Identification and Applications of Repetitive Probes for Gene Mapping in the Mouse

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ABSTRACT

Interspecific mouse hybrids that are viable and fertile provide a wealth of genetic variation that is useful for gene mapping. We are using this genetic variation to develop multilocus linkage maps of the mouse genome. As an outgrowth of this work, we have identified three repetitive probes that collectively identify 28 loci dispersed on 16 of the 19 mouse autosomes and the *X* chromosome. These loci establish a skeleton linkage map that can be used to detect linkage over much of the mouse genome. The molecular probes are derived from the mouse mammary tumor virus envelope gene, the ornithine decarboxylase gene, and the triose phosphate isomerase gene. The ability to scan the mouse genome quickly and efficiently in an interspecific cross using these three repetitive probes makes this system a powerful tool for identifying the chromosomal location of mutations that have yet to be cloned, mapping multigenic traits, and identifying recessive protooncogene loci associated with murine neoplastic disease. Ultimately, interspecific hybrids in conjunction with repetitive and single-copy probes will provide a rapid means to access virtually any gene of interest in the mouse genome at the molecular level.

INTERSPECIFIC backcrosses (IBs) are a powerful tool for multilocus mapping of the mouse genome (reviewed by GUENET 1986; AVNER *et al.* 1988). The crosses involve two species whose evolutionary distance has allowed for accumulation of DNA sequence differences. DNA sequence differences, which result in restriction fragment length polymorphisms (RFLPs) between the parents of an IB, can be used to establish the map location of molecular markers in an IB. The greater the evolutionary distance between the parents of an IB, the greater the probability and ease of finding an informative RFLP with any molecular probe.

The IB we used for mapping involved crosses of C57BL/6J females to *Mus spretus* males, followed by backcrosses of the resulting F_1 hybrid females to C57BL/6J males. Each N_2 mouse was typed for anchor loci located on each autosome and the *X* chromosome to establish the foundation of our IB linkage map. Anchor loci had previously been assigned to specific chromosomal locations after numerous independent genetic crosses between laboratory strains and stocks (summarized by DAVISSON, RODERICK and DOOLITTLE 1989; DAVISSON and RODERICK 1989). Subsequently, loci whose positions were unknown or were known solely by somatic cell hybrid analysis were positioned on the IB linkage map by comparing their segregation patterns with those of anchor loci typed in the IB.

More than 500 loci have been mapped to date in this single IB (N. G. COPELAND and N. A. JENKINS, manuscript in preparation); the average distance between molecular loci in this IB linkage map is approaching 3 centiMorgans (cM). Currently, we estimate that an additional 5000 loci can be mapped in this IB before DNA stocks are depleted. As more loci are added to the map, the average map resolution will approach 0.3 cM. In molecular terms, there will be one molecular marker on average every 300–600 kilobases (kb). This map resolution will provide molecular access to virtually any mutation of interest by chromosome walking, provided that the mutation of interest can be positioned with respect to existing molecular markers in an IB.

Our IB map was initially established using mostly single-copy probes. However, now that the IB map is established, it can be used to determine the map locations of repetitive probes as well. Identification of repetitive probes that detect several dispersed loci per Southern blot can serve as valuable tools for rapidly establishing new skeleton linkage maps of the mouse genome. A skeleton linkage map is a map that contains the minimum number of loci necessary to scan the entire mouse genome for linkage. Skeleton linkage maps can be used for several purposes, including identification of the chromosomal location of mutations that have yet to be cloned, mapping multigenic traits and potentially identifying recessive protooncogenes involved in murine neoplastic disease.

The method described here simplifies the establishment of skeleton linkage maps by identifying molec-

ular probes that detect several dispersed loci per Southern blot. Our choice of probes has focused on those probes that detect low-copy repeated sequences in mouse DNA, since probes that detect highly repeated sequences often produce complicated Southern blot hybridization patterns, making it difficult to distinguish individual bands. We have also tried to identify probes that produce autoradiographic bands that are roughly equivalent in intensity, allowing for ease and accuracy of detection. Finally, we have focused on characterizing the *M. spretus*-specific RFLPs associated with each locus, since the method is based on mating *M. spretus* mice to various laboratory strains in subsequent crosses designed to scan the genome for linkage.

Several probes were tested to determine if they fit the criteria described above. Probes for the mouse mammary tumor virus (MMTV) envelope gene, the ornithine decarboxylase gene, and the triose phosphate isomerase gene were chosen for further study; these probes were initially tested because of the reasons outlined below. First, numerous MMTV proviral integration sites had been identified in inbred strains (summarized by KOZAK *et al.* 1987), making a probe for the *MMTV envelope* (*Mtv*) gene a likely candidate for these analyses. Second, several sequences related to the *ornithine decarboxylase* (*Odc*) gene had previously been mapped in the recombinant inbred (RI) strains (RICHARDS-SMITH and ELLIOTT 1984; TAYLOR 1989), providing another candidate probe for these analyses. Third, several triose phosphate isomerase (*Tpi*) related sequences had previously been identified in the human genome (MAQUAT, CHILCOTE and RYAN 1985), making a probe for *Tpi* a likely candidate to identify a multigene family in the mouse genome as well. A total of 28 loci could be identified and mapped using the Mtv, Odc and Tpi probes. With only 100 IB N_2 progeny, it is estimated that these three probes can detect linkage over 70% of the mouse genome. The rationale for establishing a skeleton linkage map of the mouse genome based on repetitive sequences coupled with interspecific crosses and the applications of this system for gene mapping in the mouse are described.

MATERIALS AND METHODS

Mice: The C57BL/6J inbred strain is maintained at the NCI-FCRDC. The F_7, F_9, F_{10} or F_{12} generation *M. spretus* mice used for the IB were a gift from E. M. EICHER (The Jackson Laboratory, Bar Harbor, Maine). The [(C57BL/6J × *M. spretus*)F_1 × C57BL/6J] IB was performed at the NCI-FCRDC. Various subsets out of 205 N_2 progeny were used for mapping.

Probes: The MMTV envelope probe (8–21, hereafter called the Mtv probe) is a 1.7-kb envelope *Pst*I fragment cloned in pBR322 (MAJORS and VARMUS 1981); 8-21 was a gift from J. E. MAJORS (Washington University School of Medicine, St. Louis, Missouri). The ornithine decarboxylase probe (pCR6, hereafter called the Odc probe) is a 219-base pair (bp) *Pst*I fragment from the pODC934 mouse cDNA clone, subcloned in pT_3T_7-18 (RHEAUME *et al.* 1989); pCR6 was a gift from F. G. BERGER (University of South Carolina, Columbia, South Carolina). The triose phosphate isomerase probe (pHTPI-5A, hereafter called the Tpi probe) is a 1194-bp human TPI cDNA cloned in pKT218 (MAQUAT, CHILCOTE and RYAN 1985; BROWN *et al.* 1985); pHTPI-5A was a gift from L. E. MAQUAT (Roswell Park Cancer Institute, Buffalo, New York).

The map locations in the IB of several loci used to position the *Mtv*, *Odc*, and *Tpi* loci have been reported elsewhere. The loci and their corresponding probes that were mapped in our IB and previously described are: chromosome *1–En-1, Ren-2* (COPELAND *et al.* 1990); chromosome *2–Spna-2, Abl, C5, Pax-1* (SIRACUSA *et al.* 1990); chromosome *3–Ngfb, Amy-2* (BUCHBERG, JENKINS and COPELAND 1989); chromosome *4–Mup-1, Ifa* (CECI *et al.* 1989); chromosome *5–Hox-7, Kit* (HILL *et al.* 1989), *Alb-1* (MOCK *et al.* 1989); chromosome *6–Tcrb* (REGNIER *et al.* 1989), *Kras-2* (DELAPEYRIERE *et al.* 1990); chromosome *7–c, Zp2* (LUNSFORD *et al.* 1990); chromosome *9–Ldlr, Thy-1* (KINGSLEY, JENKINS and COPELAND 1989); chromosome *10–Esr, Myb, Ros-1* (JUSTICE *et al.* 1990b); chromosome *13–Tcrg, Fim-1* (JUSTICE *et al.* 1990a); chromosome *14–Hap, Plau, Tcra* (CECI *et al.* 1990). The *agouti* (*a*) locus on chromosome 2 was typed by observation of coat color (SIRACUSA *et al.* 1989, 1990).

The map locations in the IB of several additional loci used to position the *Mtv*, *Odc* and *Tpi* loci have not yet been reported. The loci and their corresponding probes that were mapped in our IB and are reported in this paper are: chromosome *6–Met*, the *Met* protooncogene probe (pUC5FL) is a 1463-bp *Bam*HI-*Sal*I mouse cDNA fragment cloned in pUC18 (IYER *et al.* 1990); pUC5FL was a gift from T. E. KMIECIK and G. F. VANDE WOUDE (NCI-FCRDC, ABL-BRP, Frederick, Maryland); chromosome *7–Gpi-1*, the glucose phosphate isomerase probe (P. FAIK and M. J. MORGAN, unpublished observation) was a gift from P. FAIK and M. J. MORGAN (The Wellcome Trust, London, England); chromosome *12–Pomc-1*, the proopiomelanocortin probe (pACYC621) is a 0.5-kb human *Ava*I/*Sal*I genomic fragment cloned in pACYC184 (A. C. Y. CHANG, personal communication); pACYC621 was a gift from A. C. Y. CHANG and S. N. COHEN (Stanford University School of Medicine, Stanford, California); *D12Nyu2* and *D12Nyu5*, the *D12Nyu2* and *D12Nyu5* probes (M13019–25 and M13030–3, respectively) are a 1.37-kb and an 850-bp *Eco*RI mouse genomic fragment, respectively (D'EUSTACHIO 1984) cloned in pUC13; M13019-25 and M13030-3 were gifts from P. D'EUSTACHIO (New York University Medical Center, New York, New York); chromosome *15–Myc*, the *myc* protooncogene probe (p104E.5) is a 0.5-kb mouse *Bam*HI-*Bgl*II fragment from exon 1 cloned in pUC8 (MUCENSKI *et al.* 1987), *Tgn*, the thyroglobulin probe (pRT57) is a 4.5-kb rat cDNA cloned in pBR322 (DI LAURO *et al.* 1985); pRT57 was a gift from R. DI LAURO (European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany); chromosome *16–Smst*, the somatostatin probe (pMST-1.4) is a 1.4-kb rat genomic fragment cloned in pBR322 (O'HARA *et al.* 1988), *Ets-2*, the *Ets-2* protooncogene probe (PH1.03) is a 1.03-kb mouse genomic fragment cloned in pGEM1 (REEVES *et al.* 1987); pMST-1.4 and PH1.03 were gifts from R. H. REEVES (Johns Hopkins University School of Medicine, Baltimore, Maryland); chromosome *17–Pim-1*, the preferred integration site-1 probe (probe A) is a 930-bp mouse genomic fragment cloned in pBR322 (CUYPERS *et al.* 1984); probe A was a gift from A. BERNS (Netherlands Cancer Institute, Amsterdam, The Netherlands), *H-2*, the histocompatibility-

TABLE 1

Listing of loci used to position the *Mtv*, *Odc* and *Tpi* loci in the IB

Chromosome	Locus[a]	Restriction endonuclease	C57BL/6J fragment sizes (kb)	*Mus spretus* fragment sizes (kb)[b]
6	*Met*	*Hin*dIII	6.0, 2.5, 1.0	5.5, 2.5, 0.8
7	*Gpi-1*	*Bgl*I	~12.0, 4.6	~19.0
12	*Pomc-1*	*Hin*dIII	9.4, 5.1	4.4[c], 1.9
12	*D12Nyu2*	*Taq*I	3.6, 3.4, 2.1	3.4, 3.1, 1.0
12	*D12Nyu5*	*Pst*I	~12.0, 3.2	~15.0, 4.2
15	*Myc*	*Taq*I	2.4	3.3
15	*Tgn*	*Bam*HI	~16.0, 9.3, 8.1, 6.5, 5.8, 4.0, 3.6, 3.4, 2.0	~14.0, 8.1, 6.5, 5.8, 3.6, 3.4, 2.9[d], 2.1[d]
16	*Smst*	*Bgl*I	~18.0	6.2
16	*Ets-2*	*Bam*HI	9.0	3.2
17	*Pim-1*	*Taq*I	3.0	2.6
17	*H-2*	*Msp*I	3.3, 2.8	4.2, 2.2
18	*Fim-2*	*Msp*I	3.4	3.8
18	*Mbp*	*Bgl*I	~19.0, ~13.0	~13.0, 3.7
X	*DXPas3*	*Taq*I	6.1, 5.0	8.0, 6.1
X	*Hprt*	*Bam*HI	10.1	6.6

[a] The mapping of these loci represent the collective efforts of D. A. SWING, C. M. SILAN, L. F. LOCK, D. J. GILBERT, M. B. CYBULSKI, B. C. CHO and A. M. BUCHBERG in the Mammalian Genetics Laboratory. References describing additional loci in the IB used to position the *Mtv*, *Odc* and *Tpi* loci that were previously published are listed in MATERIALS AND METHODS.

[b] The underlined restriction fragments identify the segregating *M. spretus* alleles followed in the IB progeny.

[c] This RFLP identifies a locus on chromosome *19* (N. A. JENKINS and N. G. COPELAND, unpublished observations).

[d] This fragment, when visible, segregated with the ~14.0-kb fragment.

2 or *IAα* probe (AαD#105) is a 950-bp mouse cDNA cloned in pBR322 (ROGERS *et al.* 1985); AαD#105 was a gift from D. S. SINGER (Immunology Branch of the National Cancer Institute, Bethesda, Maryland); chromosome *18–Fim-2*, the *Fim-2* or *c-Fms* or *Csf-1r* probe (341-pp1) is a 1.0-kb *Pvu*II mouse genomic fragment cloned in pBR322 (SOLA *et al.* 1986); 341-pp1 was a gift from J. N. IHLE (St. Jude Children's Research Hospital, Memphis, Tennessee), *Mbp*, the myelin basic protein probe (pex1) is a 3.3-kb *Hin*dIII mouse genomic fragment that contains exon 1 cloned in pUC13 (TAKAHASHI *et al.* 1985; ROACH *et al.* 1985); pex1 was a gift from H. C. MORSE III (National Institute of Allergy and Infectious Diseases, Bethesda, Maryland); the *X* chromosome–*DXpas3*, the *DXPas3* probe (66) is a random mouse genomic 3.1-kb *Eco*RI fragment cloned in pUC9 (AMAR *et al.* 1985); 66 was a gift from P. R. AVNER (Institut Pasteur, Paris, France); *Hprt*, the *Hprt* probe (pHPTλ13-in1) contains a 1.8-kb *Eco*RI/*Hin*dIII fragment from the mouse *Hprt* genomic clone 13 subcloned in sp65 (LOCK *et al.* 1986). Table 1 lists the RFLPs identifying these loci in the IB.

Southern blot analyses: Genomic DNA extractions, conditions for restriction endonuclease digestions, Southern blot analyses and autoradiography were as described (SIRACUSA *et al.* 1989) with the exceptions listed below. Hybridizations were usually performed at 62–64°; washes were usually in 0.4–0.5 × SSCP, 0.1% SDS. Agarose gels had an average thickness of 0.5 cm. The voltage (V) and running time for gel electrophoresis of the *Bam*HI digestions was 60 V for 3 days, maximizing separation of fragments in the 5–23-kb range with final gel dimensions of 20 × 17 cm. The voltage and running time for gel electrophoresis of the *Hin*dIII digestions was 80 V overnight, maximizing separation of fragments in the 0.5–23-kb range with final gel dimensions of 20 × 22 cm. The voltage and running time for gel electrophoresis of the *Kpn*I digestions was 40 V overnight, maximizing separation of fragments in the 0.5–10-kb range with final gel dimensions of 20 × 10 cm.

Statistical analyses: The map location and recombination frequencies (GREEN 1981) for the IB progeny were determined and calculated using the computer program SPRETUS MADNESS developed by D. DAVE (Data Management Services, Inc., Frederick, Maryland) and A. M. BUCHBERG (NCI-FCRDC, ABL-BRP, Frederick, Maryland).

RESULTS

Mapping methodology for establishing an IB linkage map: Segregation patterns for molecular loci are established by detecting RFLPs between the parents of the IB, C57BL/6J and *M. spretus*, with each of the probes listed (see MATERIALS AND METHODS) in Southern blot analyses. The simplest phenotype to follow in analyzing genomic DNA from the N_2 progeny is an RFLP that is *M. spretus*-specific, since C57BL/6J-specific autosomal RFLPs can be scored only by differences in hybridization intensity. We scored for the presence (one copy) or absence of *M. spretus*-specific fragments in each N_2 animal. The results produce a segregation pattern for each locus that, when compared to the segregation patterns of anchor loci already typed in the panel, identify the chromosomal location of previously unmapped loci.

The Mtv, Odc and Tpi probes identify low-copy repetitive sequences in the mouse genome: Figure 1 shows the results obtained when the Mtv, Odc, and Tpi probes were hybridized to Southern blots that contained genomic DNA from C57BL/6J and *M. spretus* mice digested with *Bam*HI, *Bgl*I, *Eco*RI, *Hin*dIII, *Msp*I, *Kpn*I, *Pst*I, *Taq*I or *Xba*I. A striking feature of the results shown in Figure 1 is that, with certain restriction endonucleases, the resulting autoradiographic intensity of most of the fragments detected with each probe is roughly equivalent, facilitat-

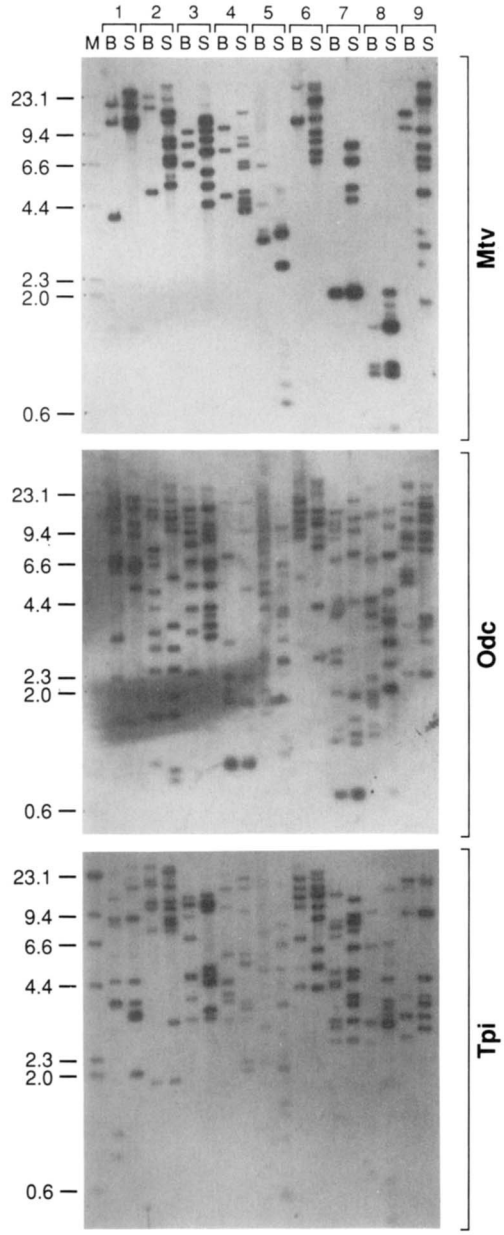

FIGURE 1.—RFLP screen of *M. spretus* and C57BL/6J genomic DNAs. Five micrograms of genomic DNA from *M. spretus* (S) and C57BL/6J (B) mice were digested with restriction endonucleases (1 = *Bam*HI, 2 = *Bgl*I, 3 = *Eco*RI, 4 = *Hin*dIII, 5 = *Msp*I, 6 = *Kpn*I, 7 = *Pst*I, 8 = *Taq*I, 9 = *Xba*I) and analyzed by Southern blot analyses using the Mtv, Odc or Tpi probe (listed on the side of each panel). *Hin*dIII digested lambda DNA (M) was included as a molecular weight marker; fragment sizes are listed in kb. The *Msp*I digestion was not complete.

ing the detection of *Mtv*-, *Odc*- and *Tpi*-related sequences in the mouse genome. Several restriction endonucleases provided clear differences in hybridization pattern between the parents of the IB and also detected many fragments in the *M. spretus* genome that were not present in the C57BL/6J genome. We tried to select those restriction endonucleases that gave the largest number of *M. spretus*-specific fragments that were equivalent in intensity; in these cases, each locus would most likely be identified by only one fragment. Southern blots of restriction endonuclease digested genomic DNA from N_2 progeny were then prepared, and each set of blots was hybridized individually with the Mtv, Odc and Tpi probes. Each probe detected several fragments that segregated independently on each Southern blot.

MMTV integration sites: The Mtv probe is derived from sequences in the envelope gene of MMTV and can be used to identify MMTV integration sites in the mouse genome. Only MMTV proviral sequences present in the *M. spretus* genome were scored in these analyses. The Mtv probe detected 4, 11 and 8 *M. spretus*-specific fragments, when *Bam*HI, *Hin*dIII and *Kpn*I, respectively, were used to cleave genomic DNA from N_2 progeny (Table 2). Comparison of the segregation patterns of the various fragments showed that the Mtv probe detected a total of ten *M. spretus*-specific *Mtv* loci. Analysis of the segregation patterns and comparisons with marker loci already typed in the panel, established that the ten MMTV integration sites were dispersed on eight autosomes (Table 3). Prior to this study, 28 *Mtv* loci had been identified in the mouse genome (summarized by KOZAK *et al.* 1987; D. P. DOOLITTLE, personal communication). The *Mtv* loci identified in our study were numbered in sequential order starting with *Mtv-29*, the locus residing on chromosome *1*.

Three of the ten *Mtv* loci were not present in all of the mice in our *M. spretus* colony at the time the IB was initiated, as determined by comparing Southern blot autoradiographic patterns from several *M. spretus* mice (data not shown). These findings are not unexpected, since the *M. spretus* parents of the IB were not fully inbred when the IB was established (see MATERIALS AND METHODS). The map positions of the MMTV integration sites that were not fixed in the *M. spretus* colony were determined by including the data only from N_2 progeny of F_1 females that transmitted the *M. spretus*-specific provirus to their offspring (see legend to Table 3).

The ornithine decarboxylase gene family: The Odc probe detects the structural gene(s) for ornithine decarboxylase as well as other related sequences in the mouse genome. The nature of these *Odc*-related sequences is unknown and could represent *Odc*-related genes or pseudogenes. The Odc probe used in these studies detected 7, 3 and 3 segregating fragments when *Bam*HI, *Hin*dIII and *Kpn*I, respectively, were used to cleave genomic DNA from N_2 progeny (Table 2). Comparison of the segregation patterns of each fragment showed that the Odc probe detected a total of eight loci. Analysis of the segregation patterns and comparisons with marker loci already typed in the panel, established that the eight *Odc* loci were dispersed on eight autosomes. The *Odc* locus that

TABLE 2

Loci abbreviations and corresponding RFLPs used for mapping *M. spretus* alleles in the IB

Locus	Chromosome	Restriction fragment sizes (kb)		
		*Bam*HI	*Hin*dIII	*Kpn*I
Mtv-29	*1*	10.5	6.3, 4.6	—[a]
Mtv-30	*2*	—	—	1.6[b]
Mtv-31	*5*	12.5[c]	5.1	5.2
Mtv-32	*5*	—	4.1[c]	7.0
Mtv-33	*6*	—	4.9, 4.3	8.9
Mtv-34	*6*	11.0	~13.0	~18.0[c]
Mtv-35	*7*	—	8.4	6.4
Mtv-36	*15*	12.0[c]	7.6	~16.5[c]
Mtv-37	*16*	—	4.0[c]	—
Mtv-38	*18*	—	6.0	7.9
Odc	*12*	~18.0[d]	—	—
Odc-1	*1*	6.4	—	4.3
Odc-2	*2*	11.0	2.5	—
Odc-4	*4*	9.0	9.3	2.7
Odc-5	*6*	~21.0[d]	—	—
Odc-7	*7*	4.9	5.0	—
Odc-14	*9*	—	—	7.7
Odc-15	*17*	~19.5[d]	—	—
Odc-13	*X*	—[e]	—[e]	—[e]
Tpi-1	*6*	~20.5	5.4[g]	5.2
Tpi-2	*3*	—	3.5	~12.5[h]
Tpi-3	*7*	—	~20.0	8.6
Tpi-4	*9*	~15.5	9.7	—
Tpi-5	*10*	—	—	6.0
Tpi-6	*10*	>27.5[f]	2.0	—
Tpi-7	*10*	—	2.2	4.2
Tpi-8	*13*	9.1	—	—
Tpi-9	*14*	—	3.8	—

[a] A "—" indicates that a restriction fragment identifying the locus could not be distinguished in the backcross progeny; this finding was usually the result of similar-sized fragments being present in the C57BL/6J and *M. spretus* genomes or multiple fragments of similar size being present in the *M. spretus* genome that could not be distinguished from each other.

[b] This fragment always appeared much lighter in intensity than the other fragments identified by the Mtv probe and required a longer autoradiographic exposure to be typed.

[c] These pairs of fragments appeared as doublets.

[d] These three fragments ran close together; the ~21.0-kb fragment was the lightest in intensity of all the *Odc* fragments.

[e] This locus was identified by a 5.6-kb *Bgl*I fragment in the *M. spretus* genome and by a 4.2-kb *Bgl*I fragment in the C57BL/6J genome.

[f] This fragment appeared lighter in intensity than other *Bam*HI fragments.

[g] A fragment of >27.5 kb was occasionally detected that segregated with the 5.4-kb fragment.

[h] This fragment was not easy to detect on 20 × 10 cm gels (see MATERIALS AND METHODS), but when visible it gave a segregation pattern identical with the segregation pattern of the 3.5-kb *Hin*dIII band. Electrophoresis of the *Kpn*I digests to a gel size equal to that for *Hin*dIII (20 × 22 cm) would most likely enhance the separation of this ~12.5-kb *Tpi* fragment and also help distinguish the ~18.0- and ~16.5-kb *Mtv* fragments (see footnote *c* above).

mapped to mouse chromosome *12* in the IB most likely represents a structural gene previously mapped to mouse chromosome *12* (COX *et al.* 1988; BERGER 1989; VILLANI, COFFINO and D'EUSTACHIO 1989). Several *Odc*-related sequences had previously been mapped in the RI strains (RICHARDS-SMITH and ELLIOTT 1984; ELLIOTT, BARLOW and HOGAN 1985; TAYLOR 1989). *Odc* loci whose positions were mapped in our IB and whose positions did not differ significantly from those previously identified in the RI strains were given the same locus symbols previously published, to be consistent with the recommendation of the International Committee on Standardized Genetic Nomenclature in the Mouse. However, further testing may determine that the *Odc* loci mapped in the IB are not allelic with the *Odc* loci mapped in the RI strains. An *Odc* locus on the *X* chromosome of *M. spretus* was also detected when *Bgl*I was used to cleave genomic DNA from N_2 mice, consistent with prior identification of the *Odc-13* locus on the *X* chromosome in inbred strains (STEPHENSON *et al.* 1988). Our analysis has localized *Odc-13* to the central region of the *X* chromosome, consistent with previous studies (V. M. CHAPMAN, personal communication). Two loci detected in our IB that did not appear to have a previously detected counterpart in the RI strains were given the numerical designations *Odc-14* and *Odc-15*.

The triose phosphate isomerase gene family: The Tpi probe detects the structural gene for triose phosphate isomerase as well as other related sequences in the mouse genome. The nature of these *Tpi*-related sequences is unknown and could represent *Tpi*-related genes or pseudogenes. The Tpi probe used in these studies detected 4, 7 and 5 segregating fragments, when *Bam*HI, *Hin*dIII and *Kpn*I, respectively, were used to cleave genomic DNA from N_2 progeny (Table 2). Comparison of the segregation patterns of each fragment showed that the Tpi probe detected a total of nine loci. Analysis of the segregation patterns and comparisons with marker loci already typed in the panel, established that the nine *Tpi* loci were dispersed on seven autosomes. The *Tpi-1* locus that mapped to mouse chromosome *6* in the IB most likely represents the structural gene previously mapped to mouse chromosome *6* (LEINWAND, KOZAK and RUDDLE 1978; MINNA *et al.* 1978; BULFIELD, BALL and PETERS 1987; PRETSCH 1988). The remainder of the loci were numbered in order starting with *Tpi-2*, the locus residing on chromosome *3*.

Summation of the map locations of *Mtv*, *Odc* and *Tpi* loci: Table 3 lists the *Mtv*, *Odc* and *Tpi* loci mapped in this study and the recombination distances between these loci and flanking loci used to position the *Mtv*, *Odc* and *Tpi* loci in the IB. The positions of the *Mtv*, *Odc* and *Tpi* loci along the length of each chromosome were estimated by comparing the IB map to the June 1990 composite locus map of the mouse (DAVISSON *et al.* 1990). The *Mtv*, *Odc* and *Tpi* loci map to 16 of the 19 mouse autosomes and the *X* chromosome (Table 3 and Figure 2). The total number of loci identified by the three probes is 28, includ-

TABLE 3

Linkage of *Mtv*, *Odc* and *Tpi* loci with genes used to position these loci in the IB

Chromosome	Pairwise loci combinations[a]	Recombinants/ total	Recombination distance[b]	Chromosome	Pairwise loci combinations[a]	Recombinants/ total	Recombination distance[b]
1	*Mtv-29[c]–En-1*	3/112	2.7 ± 1.5	*9*	*Ldlr–Odc-14*	1/172	0.6 ± 0.6
	En-1–Ren-2	9/194	4.6 ± 1.5		*Odc-14–Tpi-4*	13/180	7.2 ± 1.9
	Ren-2–Odc-1	7/195	3.6 ± 1.3		*Tpi-4–Thy-1*	22/156	14.1 ± 2.8
2	*Spna-2–Abl*	7/146	4.8 ± 1.8	*10*	*Esr–Tpi-5*	9/188	4.8 ± 1.6
	Abl–C5	7/145	4.8 ± 1.8		*Tpi-5–Myb*	1/177	0.6 ± 0.6
	C5–Mtv-30	0/111	<2.7[d]		*Myb–Tpi-6*	20/183	10.9 ± 2.3
	Pax-1–Odc-2	10/140	7.1 ± 2.2		*Tpi-6–Tpi-7*	3/199	1.5 ± 0.9
	Odc-2–a	1/150	0.7 ± 0.7		*Tpi-7–Ros-1*	1/122	0.8 ± 0.8
3	*Tpi-2–Ngfb*	34/190	17.9 ± 2.8	*12*	*Pomc-1–Odc[e]*	0/128	<2.3[d]
	Ngfb–Amy-2	8/198	4.0 ± 1.4		*Odc[e]–D12Nyu2*	13/127	10.2 ± 2.7
4	*Mup-1–Ifa*	19/122	15.6 ± 3.3		*D12Nyu2–D12Nyu5*	9/192	4.7 ± 1.5
	Ifa–Odc-4	3/121	2.5 ± 1.4	*13*	*Tcrg–Tpi-8*	2/69	2.9 ± 2.0
5	*Hox-7–Mtv-31[c]*	4/73	5.5 ± 2.7		*Tpi-8–Fim-1*	2/120	1.7 ± 1.2
	Mtv-31[c]–Kit	8/73	11.0 ± 3.7	*14*	*Hap–Tpi-9*	1/169	0.6 ± 0.6
	Kit–Mtv-32	6/203	3.0 ± 1.2		*Tpi-9–Plau*	1/166	0.6 ± 0.6
	Mtv-32–Alb-1	4/203	2.0 ± 1.0		*Plau–Tcra*	37/198	18.7 ± 2.8
6	*Mtv-33[c]–Met*	1/158	0.6 ± 0.6	*15*	*Mtv-36–Myc*	22/145	15.2 ± 3.0
	Met-Tcrb	25/189	13.2 ± 2.5		*Myc–Tgn*	6/151	4.0 ± 1.6
	Mtv-34–Tpi-1[f]	4/199	2.0 ± 1.0	*16*	*Smst–Mtv-37*	27/92	29.3 ± 4.8
	Tpi-1[f]–Odc-5	2/130	1.5 ± 1.1		*Mtv-37–Ets-2*	14/119	11.8 ± 3.0
	Odc-5–Kras-2	13/122	10.7 ± 2.8	*17*	*Odc-15–Pim-1*	3/140	2.1 ± 1.2
7	*Tpi-3–Gpi-1*	11/190	5.8 ± 1.7		*Pim-1—H-2*	5/164	3.0 ± 1.3
	Gpi-1–c	56/190	29.5 ± 3.3	*18*	*Fim-2–Mtv-38*	10/90	11.1 ± 3.3
	c–Odc-7	11/186	5.9 ± 1.7		*Mtv-38–Mbp*	13/142	9.2 ± 2.4
	Odc-7–Zp2	5/99	5.1 ± 2.2	*X*	*DXPas3–Hprt*	13/140	9.3 ± 2.5
	Zp2–Mtv-35	17/99	17.2 ± 3.8		*Hprt–Odc-13*	25/119	21.0 ± 3.7

[a] The most proximal locus is listed first. The loci were ordered by arranging the loci to obtain the least number of double or multiple crossovers.

[b] The distance is expressed in cM ± the standard error.

[c] These loci were not fixed in the *M. spretus* parents of the IB; only the N_2 progeny carrying the *M. spretus*-specific provirus and their siblings were used to determine map position.

[d] This number represents the upper 95% confidence limit.

[e] The *Tpi-1* and *Odc* loci mapped in the IB most likely represent structural loci previously mapped to mouse chromosomes *6* (LEINWAND, KOZAK and RUDDLE 1978; MINNA *et al.* 1978; BULFIELD, BALL and PETERS 1987; PRETSCH 1988) and *12* (COX *et al.* 1988; BERGER 1989; VILLANI, COFFINO and D'EUSTACHIO 1989), respectively.

ing the three *Mtv* loci (*Mtv-29*, *Mtv-31* and *Mtv-33*) that were not fixed in our *M. spretus* colony. Three sets of restriction endonuclease digestions of genomic DNA from N_2 progeny plus seven hybridizations are required to detect 27 of the 28 loci (Table 2). Hybridization of the Mtv probe to *Bam*HI digests is not required, since all of the loci detected using *Bam*HI are detected by the *Hin*dIII and *Kpn*I digests; similarly, hybridization of the Odc probe to *Hin*dIII digests is not required, since all of the loci detected using *Hin*dIII are detected by the *Bam*HI and *Kpn*I digests. The finding of several fragments that were the same size in the C57BL/6J and *M. spretus* genomes (Figure 1) suggests the presence of additional loci that were not able to be distinguished by these analyses.

DISCUSSION

The goal of the study reported here is to identify and develop a set of repetitive probes for use in gene mapping in the mouse. In the present study, we have identified and mapped the chromosomal loci distinguished by three low-copy repetitive probes. The Mtv, Odc and Tpi probes used in these analyses detected a minimum of ten, nine, and nine loci, respectively, that are dispersed on 16 of the 19 mouse autosomes and the *X* chromosome.

Use of repetitive probes for rapidly establishing skeleton linkage maps of the mouse genome: The 28 loci identified by the Mtv, Odc and Tpi probes form the core of a skeleton linkage map; only three sets of restriction endonuclease digestions and seven hybridizations are needed to determine the segregation patterns of all but one of these dispersed loci in an IB. The value of these repetitive probes is that they enable one to detect linkage to a new gene of interest using a minimum of resources and time.

The total haploid length of the female mouse genome has been estimated to be 1600 cM (DAVISSON

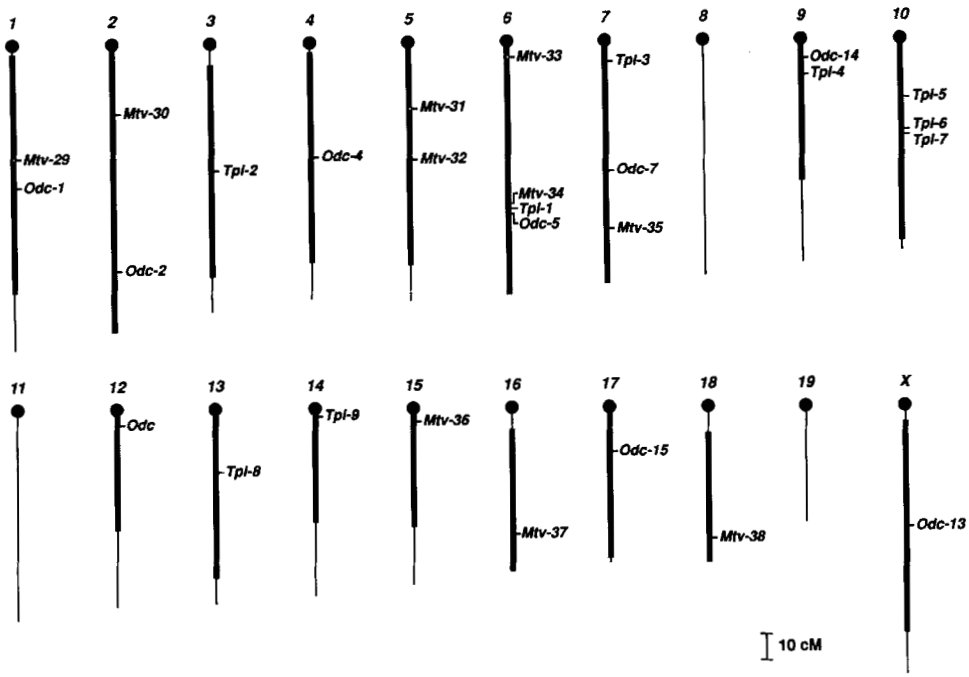

FIGURE 2.—Linkage map showing the locations of *Mtv*, *Odc* and *Tpi* loci on mouse autosomes and the *X* chromosome. The lengths of individual chromosomes are based on the June 1990 composite locus map of the mouse (DAVISSON *et al.* 1990). Map positions for most *Mtv*, *Odc* and *Tpi* loci were established by comparison with the June 1990 composite locus map of the mouse. *Mtv-36* was placed with respect to *Myc* on chromosome *15*. The positions of loci on chromosome *2* (SIRACUSA *et al.* 1990), chromosome *13* (JUSTICE *et al.* 1990a), and chromosome *14* (CECI et al. 1989) are based on maps established from our IB analysis. Chromosomal regions (± 40 cM) swept by scoring 100 N_2 progeny (see DISCUSSION) from an IB are indicated by thick lines. Dotted lines represent those loci that were not fixed in the *M. spretus* parents of the IB (*Mtv-29*, *Mtv-31* and *Mtv-33*).

and RODERICK 1989). The total haploid length of the mouse autosomal genome is 1500 cM (allowing 100 cM for the *X* chromosome). The distance on either side of a marker locus that can be scanned for linkage to an unmapped locus in a backcross can be determined directly from the recombination frequency (± the standard error) based on the number of backcross progeny examined. As a direct function of recombination frequency, the maximum number of allowable recombinants out of a total of *X* number of N_2 progeny examined would be 18 recombinants if 50 N_2 progeny were examined, 40 recombinants if 100 N_2 progeny were examined and 227 recombinants if 500 N_2 progeny were examined. These numbers give recombination distances (± the standard error) of 36 ± 6.8 cM, 40 ± 4.9 cM and 45 ± 2.2 cM, respectively, with upper 95% confidence limits remaining below 50 cM (the number expected for random assortment of loci). We have used 40 cM as the average distance scanned on either side of a marker locus when 100 backcross progeny are examined for linkage. The 27 autosomal *Mtv*, *Odc* and *Tpi* loci would thus span 72.3% of the mouse genome (Figure 2). Excluding the three *Mtv* loci (*Mtv-29*, *Mtv-31* and *Mtv-33*) that were not fixed in the *M. spretus* parents of the IB, we could still scan 70.2% of the mouse autosomal genome with the 24 remaining autosomal loci. To identify linkage of a gene to chromosome *8*, *11* or *19* (those chromosomes that do not have an *Mtv*, *Odc* or *Tpi* locus), one need only obtain a probe for each chromosome that is centrally located and polymorphic with either *Bam*HI, *Hin*dIII or *Kpn*I. Performing three sets of restriction endonuclease digestions of N_2 genomic DNAs and sequentially hybridizing the blots with these six probes (ten hybridizations maximum), it would be possible to scan 85.6% of the autosomal genome for linkage with any new gene. The number of hybridizations could be decreased by identifying probes that detect different-sized *M. spretus*-specific fragments with the same restriction endonuclease and thus could be hybridized at the same time to a single set of Southern blots. Future identification and characterization of additional repetitive probes will increase the region of the mouse genome covered by this approach and will decrease the number of blots and hybridizations required to establish skeleton linkage maps of the mouse genome.

Three other methods for linkage testing similar to the one described above have been previously established (TAYLOR and ROWE 1989; FRANKEL *et al.* 1990; JULIER *et al.* 1990). These systems are based on probes that identify low-copy repeated sequences or minisatellite sequences in the mouse genome. In one system, an MEV/1Ty strain was constructed from initial crosses of the C58/J and AKXD-14 inbred strains; the MEV/1Ty strain was specifically designed to carry 11 different ecotropic proviruses as well as three dominant visible markers that are dispersed in the mouse genome (TAYLOR and ROWE 1989). In the second system, endogenous nonecotropic murine leukemia proviruses were mapped in the inbred strains (FRANKEL *et al.* 1990). In the third system, human variable number of tandem repeat probes (human VNTRs) were examined in laboratory inbred strains and mapped in the BXD RI strains (JULIER *et al.* 1990); the human VNTRs may potentially serve as useful repetitive probes in IBs involving *M. spretus* as well, although map locations of VNTR loci in *M. spretus*

are not yet established (JULIER *et al.* 1990). The three systems provide the advantage of allowing F_1 intercrosses to be performed, since these systems are based on using laboratory strains. In contrast, the IB system allows only F_1 females to be used for subsequent crosses, since F_1 males are sterile. However, the IB system provides the advantage of almost limitless genetic variation between *M. spretus* and the inbred strains, facilitating the refinement of the map position of any gene between two previously mapped probes. In contrast, laboratory strains are ancestrally more related to each other and therefore the degree of polymorphism is more limited than between *M. spretus* and the inbred strains (FERRIS *et al.* 1983; BONHOMME *et al.* 1984).

Mapping new mutations with repetitive probes in an interspecific cross: This system will be particularly useful for mapping new mutations that have not yet been cloned. The position of the new mutation on any autosome or the *X* chromosome can be determined using this approach; however, mutations residing on the *X* or *Y* chromosomes can be detected directly from matings and would not necessarily require this approach. The mutation may be the result of spontaneous, radiation-induced, and/or chemical-induced alterations, or may be the result of a viral or transgenic insertion that is refractory to molecular cloning. The mutation can be recessive, dominant, or homozygous lethal; mating schemes can be devised to accommodate any of these possibilities. However, recessive mutations that cause lethality or sterility in homozygotes have been and remain the most difficult types of mutations to map; mutations of this type will most likely require additional crosses (than those outlined below) to determine which offspring carry the mutation of interest.

Figure 3 shows a typical breeding scheme for mapping a newly identified recessive, viable mutation (described in the legend to Figure 3). This scheme can also be adapted to map a dominant mutation (*D*). The females for the first cross could be homozygous or heterozygous for *D*. Only the resulting F_1 heterozygous *D*/+ females (as determined by examination for the *D* phenotype) would subsequently be backcrossed to males not carrying *D* to produce the N_2 progeny. N_2 mice would be either wild-type or heterozygous for *D*; the mice would be scored phenotypically for the mutant genotype. If any mutation is not completely penetrant, only the mice clearly showing the phenotype would be analyzed. Genomic DNA is then isolated from backcross progeny and screened for linkage using the repetitive probes in Southern blot analyses.

This mapping method is fast and efficient. Since DNAs from the N_2 mice are analyzed using probes that detect several dispersed marker loci per Southern blot, only a minimum number of Southern blot analyses need to be performed to provide sufficient marker loci to detect linkage over the entire mouse genome. It is not necessary to have typed the strain carrying the mutation for polymorphic marker loci, since one follows only the segregation of known *M. spretus* RFLPs and the mutant phenotype in backcross mice. The *M. spretus* mice in our colony are now a valuable resource for mapping new mutations, since these mice have been typed for the *Mtv*, *Odc* and *Tpi* loci; the restriction endonucleases and fragment sizes that identify each locus as well as the map position of each locus have already been established (Table 2). There may arise cases in which the strain carrying the mutation has a fragment indistinguishable in size from a fragment that identifies a *M. spretus*-specific allele, but this would not necessarily be a problem, since most of the *M. spretus*-specific alleles are identified by at least two restriction endonucleases. In addition, the distribution of *Mtv*, *Odc* and *Tpi* loci is such that some chromosomes have more than one locus, and these loci can provide independent confirmation of the position of a newly identified mutation on that chromosome.

Once the chromosomal location of the mutant gene is tentatively assigned, the map location can be compared to existing linkage maps, and additional probes identifying loci in the region surrounding the mutation can then be used to further refine the position of the mutant gene using the same set of backcross progeny DNAs. The power of this approach is that all of the probes used in the IB to date have detected at least one informative RFLP between the parents of

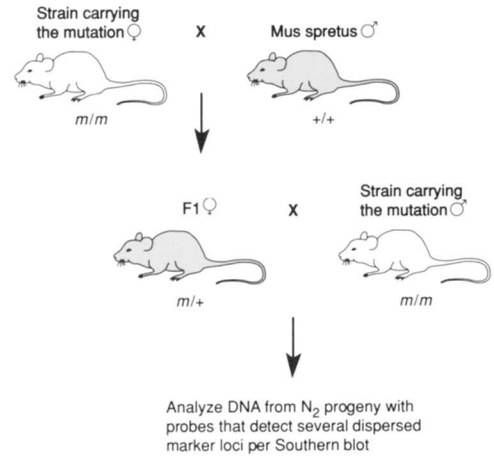

FIGURE 3.—Breeding scheme for mapping a recessive, viable mutation (*m*) by IB analysis using repetitive probes. Females homozygous for the recessive mutation of interest (*m*/*m*) are crossed to *M. spretus* (+/+) males. Heterozygous F_1 females (*m*/+) are subsequently backcrossed to homozygous (*m*/*m*) mutant males. N_2 mice are either homozygous or heterozygous for the mutation of interest; the mice are scored phenotypically for the mutant genotype. Genomic DNA is then isolated from backcross progeny and screened for linkage using the repetitive probes in Southern blot analyses.

the IB, C57BL/6J and *M. spretus*. Therefore, it is likely that these probes will detect RFLPs between laboratory mice carrying the mutation and *M. spretus* mice as well. The molecular probes used to refine the map location of the new mutation may reside at the same location as the new mutation, potentially providing a candidate gene whose alteration results in the mutant phenotype. Alternatively, the molecular probes used to refine the map location of the new mutation may reside close enough to the new mutation to be useful in chromosome walking experiments designed to clone the mutation of interest. Genomic DNAs from backcross mice carrying chromosomes that had crossovers between the mutation of interest and flanking loci identified by molecular probes would serve as useful reagents for determining the direction of chromosome walking experiments. For example, as chromosome walks extend outward from flanking molecular probes, new probes could be isolated and mapped with respect to the recombination breakpoints identified between the mutation and flanking marker loci. The finding that a recombination breakpoint has been crossed would indicate the direction to be taken for the remainder of the chromosome walk.

Use of repetitive probes for mapping multigenic traits: The ability to scan the entire mouse genome in a single cross enables the mapping of traits that result from a combination of effects of several genes. The use of repetitive probes facilitates the mapping of multigenic traits in interspecific crosses. The only criterion is that the inbred strain and *M. spretus* differ in the multigenic trait to be analyzed. The proportion of offspring from an IB that would exhibit the trait and appear identical in phenotype to one of the parents of the IB is $1/2^n$, where n is the number of genes responsible for the trait. The remainder of the offspring may exhibit a range in phenotypes intermediate between those of the IB parents or may exhibit almost identical phenotypes to each other (if the multigenic effects are additive). N_2 progeny are then typed for the trait and also typed for the marker loci identified by the Mtv, Odc and Tpi probes; one looks for a correlation between the range in phenotypes and the alleles at each locus. Those regions showing linkage can be compared to the composite IB linkage map; molecular probes previously mapped to these regions can then be used to refine the location of the genes involved in the trait and provide molecular access points to the genes of interest.

Use of repetitive probes to identify recessive protooncogenes associated with murine neoplastic disease: Hybrid mice derived from interspecific crosses can potentially be used to identify recessive protooncogenes associated with murine disease. In humans, recessive protooncogenes are often identified by reduction to homozygosity of polymorphic alleles in tumors (reviewed by HANSEN and CAVENEE 1987). In mice, this approach has been problematic, since most murine tumors studied to date have occurred on relatively uniform host genetic backgrounds. By studying tumors that arise in interspecific F_1 hybrid mice (Figure 4), it may be possible to identify recessive protooncogenes associated with murine disease by screening tumors for reduction to homozygosity of polymorphic alleles, similar to what is being done for human tumors.

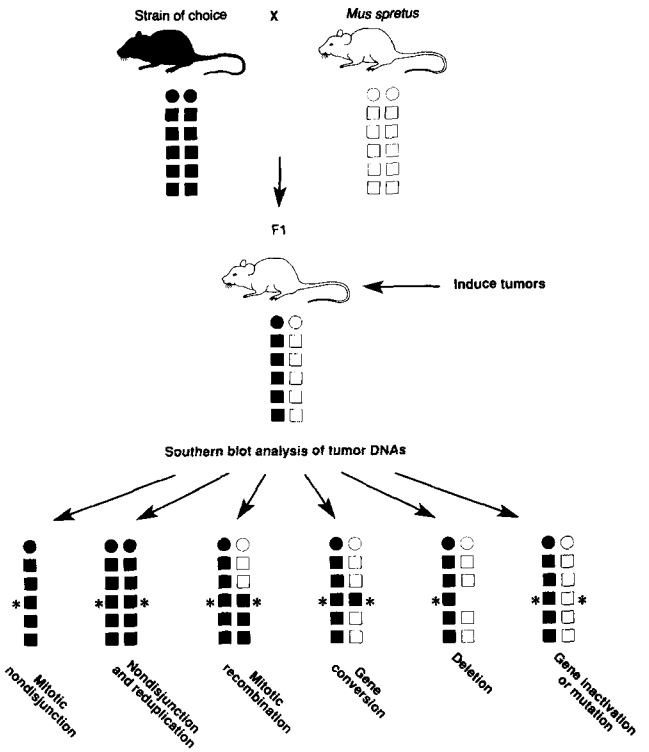

FIGURE 4.—Scheme for using interspecific crosses to identify recessive protooncogenes in murine neoplastic diseases. For comparison, only one pair of chromosomes is shown for each parent. *M. spretus* alleles are indicated by light boxes; laboratory inbred strain alleles are shown as dark boxes. The location of a mutation in a hypothetical recessive protooncogene is indicated by an asterisk.

Tumors in interspecific F_1 hybrid mice may arise spontaneously or may be induced by viral insertional mutagenesis, chemical carcinogens, or X-irradiation. In the case of recessive protooncogenes, inactivation of the first allele could occur spontaneously or be mutagen induced (Figure 4). The second allele could subsequently be inactivated by several different mechanisms, including mitotic nondisjunction, nondisjunction and chromosome reduplication, mitotic recombination, gene conversion, chromosome deletion, and a second independent mutation. Many of these processes can be scored by reduction to homozygosity of polymorphic alleles, using Southern blot analysis and hybridization with repetitive probes. Unique single-copy probes can then be used to refine the chromosomal location of the recessive protooncogene and

may subsequently serve as useful molecular starting points for isolating the gene of interest.

The ability to identify recessive protooncogenes by this approach relies on the frequency of events that result in a reduction to homozygosity of polymorphic alleles versus events that do not. For example, inactivation of the second allele by a second independent mutation will not be identified by this approach (Figure 4). In human tumors, the frequency of events that result in reduction to homozygosity of polymorphic alleles is high (HANSEN and CAVENEE 1987). Thus, it is hoped that this approach will facilitate identification and cloning of recessive protooncogenes in murine tumors as well.

The method proposed here can examine a limited amount of the mouse genome for alterations involved in oncogenesis. Identification of additional repetitive probes fulfilling the criteria outlined in the introduction will enable larger regions of the genome to be scanned. These multilocus families can be mapped in the same IB used in the analyses described here, increasing the power of this approach for gene mapping studies in the mouse.

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