplantation development lie between the proximal breakpoint of p^{2MNURf} and the distal breakpoint of $p^{4THO\text{-}II}$, a distance estimated to be >3 cM.

The region from *D7H15F37S1* to *Znf127* is homologous to human chromosome *15q11-q13*, a segment that includes the Prader-Willi (PWS) and Angelman Syndrome (AS) "critical regions" (NICHOLLS *et al.* 1993). A number of loci within this region have been shown to be imprinted in humans. For example, the expression of the *SNRPN* locus is imprinted (LEFF *et al.* 1992), and *ZNF127* carries a methylation imprint (DRISCOLL *et al.* 1992). The expression of neither *HPVE6A* nor *PAR-2*, a gene of unknown function in the interval, however, is imprinted in cultured human fibroblasts or lymphoblasts (NAKAO *et al.* 1994). The AS critical region encompasses ~1000 kb as estimated from a study of deletion breakpoints in human patients (REIS *et al.* 1994). Another study, analyzing deletion breakpoints ~200 kb apart in a single patient, may serve to narrow the critical region for the AS gene(s) even further (BUXTON *et al.* 1994). The homologous region in the mouse lies just distal to the *Gabrb3* locus. It is known that *Snrpn* expression is imprinted in the mouse (LEFF *et al.* 1992), but that *Gabrb3, Gabra5,* and *Gabrg3* are not imprinted (CATTANACH *et al.* 1992; NICHOLLS *et al.* 1993; CULIAT *et al.* 1994). The p^{30PUb} deletion, whose proximal breakpoint lies between *Gas2* and *l(7)1Rl* and whose distal breakpoint lies between *Hpve6a* and *Snrpn/*

Znf127, is the most distally extending *p* deletion of those tested. It is noteworthy that p^{30PUb} can be passed through either male or female carriers without causing lethality or any readily apparent phenotype (L. B. RUSSELL, unpublished data), which suggests two alternative hypotheses: (1) the region in mouse covered by the the distal end of the p^{30PUb} deletion is not imprinted, and a homologous region of imprinted loci in the mouse may be found only distal to the p^{30PUb} distal breakpoint; or (2) some or all of an AS-gene may indeed be deleted in p^{30PUb}, but the corresponding AS phenotype may not be as readily apparent in mice. We have previously suggested (NICHOLLS *et al.* 1993) that, because none of the distally extending deletions (including p^{30PUb}) includes *Znf127* (or *Snrpn*, as reported here), heterozygous deletion of the homologous imprinted region in the mouse may be lethal during prenatal or neonatal development and hence would preclude the recovery from the specific-locus test of deletions extending to any of the imprinted genes.

The region of chromosome 7 included in the $p^{46DFiOD}$ deletion is currently the target for the induction and recovery of *N*-ethyl-*N*-nitrosourea (ENU)-induced presumed point mutations that have some effect on the normal development of the animal (RINCHIK *et al.* 1995; E. M. RINCHIK, unpublished data). The mutagenesis strategy being employed is similar to the strategy used to generate a number of such mutations within the 6- to 11-cM region of chromosome 7 included in the more distally mapping c^{26DVT} deletion (RINCHIK and RUSSELL 1990; RINCHIK and CARPENTER 1993; RINCHIK *et al.* 1993a; POTTER *et al.* 1995). The existence of a highly developed original complementation map of the region surrounding the albino (*c*) locus (RUSSELL *et al.* 1982), along with constantly evolving molecular deletion maps of subregions of the complex (*e.g.*, RINCHIK and RUSSELL 1990; SHARAN *et al.* 1991; KELSEY *et al.* 1992; KLEBIG *et al.* 1992; RINCHIK *et al.* 1993b; FAUST *et al.* 1995), has greatly facilitated the genetic and molecular analyses of these new *c*-region mutations (RINCHIK and CARPENTER 1993; RINCHIK *et al.* 1993a; POTTER *et al.* 1995). Similarly, the availability of a well-resolved subset of the *p* deletions has been instrumental in identifying candidates for *cp1*, a gene required for normal palate development (CULIAT *et al.* 1993). We expect that having a molecular and genetic deletion/complementation map of the region surrounding *p*, as presented here, will similarly facilitate the localization of ENU-induced mutations with respect to both deletion breakpoints and molecularly defined loci, thereby providing a highly useful framework on which to expand the genetic and phenotypic analysis of the [*Myod1-Ldh3*]–[*Snrpn-Znf127*] region of this chromosome.

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