A Molecular Genetic Linkage Map of Mouse Chromosome *9* With Regional Localizations for the *Gsta*, *T3g*, *Ets-1* and *Ldlr* Loci

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ABSTRACT

A 64-centiMorgan linkage map of mouse chromosome *9* was developed using cloned DNA markers and an interspecific backcross between *Mus spretus* and the C57BL/6J inbred strain. This map was compared to conventional genetic maps using six markers previously localized in laboratory mouse strains. These markers included *thymus cell antigen-1*, *cytochrome P450-3*, *dilute*, *transferrin*, *cholecystokinin*, and the *G-protein alpha inhibitory subunit*. No evidence was seen for segregation distortion, chromosome rearrangements, or altered genetic distances in the results from interspecific backcross mapping. Regional map locations were determined for four genes that were previously assigned to chromosome *9* using somatic cell hybrids. These genes were *glutathione S-transferase Ya subunit* (*Gsta*), the *T3 gamma subunit*, the *low density lipoprotein receptor*, and the *Ets-1* oncogene. The map locations for these genes establish new regions of synteny between mouse chromosome *9* and human chromosomes *6*, *11*, and *19*. In addition, the close linkage detected between the *dilute* and *Gsta* loci suggests that the *Gsta* locus may be part of the *dilute/short ear* complex, one of the most extensively studied genetic regions of the mouse.

THE construction of genetic linkage maps is a crucial first step in the structural and functional characterization of mammalian genomes. Linkage maps provide the necessary tools for positioning new genes, for establishing correspondence between cloned genes and previously described mutations, and for identifying animal models of human genetic diseases.

A long history of genetic experiments has already led to the development of linkage maps of the mouse genome (see for example, DAVISSON *et al.* 1988). These maps contain hundreds of different mutations known only by their phenotypic effects on behavior, coat color, skeletal development, immune function, or incidence of different diseases (reviewed by GREEN 1981). As more molecular markers have been placed on the mouse linkage map, it has become increasingly possible to identify candidate genes for existing mouse mutations and to identify relationships between these mutations and genetic syndromes in humans. For example, genetic linkage experiments recently helped establish the correspondence between the mouse *mdx* mutation and the human gene that is defective in Duchenne's muscular dystrophy (RYDER-COOK *et al.* 1988). In addition, close linkages between the *Kit* proto-oncogene and the mouse *dominant-white spotting* (*W*) mutation, and between the *Pax-1* gene and the mouse *undulated* mutation, have recently led to experiments demonstrating a direct relationship between the cloned genes and the corresponding mutations (CHABOT *et al.* 1988; GEISSLER, RYAN and HOUSMAN 1988; BALLING, DEUTSCH and GRUSS 1988).

In order to expand the power of genetic linkage studies in the mouse, it is critical to determine the map location of additional molecular markers. The recent advent of interspecific backcross analysis provides a convenient method for simultaneous linkage mapping of large numbers of cloned genes (reviewed by AVNER *et al.* 1988). This system takes advantage of the high degree of DNA sequence divergence between laboratory mouse strains and the distantly related species *Mus spretus*. Although *M. spretus* diverged from *Mus domesticus* and *Mus musculus* several million years ago (BONHOMME *et al.* 1984), *M. spretus* animals will still interbreed with laboratory mouse strains to produce fertile F_1 females and sterile F_1 males. Meiotic crossovers in the F_1 females can be readily analyzed by backcrossing to laboratory mouse strains and monitoring the inheritance of *M. spretus*-specific restriction fragment length polymorphisms (RFLPs) for various cloned DNA probes.

Here we report the use of the *M. spretus* interspecific backcross system to assemble a molecular genetic linkage map of mouse chromosome *9*. Previous studies have identified a large number of interesting mutations that map to chromosome *9*, including genes that affect prenatal and postnatal development, neurolog-

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ical functions and behavior, susceptibility to viral infection, and incidence of diabetes (GREEN 1981; RUSSELL 1971; PROCHAZKA *et al.* 1987). The RFLP linkage map covers most of the genetic length of this chromosome and provides regional localizations for four genes previously mapped to mouse chromosome *9* by somatic cell hybrids. These results suggest new regions of homology between mouse and human chromosomes, and provide a foundation for future molecular studies of many genes and mutations on mouse chromosome *9*.

MATERIALS AND METHODS

Mice: Interspecific backcross progeny were generated by mating (C57BL/6J × *M. spretus*)F_1 females and C57BL/6J males as previously described (BUCHBERG *et al.* 1988). One hundred seventy-six backcross progeny were analyzed in these studies. One hundred sixty of the progeny were typed for all ten probes (see RESULTS).

DNA isolation and hybridization analysis: DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and probe hybridization were performed essentially as described in JENKINS *et al.* (1982). All blots were prepared with Zetabind nylon membrane (AMF-Cuno). Probes were prepared by random-hexanucleotide priming (FEINBERG and VOGELSTEIN 1984). Washing was done to a final stingency of 1 × SSPC, 0.1% SDS, 65°.

Probes: The *cholecystokinin* (*Cck*) probe was a 1.7-kilobase (kb) *Bam*HI/*Eco*RI fragment of a mouse genomic clone (Cck.b5; FRIEDMAN *et al.* 1989). The *dilute* (*d*) probe was a 2.5-kb partial mouse cDNA clone (pGT104-2; P. SEPARAK, N. G. COPELAND and N. A. JENKINS, unpublished data). The *ets oncogene* (*Ets-1*) probe was a 3-kb *Hin*dIII fragment from a mouse genomic clone (mEts100a; R. REEVES, unpublished results). The *G protein alpha inhibitory subunit* (*Gnai-2*) probe was a 1.34-kb full length mouse cDNA clone (pGM1.3; SULLIVAN *et al.* 1986). The *glutathione S-transferase Ya subunit* (*Gsta*) probe was a 0.5 kg-*Bgl*II/*Eco*RI fragment from the 3′ end of a rat liver Ya cDNA clone (DANIEL *et al.* 1983). The *low density lipoprotein receptor* (*Ldlr*) probe was a 1.2-kb *Xba*I fragment from a Chinese hamster genomic cosmid clone (pV79-1; SEGE, KOZARSKY and KRIEGER 1986). The *cytochrome P3-450* (*P450-3*) probe was a *Bam*HI/*Hin*dIII subclone from the 3′-end of a full-length mouse cDNA clone (pP$_3$450FL; KIMURA, GONZALEZ and NEBERT 1984). The *T3 gamma subunit* (*T3g*) probe was a 0.8-kb *Eco*RI/*Hin*dIII fragment of a mouse cDNA clone (pB10.AT3-gamma-1; KRISSANSEN *et al.* 1987b). The *thymus cell antigen-1* (*Thy-1*) probe was a 0.7-kb *Pst*I fragment from a partial mouse cDNA clone isolated by M. DAVIS (pTM-8; see HIRAKI *et al.* 1986). The *transferrin* (*Trf*) probe was a 2.3-kb mouse cDNA clone (pMTF-5; CHEN and BISSELL 1987).

Statistical analysis: Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns ("pedigree analysis," AVNER *et al.* 1988) using the computer program "Spretus Madness" developed by D. DAVE (Data Management Services, Inc., Frederick, MD) and A. M. BUCHBERG (BRI-Basic Research Program, Frederick, MD). All reported gene orders are at least 50-fold more likely than alternative orders as calculated by maximum likelihood analysis (BISHOP 1985). The distribution of nonrecombinant, single-, double-, and triple-recombinant chromosomes was compared to a Poisson distribution, using the chi-square test of goodness of fit.

RESULTS

The ten different genes analyzed in the current study are defined and summarized in Table 1. The *d*, *Thy-1*, and *Trf* loci have been extensively mapped in laboratory mouse strains and are considered anchor loci for existing linkage maps of mouse chromosome *9* (see DAVISSON *et al.* 1988). The *P450-3*, *Gnai-2*, and *Cck* genes have been less extensively mapped, but their approximate chromosomal positions are known (HILDEBRAND *et al.* 1985; BLATT *et al.* 1988; FRIEDMAN *et al.* 1989). The *Ets-1*, *Gsta*, *Ldlr*, and *T3g* genes have been assigned to chromosome *9* only using somatic cell hybrids (WATSON *et al.* 1986; CZOSNEK *et al.* 1984; WANG *et al.* 1988; KRISSANSEN *et al.* 1987b).

C57BL/6J and *M. spretus* DNA samples were digested with several different restriction enzymes and analyzed by Southern blot hybridization. Each of the ten probes recognized RFLPs that distinguished C57BL/6J and *M. spretus* DNA genotypes (Table 1), confirming the high degree of DNA sequence polymorphism between these two species.

The backcross progeny were analyzed for the presence of C57BL/6J or *M. spretus* specific alleles of each gene. As expected, all of the progeny from the (C57BL/6J × *M. spectrus*)F_1 × C57BL/6J mating were either homozygous for C57BL/6J alleles or heterozygous for C57BL/6J and *M. spretus* alleles. We did not observe any novel genotypes or any significant deviations from the expected 50:50 transmission ratio of C57BL/6J and *M. spretus* alleles from the F_1 females to the backcross progeny.

Table 2 summarizes the recombination frequencies observed among the various genes. Gene order was determined by "pedigree analysis" (AVNER *et al.* 1988) and confirmed by maximum likelihood analysis (BISHOP 1985). Figure 1 shows the different chromosome classes observed. The only loci that could not be ordered with respect to one another were the *Thy-1* and *T3g* genes [0 recombinant chromosomes in 173 animals, upper 95% confidence interval, 1.7 centi-Morgans (cM)]. The estimated distances (±SE) between adjacent loci were: *Ldlr*–(12.3 ± 2.6 cM)–*Ets-1*– (9.8 ± 2.3 cM)–[*Thy-1*, *T3g*]–(4.6 ± 1.6 cM)–*P450-3*–(11.6 ± 2.4 cM)–*d*– (1.2 ± 0.8 cM)–*Gsta*–(8.7 ± 2.1 cM)–*Trf*–(3.5 ± 1.4 cM)–*Gnai-2*–(11.6 ± 2.4 cM–*Cck*. These data establish a 64 cM map of mouse chromosome *9* and provide detailed regional assignments for the *Ldlr*, *Ets-1*, *T3g*, and *Gsta* genes (see Figure 2B and discussion below).

Note that all genetic distances have been calculated directly from observed recombination frequencies, rather than from genetic mapping functions which correct for postulated multiple recombination events between adjacent makers. For the small genetic intervals in this map, the two methods would give roughly comparable results. Even for larger intervals, how-

TABLE 1

Loci and restriction fragment length polymorphisms for interspecific backcross mapping

Locus	Gene name	Enzyme	C57BL/6J fragments (kb)	*M. spretus* fragments (kb)
A. Anchor loci on conventional maps				
d	*Dilute* (coat color)	*Eco*RI	8.9, 5.9, 3.6, 2.75, 1.9, 1.45	8.9, 7.9, 5.3, 3.6, 1.9, 1.45
Thy-1	*Thymus cell antigen-1*	*Eco*RI	8.4	6.2
Trf	*Transferrin*	*Eco*RI	9.0, 3.3, 3.2, 2.35, 2.1, 1.83	10.5, 3.3, 3.2, 2.4, 2.1, 1.83
B. Previously mapped genes				
Cck	*Cholecystokinin*	*Taq*I	4.1	2.7
Gnai-2	*G-protein, inhibitory alpha subunit*	*Msp*I	3.1, (1.4, .7, .6, .5)	2.5, (1.4, .7, .6, .5)
P450-3	*Cytochrome P3-450, polycyclic hydrocarbon inducible*	*Eco*RI	10.5, (3.6)	14.4, (3.6)
C. Genes with previously unknown regional map positions				
Ets-1	*E26 avian leukemia virus oncogene, 5′ domain*	*Msp*I	7.2, 6.4	11, 6.4
Gsta	*Glutathione S-transferase, Ya subunit*	*Taq*I	15, 11, 8.8, 7.3, 3.4	8.1, 6.4, 5.5, 4.4, 4.0, 3.4
Ldlr	*Low density lipoprotein receptor*	*Pvu*II	2.2	1.75
T3g	*T3 antigen, gamma subunit*	*Taq*I	2.25, (2.0), 1.85, 1.2, 1.1, .54, .45	5.1, 3.2, 1.7, 1.2, .54, .45

The ten genes mapped in this study are shown, together with the restriction fragment length polymorphisms used for determining backcross distribution patterns. *M. spretus*-specific restriction fragments are underlined. Weakly hybridizing fragments are shown in parentheses.

TABLE 2

Genetic distances between loci measured by interspecific backcross analysis

	Ets-1	*Thy-1*	*T3g*	*P450-3*	*d*	*Gsta*	*Trf*	*Gnai-2*	*Cck*
Ldlr	12.3 ± 2.6 (20/162)	22.9 ± 3.2 (39/170)	22.7 ± 3.2 (39/172)	27.6 ± 3.4 (47/170)	39.4 ± 3.7 (67/170)	40.1 ± 3.7 (69/172)	45.9 ± 3.8 (78/170)	48.0 ± 3.8 (82/171)	50.3 ± 3.8 (86/171)
Ets-1	—	9.8 ± 2.3 (16/163)	9.8 ± 2.3 (16/164)	14.7 ± 2.8 (24/163)	26.4 ± 3.5 (43/163)	27.4 ± 3.5 (45/164)	36.8 ± 3.8 (60/163)	39.6 ± 3.8 (65/164)	41.7 ± 3.9 (68/163)
Thy-1	—	—	0.0–1.7 (0/173)	4.6 ± 1.6 (8/173)	16.2 ± 2.8 (28/173)	17.3 ± 2.9 (30/173)	26.0 ± 3.3 (45/173)	29.7 ± 3.5 (51/172)	37.2 ± 3.7 (64/172)
T3g	—	—	—	4.6 ± 1.6 (8/173)	16.2 ± 2.8 (28/173)	17.1 ± 2.8 (30/175)	26.0 ± 3.3 (45/173)	30.1 ± 3.5 (52/173)	37.4 ± 3.7 (65/174)
P450-3	—	—	—	—	11.6 ± 2.4 (20/173)	12.7 ± 2.5 (22/173)	21.4 ± 3.1 (37/173)	25.0 ± 3.3 (43/172)	34.9 ± 3.6 (60/172)
d	—	—	—	—	—	1.2 ± 0.8 (2/173)	9.8 ± 2.3 (17/173)	13.4 ± 2.6 (23/172)	25.6 ± 3.3 (44/172)
Gsta	—	—	—	—	—	—	8.7 ± 2.1 (15/173)	12.7 ± 2.5 (22/173)	24.7 ± 3.3 (43/174)
Trf	—	—	—	—	—	—	—	3.5 ± 1.4 (6/172)	15.7 ± 2.8 (27/172)
Gnai-2	—	—	—	—	—	—	—	—	11.6 ± 2.4 (20/172)

For each pairwise combination of genes, recombination frequencies were calculated as the number of backcross animals that showed discordant inheritance of C57BL/6J- and *M. spretus*-specific alleles divided by the total number of animals typed for both loci (see numbers in parentheses). The recombination frequencies are reported as genetic distances in cM (±SE) (1 cM = 1% recombination frequency). The distance between the *Thy-1* and *T3g* genes is reported from zero to the upper 95% confidence interval.

ever, simple recombination frequencies may provide fairly reliable estimates of genetic distance in this system. Mapping functions assume that recombination events are distributed randomly among chromosomes according to the Poisson distribution, with no interference between multiple crossover events on a single chromosome (see for example, SUZUKI, GRIFFITHS and LEWONTIN 1981). If this were true for the 104 recombination events shown in Figure 1, we would expect to observe approximately 84, 54, 18 and 4 chromosomes with 0, 1, 2 and 3 recombination events, respectively. The observed distribution of 68, 80, 12 and 0 chromosomes with 0, 1, 2 and 3 recombination events differs significantly from the predicted distribution, with single-recombinant chromosomes more common than expected, and multiple-recombinant chromosomes less common ($\chi^2 = 21$, $P < 0.001$). Similar results have been seen in interspecific backcross maps of several other mouse chromosomes (SIRACUSA *et al.* 1989; J. D. CECI, L. D. SIRACUSA, N. A. JENKINS and N. G. COPELAND, unpublished data). The scarcity of chromosomes with multiple recombination events simplifies the determination of gene order and genetic distances by backcross mapping.

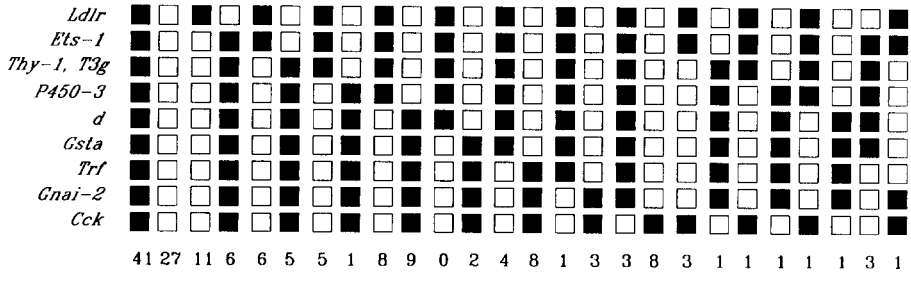

FIGURE 1.—Summary of the results of interspecific backcross mapping. The ten genes mapped in these studies are shown at the left. Each column represents a particular type of chromosome transmitted from the (C57BL/6J × *M. spretus*)F_1 parents to the backcross offspring. The black boxes represent the presence of a C57BL/6J allele and the white boxes represent the presence of a *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom. Only the 160 chromosomes that were typed for all ten markers are shown.

DISCUSSION

The current studies provide another example of the utility of chromosome mapping using interspecific backcross analysis. Every probe used in these studies detected RFLPs that distinguished the C57BL/6J and *M. spretus* genotypes. Each gene could be rapidly mapped with respect to the others by determining its segregation pattern on a common set of backcross animals.

An important issue is whether the genetic map of chromosome *9* determined from these experiments is comparable to the maps generated from crosses among standard laboratory strains of mice. Figure 2 shows a comparison of the two types of maps. Six of the ten loci used in the current experiments have also been mapped in laboratory mouse strains. The *Thy-1*, *d*, and *Trf* loci have been extensively mapped in conventional crosses and are considered anchor loci for the standard map. The *P450-3*, *Gnai-2* and *Cck* genes have not been mapped as extensively as the anchor loci, but their approximate positions are known. All six genes occupy comparable positions on the interspecific backcross map and the conventional genetic map (compare Figure 2, A and B). Thus, we see no evidence for any rearrangements, inversions, or distance distortions in the interspecific backcross map of chromosome *9*.

Crosses between *M. spretus* and laboratory mouse strains are now being used to develop maps of many mouse chromosomes. To date, the only evidence for significant rearrangements between the genomes of *M. spretus* and conventional laboratory mice involves a region of the proximal portion of chromosome *17* (HAMMER, SCHIMENTI and SILVER 1989). In addition, one group has reported a region of possible recombination enhancement on chromosome *4* in a *M. spretus* cross (NADEAU *et al.* 1986), and several workers have noted preferential transmission of *M. musculus* or *M. spretus* genes from F_1 females to backcross progeny (BIDDLE 1987; SIRACUSA *et al.* 1989). Although these results indicate the need for caution in interpreting the results of interspecific backcross mapping, the chromosome *9* data provide an example of full compatibility between genetic maps derived from conventional and interspecific crosses.

The current studies have allowed us to assign regional map locations for four genes previously assigned to mouse chromosome *9* using somatic cell hybrids. The *T3g* gene encodes the gamma subunit of the T3 complex, a cell-surface molecule associated with T cell receptors. Genomic cloning experiments suggest that this gene is found within a few kilobases of the gene for another subunit of the T3 complex, the *T3 delta* (*T3d*) gene (SAITO *et al.* 1987). On the interspecific backcross map, the *T3g* gene maps very near the *Thy-1* locus, which also encodes a cell-surface molecule expressed on T cells. The *T3d* gene, although not typed directly, must also map to this position. The close linkage of the *Thy-1* gene with the genes for the *T3g/T3d* loci extends a region of suspected chromosome homology between mouse chromosome *9* and human chromosome *11* (see Figure 2). All three of these genes have previously been mapped to the long arm of human chromosome *11* (KRISSANSEN *et al.* 1987a; VAN DEN ELSEN *et al.* 1985; VAN RIJIS *et al.* 1985).

The region of homology between mouse chromosome *9* and human chromosome *11* has also been extended by the regional localization of the *Ets-1* gene. The *Ets-1* gene is one of two mouse homologs of a gene first identified in the avian E26 virus, a replication-defective retrovirus that causes myeloblastosis and erythroblastosis and that also contains a portion of the *Myb* gene. Previous studies have shown that the human analog of the *Ets-1* gene is located on human chromosome *11* at band *11q23-q24* (DE TAISNE *et al.* 1984). (A second homolog of the *Ets* gene maps on mouse chromosome *16* and human chromosome *21*; WATSON *et al.* 1986). The current data suggest that the mouse *Ets-1* gene is located on the proximal third of mouse chromosome *9*, approximately 10 cM from the *Thy-1* locus. Prior to these studies, the most proximal gene on chromosome *9* whose human analog was known to map to chromo-

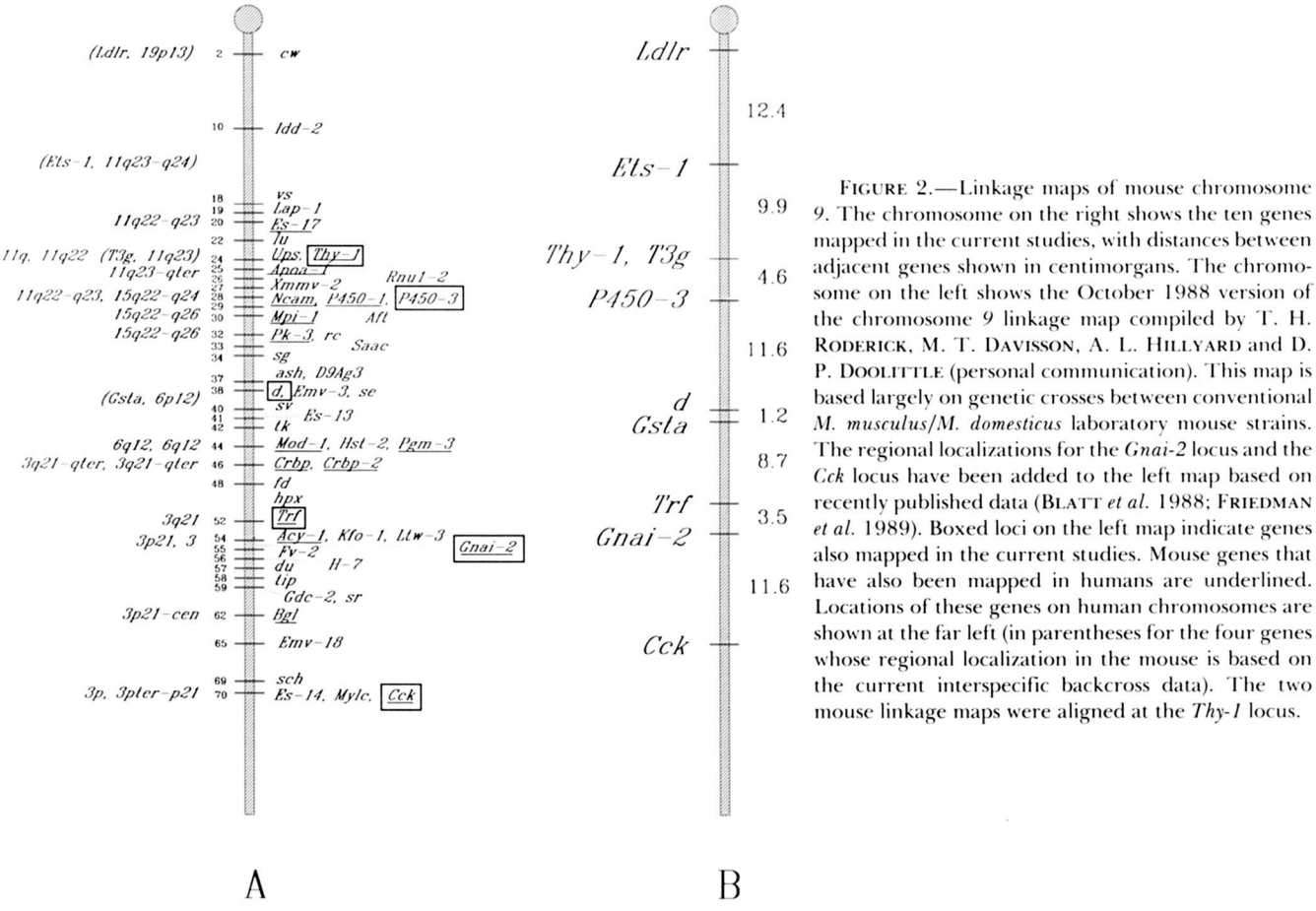

FIGURE 2.—Linkage maps of mouse chromosome 9. The chromosome on the right shows the ten genes mapped in the current studies, with distances between adjacent genes shown in centimorgans. The chromosome on the left shows the October 1988 version of the chromosome 9 linkage map compiled by T. H. RODERICK, M. T. DAVISSON, A. L. HILLYARD and D. P. DOOLITTLE (personal communication). This map is based largely on genetic crosses between conventional *M. musculus/M. domesticus* laboratory mouse strains. The regional localizations for the *Gnai-2* locus and the *Cck* locus have been added to the left map based on recently published data (BLATT *et al.* 1988; FRIEDMAN *et al.* 1989). Boxed loci on the left map indicate genes also mapped in the current studies. Mouse genes that have also been mapped in humans are underlined. Locations of these genes on human chromosomes are shown at the far left (in parentheses for the four genes whose regional localization in the mouse is based on the current interspecific backcross data). The two mouse linkage maps were aligned at the *Thy-1* locus.

some *11* was the *Es-17* gene (approximately 4 cM proximal to *Thy-1* on mouse chromosome *9*; see Figure 2).

The new *Ets-1* mapping data extend the total known region of mouse chromosome *9*/human chromosome *11* homology to at least 14 cM (the estimated distance from *Ets-1* to *Ncam*). The expansion of this homology segment substantially increases the probability that mouse chromosome *9* contains the homolog of a gene that causes ataxia telangiectasia in humans. Ataxia telangiectasia is a syndrome of unknown cause that is associated with cerebellar ataxia, dramatic increases in susceptibility to cancer and ionizing radiation, and other chromosomal, immunological, and endocrine defects (see GATTI and SWIFT 1985). The gene for one form of this syndrome was recently shown to map near the *THY1* locus on human chromosome *11* (GATTI *et al.* 1988). Based on the current mouse data, both the *T3g* and the *Ets-1* genes represent additional probes that should be tested for linkage to the ataxia telangiectasia gene in humans.

An important new region of mouse/human chromosome homology is also provided by the regional mapping of the *LDL receptor* gene. In humans, the *LDLR* gene is located on chromosome *19* (FRANCKE, BROWN and GOLDSTEIN 1984), a chromosome that contains no other genes previously located on mouse chromosome *9* (LALLEY *et al.* 1987). The current data show that the mouse *Ldlr* gene maps to the extreme proximal end of mouse chromosome *9*. As more genes are mapped, it seems likely that other human chromosome *19* genes will be found that map to this region.

In both humans and rabbits, mutations in the *LDL receptor* gene disrupt clearance of low density lipoprotein from the plasma and lead to elevated levels of serum cholesterol and premature atherosclerosis (BROWN and GOLDSTEIN 1986). This defect has been shown to be the underlying cause of familial hypercholesterolemia, one of the most common genetic diseases in humans. In mice, the *Ldlr* gene is located in a region of chromosome *9* that is devoid of any mutations with known effects on lipoprotein metabolism or atherosclerosis. In fact, the only mutation previously located within this region is one that causes curly whiskers. The LDL receptor should provide an important new molecular marker for further linkage studies of this region of chromosome *9*.

It is interesting to note that a diabetes-susceptibility gene maps between the newly localized LDL receptor gene and the *Ets-1* oncogene on chromosome *9* (Figure 2). The *Idd-2* locus is one of three recessive genes

that increase susceptibility to insulin-dependent diabetes in the nonobese diabetic (NOD) mouse (PROCHAZKA *et al.* 1987). In humans, susceptibility to diabetes is also thought to be influenced by genetic factors, but susceptibility genes have not been precisely localized. The new locations of the *Ldlr* and *Ets-1* genes suggest that an analog of the mouse *Idd-2* gene may be located on human chromosome *19* or *11*.

The final gene mapped in these studies is the mouse analog of the Ya subunit of rat glutathione-*S*-transferase. The *Gsta* gene had previously been assigned to mouse chromosome *9* using somatic cell hybrids (CZOSNEK *et al.* 1984). In those studies, a rat Ya cDNA probe detected several different restriction fragments, all of which cosegregated with mouse chromosome *9* markers. The large number of hybridizing bands suggested that there were several different *Gsta* genes on chromosome *9*, some of which appeared to be clustered. The Ya probe also detected five different polymorphic bands that distinguished *M. spretus* and C57BL/6J DNA (Table 1). No recombinants among the different RFLPs were detected in the backcross progeny, suggesting that all *Gsta* related sequences mapped to the same region of chromosome *9*.

Our data suggest that the *Gsta* locus maps substantially distal to the region of known homology between mouse chromosome *9* and human chromosome *11*. These result were somewhat surprising, since the mouse *Gsta* gene is frequently listed as the homolog of the *GST3* gene on human chromosome *11* (see, for example, LALLEY *et al.* 1987). Recently, a human *GST2* cDNA clone has been isolated that is highly homologous to the rat Ya probe and that maps to the proximal short arm of human chromosome *6* (BOARD and WEBB 1987). We suggest that this *GST2* gene, not the *GST3* gene on human chromosome *11*, is the human homolog of the mouse *Gsta* gene on chromosome *9*. (Both the human *GST2* gene and the mouse *Gsta* gene belong to the alpha class of glutathione-*S*-transferase; the human *GST3* gene appears to belong to the distinct pi class. See SUZUKI and BOARD 1984; MANNERVIK *et al.* 1985; SUZUKI *et al.* 1987). Note that the mouse *Gsta* gene maps in a region adjacent to two other mouse chromosome *9* genes whose human analogs are on chromosome *6* (*Mod-1* and *Pgm-3*). If the current interpretation of homology relationships is correct, the region of synteny between mouse chromosome *9* and human chromosome *6* extends approximately 5 cM further proximal than previously suspected.

The *Gsta* locus appears to be closely linked to the *dilute* locus, with only two recombinations in 173 animals. This result suggests that the *Gsta* locus may fall within the *dilute/short ear* complex, one of the most extensively characterized genetic regions in the mouse genome (reviewed in RINCHIK *et al.* 1985). Both the *dilute* and the *short ear* genes were included in specific locus mutagenesis experiments designed to measure the frequency of generating new alleles of existing mutations after irradiation of the germline. These experiments led to isolation of hundreds of different radiation-induced *dilute* and *short ear* mutations, many of which were found to be deficiency mutations that included surrounding genetic factors. Extensive complementation mapping between these various mutations has identified more than a dozen different functional units in a 4 to 6 cM interval surrounding *dilute* and *short ear* (RUSSELL 1971; RUSSELL and MONTGOMERY 1987). These units include the original *dilute* and *short ear* genes, the *ash* coat-color locus, a number of genes required for prenatal or neonatal development, and a gene essential for normal hearing and locomotor control [the *Snell's waltzer* (*sv*) locus].

The new map location of the *Gsta* locus suggests that the *Gsta* gene may correspond to one of the functional units previously identified by complementation mapping of the *dilute/short ear* complex. It should be possible to test this hypothesis by examining the presence or absence of *Gsta* sequences in different deficiency chromosomes. Preliminary results suggest that *Gsta* sequences are deleted in at least some of the *dilute/short ear* mutations (our unpublished data). This provides another example of the predictive power of interspecific backcross mapping and suggests a new starting point for additional molecular studies of the genes within the *dilute/short ear* complex.

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