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Genetic Map of the Region around *grizzled* (*gr*) and *mocha* (*mh*) on Mouse Chromosome 10, Homologous to Human 19p13.3

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Grizzled (_gr_) is a recessive mouse mutation resulting in a gray coat color and reduced perinatal viability. _Mocha_ (_mh_) is one of several recessive mouse mutants characterized by platelet storage pool disorder, pigment abnormalities, reduced fertility, kidney function deficiencies, and, in some mutants, inner ear and natural killer cell deficiencies. Murine platelet storage pool deficient mutants may be models for Chediak–Higashi and Hermansky–Pudlak syndromes in humans. The genes for _gr_ and _mh_ are very closely linked to each other (0 ± 1.2 cM). However, their relative position with respect to molecular markers was previously unknown. Thus, genetic mapping of the _gr_ locus will also yield information about the _mh_ location. To map these two genes genetically, we have performed an intersubspecific backcross of _grizzled_ mice with _Mus musculus castaneus_. In 539 progeny tested, we found no recombination between the _gr_ gene, the gene for anti-Muellerian hormone (_Amh_), and the microsatellite markers _D10Mit7, D10Mit21,_ and _D10Mit23_. One recombination event for each of the flanking markers Basigin (_Bsg_) and _D10Mit22_ was identified. These closely linked markers should provide entry points for positional cloning of the _gr_ and _mh_ genes. The region linked to _grizzled_ is homologous to a gene-rich region on human Chromosome 19p13.3. © 1994 Academic Press, Inc.

INTRODUCTION

Grizzled (*gr*) is a recessive coat color mutation (Bloom and Falconer, 1966), initially identified in 1950. Homozygous *gr* mice are missing the yellow pigment (phaeomelanin) but not the black pigment (eumelanin), giving rise to a gray coat color. Homozygous *gr/gr* mice are initially about 25% smaller than their littermates, but reach normal weight after weaning; 50–60% die perinatally for unknown reasons (Bloom and Falconer, 1966). Other mouse coat color mutations that have been identified are mostly associated with other functional defects, in the generation, migration, and regulation of melanosomes and in the enzymatic pathways of coat color development, e.g., the *brown, agouti, steel,* and *dilute* mutations (Bultman *et al.*, 1992; Jenkins *et al.*, 1989; Michaud *et al.*, 1993; Miller *et al.*, 1993; Morrison and Takahashi, 1993; Shibahara *et al.*, 1992; Winder *et al.*, 1993). However, the phaeomelanin pathway that is affected in *grizzled* is much less understood (see Hearing, 1993 for review).

Mocha (*mh*) is one of about a dozen mutations that show platelet storage pool deficiencies, characterized by coat color and eye pigment dilution, decreased granule contents in platelets with a resulting increase in bleeding time, and a lysosomal enzyme secretion defect in the kidney (Novak *et al.*, 1984; White *et al.*, 1992). In addition, some of these mutants have inner ear or natural killer cell abnormalities (Clark *et al.*, 1981; Swank *et al.*, 1991). The latter may represent models for the human disorders Hermansky–Pudlak syndrome and Chediak–Higashi disorder in which individuals have similar deficits (Belohradsky and Laminger, 1992; Brandt and Swank, 1976; Burkhardt *et al.*, 1993; Clark *et al.*, 1981; White, 1987). The mutations *pallid, muted,* and *mocha* show an incompletely penetrant otolith deficiency in the inner ear and a slowly progressive degeneration of the cochlea (Rolfsen and Erway, 1984; Swank *et al.*, 1991). The otolith defect can be prevented by increasing Mn^{2+} or Zn^{2+} concentrations in the diet of the pregnant dams (Erway *et al.*, 1986; Rolfsen and Erway, 1984). In addition, *mh/mh* mice are hyperactive, tilt their head to one side, make a characteristic noise that resembles chirping (unpublished observation), and show a unique persistent hypersynchronized electrocorticogram (Noebels and Sidman, 1989). The coat color of *mh/mh* is a result of dilution of both the yellow and the black pigments. Mice mutant for a second allele of *mocha*, mh^{2J}, are available from the Jackson Laboratory (Bar Harbor, ME) where they were discovered, but this allele has not been published. mh^{2J}/mh^{2J} homozygote mice are less severely affected than *mh/mh* mice. They share the coat color defect and platelet storage pool deficiency, but not the hyper-

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synchronized electrocorticogram (Noebels and Sidman, 1989), and the inner ear is much less affected (Y. Raphael and M.B., unpublished observation). In summary, the underlying defect in this class of mutations seems to affect function and morphology of several membranous organelles.

We are interested in identifying the defect in both the *gr* and the *mh* mutations, which have been localized to the central region of mouse Chromosome 10 (see Taylor *et al.*, 1993 for summary of linkage information). As a first step it is necessary to obtain accurate mapping data. No closely linked molecular markers have been reported. The genes for *mh* and *gr* are extremely closely linked to each other: by repulsion intercrosses and further testcrosses, Lane and Deol (1974) have tested 256 meioses without finding recombination, placing *mh* 0 cM from *gr* (upper 95% confidence limit 1.2 cM). Since *mh*/*mh* mice are sterile, and mh^{2J}/mh^{2J} mice were initially not available and are semisterile, we started a genetic backcross using *gr*/*gr* animals. In this study we report the identification of several markers that are closely linked to *grizzled* that will be useful for positional cloning of the gene. Because of the close linkage of *mh* and *gr*, these backcross data will also be relevant for the localization of the *mh* gene.

MATERIALS AND METHODS

Intraspecific backcross. An intersubspecific backcross with *Mus musculus castaneus* [(JIGR/Dn-*gr/gr* × CASA/Rk)F1 × JIGR/Dn-*gr/gr*] was performed. F1 animals were also generated by mating JIGR/Dn-*gr+/+ji* animals with CASA/Rk. F1 animals from that cross were testcrossed to JIGR/Dn-*gr+/+ji* animals to discriminate between *ji*/+ and *gr*/+ F1 offspring; only those carrying a *gr* allele were used in the experiments reported here. The backcross offspring was scored for coat color (gray or agouti) at weaning, and tissues (lung, liver, spleen) were removed and frozen for subsequent DNA analysis. JIGR/Dn-*gr/gr*, JIGR-*gr+/+ji* and CASA/Rk animals were obtained from the Jackson Laboratories. Animal experiments were reviewed and approved by the local UCUCA/ULAM committees.

DNA preparation. Small-scale DNA preparation was performed from an aliquot (1/10) of frozen lung tissue using a salting out method (Miller *et al.*, 1988). In short, the tissue was ground using disposable pellet pestles (VWR Scientific, Philadelphia, PA); 1 ml of TNES (10 m*M* Tris–HCl, pH 7.5, 400 m*M* NaCl, 100 m*M* EDTA, pH 8.0, and 0.6% SDS) was added, as well as 10 μl of a solution containing 10 mg/ml Proteinase K (Boehringer Mannheim, Germany). The tissue was dissolved overnight in a rocking 55°C incubator; 330 μl of a saturated NaCl solution was added, after which the mixture was shaken vigorously and centrifuged at 14,000*g* for 10 min. The supernatant was transferred to a new tube, 3 ml of 100% ethanol was added, and the precipitated DNA was spooled onto a bent glass Pasteur pipe. The DNA was dissolved in 200 μl of TE. PCR reactions for SSLP analysis were performed using 0.1–0.2 μl of this DNA solution. Larger amounts of DNA for RFLP analysis of recombinants were isolated from spleen by using a regular size mortar and grinding the frozen tissue under liquid nitrogen, followed by the salting out extraction method described above, scaled up by a factor of 10.

SSLP markers. All simple sequence length polymorphisms (SSLPs) were scored using published primers (Dietrich *et al.*, 1992) or primers from the updates obtained by electronic mail from the MIT Mouse Genome group (genome_database@genome.wi.mit.edu). The reference (Dietrich *et al.*, 1992) will be used in the text to refer to both the published markers as well as the updated information. All primer pairs were obtained from Research Genetics (Huntsville, AL). Forward primers were radioactively labeled with ^{32}P using polynucleotide kinase (Promega), and PCR was performed under the conditions described by Dietrich *et al.* (1992). Alleles were separated by denaturing polyacrylamide gel electrophoresis.

RFLP analysis. Molecular probes used for hybridization, the corresponding references, and the polymorphic fragment sizes observed are listed in Table 1. We routinely checked a blot prepared from 20 μg genomic DNA from JIGR/Dn and CASA/Rk animals, digested with 6 restriction enzymes (*Msp*I, *Taq*I, *Pst*I, *Bam*HI, *Bgl*II, and *Eco*RI), and found polymorphisms with each marker tested. Southern blots of 46 recombinant animals were prepared from 20 μg genomic DNA digested with *Bam*HI, *Msp*I, and *Bgl*II. Probes were radioactively labeled by random priming (Feinberg and Vogelstein, 1983). Hybridization was performed at 65°C in 0.5 *M* Na phosphate, pH 7.2, 7% SDS, and 0.2 m*M* EDTA (Church and Gilbert, 1984) and washing at 65°C in 0.2× SSC, 0.2% SDS. Since the probe for *Amh* cross-reacts to other related genes, we used a *Bam*HI polymorphism between *M. m. castaneus* and other inbred strains that was known to be linked to the *Amh* gene on Chromosome 10, rather than to *Amh*-related genes on other chromosomes (King *et al.*, 1991).

RESULTS

Viability and Fertility of *gr*/*gr* Animals

To map the *gr* gene genetically, we performed an intersubspecific backcross of *grizzled* animals with the CASA/Rk strain of *M. m. castaneus* (see Materials and Methods for details). We expected a reduced number of *gr*/*gr* homozygote offspring since Bloom and Falconer (1966) had shown a 50–60% reduced viability of *gr*/*gr* animals, especially males. We observed 315/535 (59%) *gr*/+ and 220/535 (41%) *gr*/*gr* animals. While this indicates reduced viability ($P < 0.005$), lethality was less than expected. Our data are compatible with a 30% reduction in viability. We could not confirm a preferential lethality of male homozygotes (52% male and 48% female *gr*/*gr* animals were born). The different background, introduced from CASA/Rk, may have increased the viability of *gr*/*gr* animals by heterozygote advantage. Therefore, the perinatal lethality associated with *gr* can vary with the genetic background. Once born, *gr*/*gr* animals had no obvious reduced viability within the first year.

Homozygote *gr*/*gr* females often did not rear their young. Therefore, the majority of offspring was derived from female heterozygote mothers, and thus our backcross data are mostly from female meioses (452/539 = 84% of all meioses scored). For the whole region analyzed we did not find a significant difference between male and female distances. Data from female and male meioses were therefore combined.

High-Resolution Genetic Mapping of *gr*

DNA from all 539 backcross progeny was scored for segregation of the markers *D10Mit31* and *D10Mit8*. These markers were chosen because they flank the *gr* gene, were easily and reproducibly scored, and are close enough (about 10 cM apart) to exclude double recombi-

TABLE 1

Gene Loci Tested in the *gr* Backcross by RFLP Analysis

Locus	Probe used	Reference	Restriction enzymes		Restriction fragment size (kb)	
			Not polymorphic	Polymorphic	CASA	JIGR
Amh	pMIS330.5-2	King *et al.* (1991)	*Bgl*II, *Msp*I	*Bam*HI, *Eco*RI, *Pst*I, *Taq*I	2.5	4.6
Bsg	pBsg (total mouse cDNA)	Miyauchi *et al.* (1990)	*Eco*RI, *Pst*I	*Bam*HI, *Bgl*II, *Msp*I, *Taq*I	~13	~18
Bcr	1 kb from 3′ end of mouse gene	Justice *et al.* (1990) and A. DeKlein, unpubl.		*Bam*HI, *Bgl*II, *Eco*RI,* *Msp*I, *Pst*I,* *Taq*I	5.4 1.2	5.0 2.3
Pfkl	Clone 19 (total cDNA)	D. Levanon and Y. Groner, unpubl. (available from ATCC)	*Bgl*II, *Pst*I	*Bam*HI, *Eco*RI	3.7	8.5
S100b	0.95-kb unique *Xba*I/*Eco*RI fragment	Jiang *et al.* (1993)	*Bam*HI, *Eco*RI, *Msp*I, *Pst*I, *Taq*I	*Bgl*II	7.8	9.0
Pah	LX/mPAH	Ledley *et al.* (1990)	*Bam*HI	*Msp*I	7.4 7.7	~15
Pmch	Total rat cDNA	Thompson and Watson (1990)	*Bgl*II, *Eco*RI, *Taq*I	*Bam*HI*, *Msp*I, *Pst*I	~16	9

Note. Enzymes that were used in this study are underlined. An asterisk indicates that a polymorphism was found for that enzyme that could not be well resolved under our gel conditions (0.8% agarose gel). Restriction fragment sizes for only the polymorphic bands that were used are listed.

nants due to interference. All nonrecombinant animals with an *agouti* coat were heterozygous for both alleles, and all nonrecombinant animals with a gray coat were homozygous for these markers. This reflects the fact that the coat color phenotype is fully penetrant and that double crossover events are excluded in the interval between *D10Mit8* and *D10Mit31*.

We found 46 animals to be recombinant in that interval. DNA from these recombinant animals was scored for the SSLP markers *D10Mit7*, *D10Mit21*, *D10Mit22*, *D10Mit23*, *D10Mit42*, *D10Mit65*, and *D10Mit91*. In addition, Southern blot analysis with seven different cloned molecular markers was perfomed (Table 1), using restriction enzymes found to reveal RFLPs between JIGR/Dn and CASA/Rk (see Materials and Methods). The genotypes of these recombinant animals are shown in Fig. 1. The recombination fractions between all loci were calculated and are listed in Table 2. No recombinants were observed between *gr* and *Amh*, *D10Mit7*, *D10Mit21*, and *D10Mit23* (0 ± 0.4 cM for 95% confidence interval). One flanking recombinant places *D10Mit22* distal to this cluster, and two recombinants place *D10Mit42* further distal. The gene for *Basigin* (*Bsg*) (Miyauchi *et al.*, 1990) flanks the *grizzled* gene on the proximal side. Figure 2 illustrates the genetic map constructed from our data. Since no double crossover events were observed in the interval studied, gene orders could be assigned unambiguously: any change in gene order from the one shown would have resulted in introducing double crossover events. Thus, we have now established markers flanking the *gr* locus at 0.2-cM intervals and have found four markers that are non-recombinant with the *grizzled* gene. These should give us entry points for positional cloning.

Map Positions of Pah, Pmch, and D10Mit65 near D10Mit8

Our data also refine and confirm other details of the mouse Chromosome 10 map. The phenylalanine hydroxylase (*Pah*) gene, which has previously been located close to *D10Mit8* (Copeland *et al.*, 1993b), was nonrecombinant with this marker in our cross. However, since only animals recombinant in the *D10-Mit31–D10Mit8* interval were tested, we cannot exclude the possibility that *Pah* maps distal to *D10Mit8*. Similarly, we found no recombinants between *D10Mit8* and the gene for promelanin-concentrating hormone (*Pmch*). *Pmch*, like *Pah*, is located on human Chromosome 12q23–q24 in a linkage group conserved with mouse Chromosome 10. *Pmch* has previously been reported to map to mouse Chromosome 10, but no data were given (Pedeutour *et al.*, 1992). Our results clearly demonstrate linkage of *Pmch* to *D10Mit8* on mouse Chromosome 10 and map it at or distal to *D10Mit8*. *D10Mit65* is located 0.9 ± 0.5 cM proximal to *D10Mit8*, whereas these two markers were previously not resolved (Dietrich *et al.*, 1992). *D10Mit91* is located 0.2 $\pm$ 0.2 cM distal to *Bcr*.

Allele Sizes of SSLP Markers in JIGR

The background strain of *gr*, JIGR, is not well defined. It arose by forced heterozygous matings between animals heterozygous for both *gr* and *ji* (i.e., genotype *ji*+/+*gr*). The *ji* gene is closely linked to the *gr* gene. The *gr* mutation arose in a heterozygote mouse between an undefined strain and the A strain (Bloom and Falconer, 1966), whereas *ji* arose on the Bagg albino

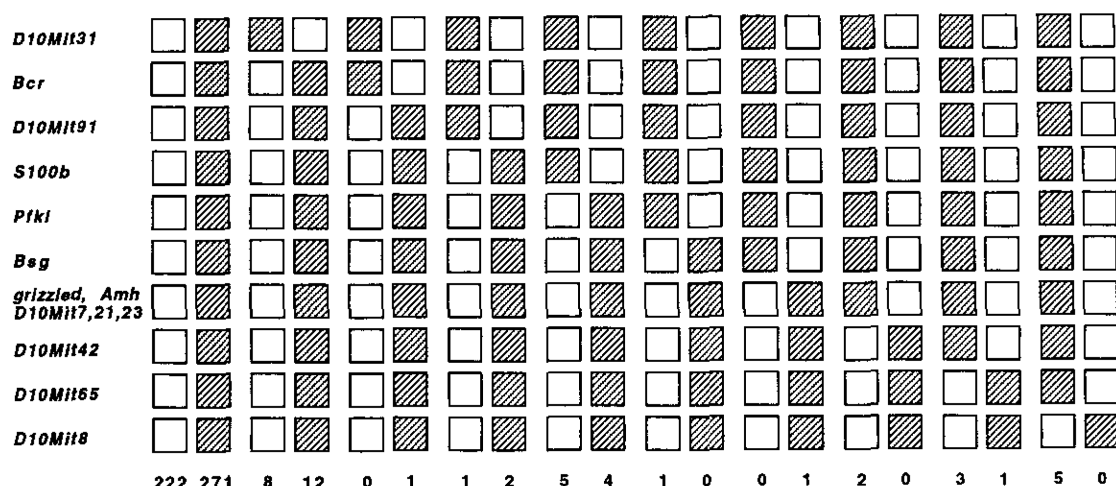

FIG. 1. Analysis of intersubspecific backcross haplotypes. Each column represents a chromosomal haplotype that was inherited from the (JIGR-*gr/gr* × CASA/Rk)F1 parent. The number of backcross progeny of each haplotype is indicated below each column. Open boxes represent the JIGR alleles and hatched boxes the CASA/Rk alleles as determined by Southern blotting or PCR for the markers indicated to the left.

strain (DeOme, 1941; Green, 1968). The A strain is an early progenitor of A/J, whereas Bagg albino later gave rise to BALB/C. It was of interest to compare allele sizes since some SSLPs differentiate between inbred mouse strains (Dietrich *et al.*, 1992). All allele sizes found were homozygous in JIGR and were identical to the alleles of A/J, except for *D10Mit31*, which has allele sizes of 152 bp for JIGR and 154 bp for A/J and BALB/C; 152 bp is typical of other inbred strains, such as C57BL/6J and DBA/2J (Dietrich *et al.*, 1992).

D10Mit22 Alleles Are Not Identical in All CASA/Rk Animals and Are Different from CAST/Ei

CASA/Rk and CAST/Ei are separately inbred lines that were started from an identical small founder colony of *M. m. castaneus* at the Jackson Laboratory. Most alleles of CASA/Rk determined in our laboratory were identical or very similar in size to those of CAST/Ei reported by Dietrich *et al.* (1992). However, we observed two different inconsistencies for *D10Mit22*. *D10Mit22* has an allele size of 148 bp in all reported inbred *M. m. musculus/domesticus* strains and of 154 bp in CAST/Ei (Dietrich *et al.*, 1992). We found an allele size of 150 bp for the CASA/Rk chromosome in most heterozygous backcross offspring. However, some otherwise clearly heterozygous mice were homozygous for the 148-bp allele of *D10Mit22*. After testing all animals of these litters, we concluded that at least one of the founder CASA/Rk chromosomes (which are no longer available) had an allele size of 148 bp rather than 150 bp. Offspring from that animal were thus uninformative. This included one of the two animals that were recombinant between *D10Mit42* and *grizzled*. Occasional differences

TABLE 2

Number of Recombinants and Recombination Fraction between Loci on Mouse Chromosome 10

	Mit31	*Bcr*	*Mit91*	*S100b*	*Pfkl*	*Bsg*	*Amh, gr, Mit7, 21, 23*	*Mit42*	*Mit65*	*Mit8*
Mit31	—	3.7 ± 0.8	3.9 ± 0.8	4.5 ± 0.9	6.1 ± 1.0	6.3 ± 1.0	6.5 ± 1.1	6.9 ± 1.1	7.6 ± 1.1	8.5 ± 1.2
Bcr	20	—	0.2 ± 0.2	0.7 ± 0.4	2.4 ± 0.7	2.6 ± 0.7	2.8 ± 0.7	3.2 ± 0.8	3.9 ± 0.8	4.8 ± 0.9
Mit91	21	1	—	0.6 ± 0.3	2.2 ± 0.6	2.4 ± 0.7	2.6 ± 0.7	3.0 ± 0.7	3.7 ± 0.8	4.6 ± 0.9
S100b	24	4	3	—	1.7 ± 0.6	1.9 ± 0.6	2.0 ± 0.6	2.4 ± 0.7	3.2 ± 0.8	4.1 ± 0.9
Pfkl	33	13	12	9	—	0.2 ± 0.2	0.4 ± 0.3	0.7 ± 0.4	1.5 ± 0.5	2.4 ± 0.7
Bsg	34	14	13	10	1	—	0.2 ± 0.2	0.6 ± 0.3	1.3 ± 0.5	2.2 ± 0.7
Amh, gr, Mit7, 21, 23	35	15	14	11	2	1	—	0.4 ± 0.3	1.1 ± 0.4	2.0 ± 0.6
Mit42	37	17	16	13	4	3	2	—	0.7 ± 0.4	1.7 ± 0.6
Mit65	41	21	20	17	8	7	6	4	—	0.9 ± 0.5
Mit8	46	26	25	22	13	12	11	9	5	—

Note. The lower left shows the number of recombinations between each marker pair in the 539 animals tested. The top right shows the recombination fraction (in % ± SE). The five loci *gr*, *Amh*, *D10Mit7*, *D10Mit21*, and *D10Mit23* were not separated by recombination and are listed together. All SSLP markers are abbreviated (*Mit7* for *D10Mit7*, etc.).

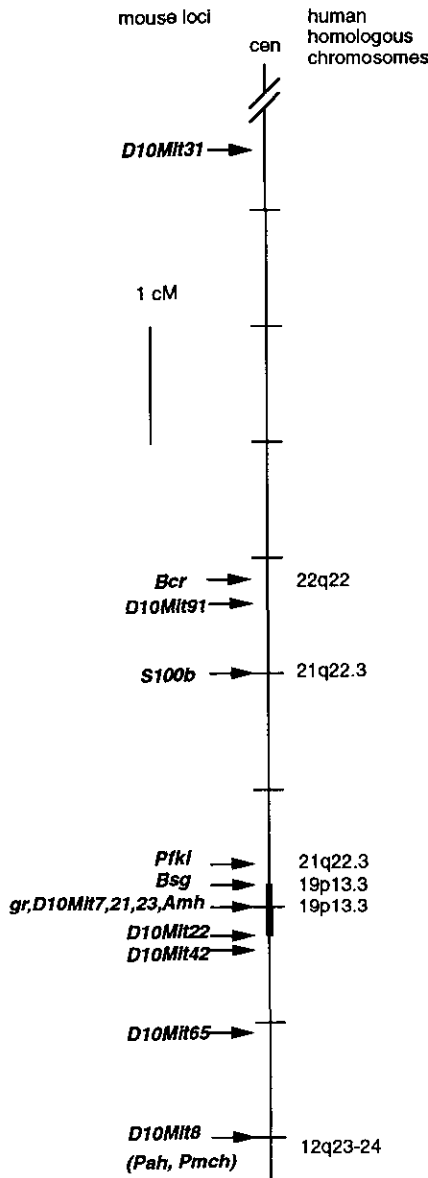

FIG. 2. Genetic map of the central region of mouse Chromosome 10. Markers scored in our cross are indicated to the left, human homologous chromosomes when known to the right. The region to which the *gr* gene has been localized is indicated as a thickened area on the map.

between the two *M. m. castaneus* strains of common origin are expected, as are occasional individual differences between animals or chromosomes in newly inbred lines. However, the fact that both unusual observations, a difference between CASA/Rk and CAST/Ei and heterogeneity of allele sizes within CASA/Rk, were found for the same marker and only for that one marker is unexpected. It might suggest that *D10Mit22* has a higher mutation rate than other SSLPs.

Only litters that showed the CASA/Rk 150-bp allele of *D10Mit22* could therefore be scored unequivocally. Since we did not score *D10Mit22* in all animals and could not determine what percentage of animals would have been informative, *D10Mit22* is not included in Table 2. However, some animals that were recombinant for flanking markers were informative for *D10Mit22*, as determined by testing nonrecombinant litter mates. One of the two animals that had a recombination break between *gr* and *D10Mit42* was also recombinant between *gr* and *D10Mit22*, whereas a second animal that was recombinant between *gr* and *D10Mit42* was not informative for *D10Mit22*. *D10-Mit22* was placed unequivocally on the map using the information provided by Dietrich *et al.* (1992). They give the order (*D10MIT7,21,22,23–D10Mit42*). We find (*gr, D10Mit7,21,23–D10Mit22, 42*), and therefore the combined order is [proximal–(*gr, D10Mit7, D10Mit21, D10Mit23*)–*D10Mit22*–*D10Mit42*–distal], as shown in Fig. 2.

DISCUSSION

We have located the *gr* gene on a small region of mouse Chromosome 10 that is homologous to human Chromosome 19p13.3. We could not identify any candidate gene that is likely to be involved in the *gr* phenotype. The only gene found to be nonrecombinant with *grizzled* is the *Amh* gene (anti-muellerian hormone, also called müllerian inhibiting substance or MIS). *Amh* affects the development of male genitalia (Behringer *et al.*, 1990; Lee and Donahoe, 1993) and is therefore unlikely to be involved in coat color development. While no candidate gene is identified for *gr*, our map has identify closely linked markers that provide important entry points for the search for candidate genes by positional cloning. It is worth noting that a third gene locus, *jittery* (*ji*), also has been mapped near *gr* and *mh* (Taylor *et al.*, 1993) and thus might map into the same region. Experiments to test that hypothesis are in progress.

Relevance of the Homology to Human Chromosome 19p13.3

In the central region of mouse Chromosome 10, conserved linkage groups with human chromosomes are short, only several centimorgans in size (Taylor *et al.*, 1993). The *Amh* gene, the most closely linked marker to *gr* and *mh*, has been mapped to human Chromosome 19p13.3 (Cohen *et al.*, 1987). The distal tip of Chromosome 19p13.3 is extremely gene-rich (Mohrenweiser *et al.*, 1994), and progress has been made to completely clone this region in overlapping cosmids (Brandriff *et al.*, 1994). These facts can now be used in prescreening candidate genes before mapping them in our cross: all genes that map to mouse Chromosome 10 *and* human 19p13.3 will be genetically close to *gr* and *mh* and thus will be useful as candidate genes and additional markers. One example is the gene for lamin B2 (Biamonti *et al.*, 1992; Zewe *et al.*, 1991). Many other genes from

19p13.3 have not yet been mapped in the mouse (Mohrenweiser *et al.*, 1994), and should now be tested.

Position of mocha and Potential Candidate Genes

Since *mocha* is part of a family of similar mutations with platelet storage pool deficiency, identification of one member of this family could help to find other members of this class. Interestingly, a gene involved in one of these mutations, *pallid*, has been reported (White *et al.*, 1992). The gene for protein 4.2, an intracellular matrix protein expressed in erythrocytes and other tissues, maps genetically close to the *pallid* mutation. In humans, protein 4.2 mutations do not give rise to storage pool deficiencies, but instead to hemolytic anemias (Bouhassira *et al.*, 1992; Iwamoto *et al.*, 1993; Jarolim *et al.*, 1992; Rybicki *et al.*, 1988, 1993). Differences on Southern blots and a slightly smaller transcript in one of three tissues analyzed were observed between *pallid* and control mice, and it was concluded that *pallid* results from a mutation in protein 4.2. However, since *pallid* arose on a wild-caught mouse, caution has to be exercised in claiming this to be the cause of the *pallid* mutation until a functional mutation in protein 4.2 has been identified.

If indeed protein 4.2 is mutated in *pallid*, any gene that interacts with protein 4.2. might be considered a candidate gene for *mocha*. It was thus of great interest that a new form of ankyrin, *Ankyrin3*, was recently mapped to mouse Chromosome 10 (Peters *et al.*, 1993). In this abstract, mapping of *Ankyrin3* 5 cM proximal to *Amh* was reported and the gene was postulated to be a candidate for *mh*. However, our data clearly place *gr* 0 ± 0.2 cM from *Amh* and are thus not compatible with a location of *gr* 5 cM proximal to *Amh*. Given our data and the close proximity of *gr* and *mh*, it is unlikely that *mh* maps 5 cM proximal to *Amh* and thus unlikely that *Ankyrin3* is the *mh* gene. An alternative explanation for this discrepancy is that *gr* or *mh* are associated with a several-centimorgans-long inversion that suppresses recombination. While we cannot exclude this possibility for *mh*, preliminary data with those markers that are at 0 cM from *gr* indicate that they are also physically close (D.K. and M.B., unpublished observation), arguing against an inversion around *gr*. A more likely alternative explanation is that *mocha* is not a mutation in the *Ankyrin3* gene.

Comparison of Map with Other Published Maps of this Region.

The order of markers found here is consistent with those published in other genetic maps from this chromosome (Copeland *et al.*, 1993a, b; Justice *et al.*, 1990; Taylor *et al.*, 1993). However, none of these studies has previously mapped molecular markers relative to *grizzled*. The combination of markers used in our cross has also not been published previously. Our results are entirely consistent with previous maps but refine the location of many markers, including the *gr* locus and many SSLPs, relative to markers scored by RFLP analysis. We find *Bsg* and *Amh* to be located closer to *Pfkl* than in other maps (Copeland *et al.*, 1993b; Taylor *et al.*, 1993). The distance between *D10Mit31* and *D10Mit8* as found by Dietrich *et al.* (1992), in a CAST/Ei × Ob intercross, was 12.7 cM. In a recent cross involving *Mus musculus molossinus* (MOLD/Rk), Irving *et al.* (1994) found significantly shorter distances in the proximal part of the same region, but all marker orders and other distances are consistent with those found here. Irving *et al.* (1994) found a distance of only 4.5 cM between *D10Mit20* and *D10Mit7*, whereas we find 6.5% recombination between *D10Mit31* and *D10Mit7*. Since *D10Mit20* is about 6 cM proximal to *D10Mit31* (Dietrich *et al.*, 1992), the difference adds up to about 12.5 cM compared to 4.5 cM, which is highly significant and probably due to differences between *M. m. castaneus* and *M. m. molossinus*. The distance between *D10Mit31* and *D10Mit8* found in our cross, 8.5% recombination fraction or 8.9 cM, is thus intermediate between the distances found in these other two crosses.

In summary, we have placed the *gr* and by inference the closely linked *mh* gene precisely on the molecular map of mouse Chromosome 10, refined the order and distance of many genetic markers, and established that this region is homologous to human Chromosome 19p13.3. These results will facilitate molecular cloning of the *gr* and *mh* genes.

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