A GENETIC MAP OF MOUSE CHROMOSOME 12 COMPOSED OF POLYMORPHIC DNA FRAGMENTS

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Three sets of genes related to the immune system are linked on mouse chromosome 12. The *Igh* complex, which spans 8 centimorgans (cM),[1] encodes all of the elements of immunoglobulin heavy chains. Many DNA segments within this complex have been isolated as recombinant DNA clones, and polymorphisms associated with the complex have been defined at both the protein and the DNA levels (1–5). The T lymphocyte alloantigens Tpre, Tthy, Tind, and Tsu are encoded by a gene cluster closely linked to *Igh*. Serological analyses indicate that each of these markers is expressed by a distinct functional subset of T lymphocytes, and suggest a close association between these markers and the T cells' receptors for foreign antigens (6, 7). The unique transplantation antigen of the MethA sarcoma is encoded by a locus on the distal portion of the chromosome. The antigen is found only on the MethA sarcoma, a fibroblastoid cell, and not on lymphoid or myeloid cells. Antibodies to the antigen cross-react with an immunoglobulin-like T cell factor encoded at *Igh*, however, raising the possibility of structural homology between the two molecules (8–10).

Attempts to define the genetic organization of this chromosomal region more precisely and to relate the organization of the genes on the chromosome to their expression in the immune system of the mouse are hampered by a scarcity of genetic markers beyond these three. We have undertaken a search for such markers. Here, we report: first, the assignment of two genes, *c-fos* and a locus defined by the cDNA probe pCl.2, to the chromosome by analysis of somatic cell hybrids; second, the development of a procedure to identify mouse-specific DNA fragments in recombinant DNA libraries derived from mouse × Chinese hamster somatic cell hybrids and its use to isolate five DNA fragments of unknown function specific for chromosome 12; and third, the organization of these markers into a tentative linkage map on the basis of DNA-level polymorphisms associated with them.

Materials and Methods

Mice. CXJ recombinant inbred (RI) strains (11) were a gift from Drs. Ruth Epstein and Melvin Cohn. All other inbred and RI strains were obtained from The Jackson Laboratory, Bar Harbor, ME.

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[1] *Abbreviations used in this paper:* cM, centimorgan; Igh, immunoglobulin heavy chains; kb, kilobase; RI, recombinant inbred, SDS, sodium dodecyl sulfate.

Cell Lines. MethA(a), a line adapted to culture from the MethA tumor (8), E36, a Chinese hamster fibroblastoid cell line, and MethA × E36 somatic cell hybrids MAE28 and MAE32 were maintained in culture and characterized for chromosomal composition as described previously (9).

DNA. Genomic DNA was purified from mouse livers or from cultured cells, digested with restriction endonucleases, fractionated by agarose gel electrophoresis, and analyzed by filter hybridization with radiolabeled DNA probes exactly as described previously (12). The plasmid pCl.2, containing a cDNA copy of a mouse liver mRNA species of uncertain function, was a gift from Dr. Jonathan Seidman. The plasmid p*fos*-1, from Dr. Inder Verma, contains a 1.3 kilobase (kb) fragment of the FBJ osteosarcoma virus oncogene (13).

Isolation of Chromosome 12–specific Genomic DNA Fragments. DNA from the MAE28 somatic hybrid cell line was digested to completion with EcoRI, ligated with purified λwes phage DNA arms, and packaged in vitro to generate a partial genomic library (14). DNA from phage plaques was transferred onto nitrocellulose filters (BA85, Schleicher and Schuell, Keene, NH) (15). Filters were baked 3 h at 80°C under vacuum. 1–10 filters (100 mm diameter) were incubated 3 h at 65°C in 30 ml of 6× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 1× Denhardt's solution (0.02% polyvinylpyrrolidone, 0.02% Ficoll 400, 0.02% bovine serum albumin), 0.1% sodium dodecyl sulfate (SDS), 0.3 mg/ml sonicated heat-denatured (100°C, 5 min) salmon testes DNA (Sigma Chemical Co., St. Louis, MO), then incubated further for 12–18 h at 65°C in 30 ml of the same solution plus 1.5 μg radiolabeled (16) MethA DNA and 3,000 μg unlabeled sonicated E36 DNA (both heat denatured as above). Filters were washed at R.T. in 2X SSC-0.1% SDS, then twice for 30 min at 65°C in 0.3× SSC-0.1% SDS, blotted dry and subjected to autoradiography at −70°C (Kodak XAR film, DuPont "Cronex" intensifying screens) for 12–72 h. Presumptive mouse-specific recombinant phage were plaque-purified and re-screened. DNA from each phage was digested with EcoRI, and the insert purified by sucrose gradient centrifugation (14).

To obtain unique-sequence subclones, purified insert DNA was partially digested with AluI plus RsaI to generate a mixture of overlapping, blunt-ended DNA fragments. These were cloned into the HincII site of the M13mp7 vector (14, 17). Recombinant (β-galactosidase-minus) plaques were screened by filter hybridization to identify ones lacking reiterated sequences, i.e., that failed to hybridize with radiolabeled MethA DNA. Hybridization conditions were exactly as described above, except that unlabeled E36 DNA was omitted from the hybridization mix. The double-stranded (RFII) form of each M13 recombinant DNA was purified from infected JM103 cells by a standard cleared lysate procedure (14).

Results

Assignment of fos and pCl.2 to Chromosome 12. Mouse genes for which recombinant DNA probes exist can be localized to chromosomes, using Southern blotting to screen panels of mouse × Chinese hamster somatic cell hybrids (18). This strategy was used to search for markers of chromosome 12. Two were found: the oncogene *c-fos* (Fig. 1 *A*); and the gene family defined by the cDNA clone pCl.2 (Fig. 1 *B*).

Chromosome 12–specific DNA Clones. Assigning known genes to chromosomes, however, is an inefficient means for constructing dense genetic maps of chromosomal regions of interest. Methods were therefore developed to clone mouse genomic DNA fragments from mouse × Chinese hamster somatic cell hybrids, in order to generate markers of chromosome 12 specifically.

EcoRI fragments of genomic DNA from the MAE28 somatic hybrid cell line, which contains a full Chinese hamster (E36) chromosome complement plus mouse chromosomes 12 and X (9), were cloned in λwes. To identify presumptive mouse-

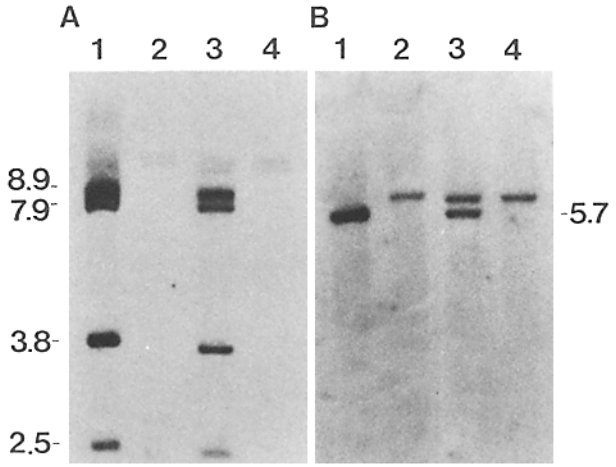

FIGURE 1. Assignment of *c-fos* and the pCl.2 gene family to mouse chromosome 12. 25-μg samples of mouse (MethA, track *1*), Chinese hamster (E36, track *2*), and somatic cell hybrid (MAE28, track *3*; MAE32, track *4*) DNA were digested with EcoRI endonuclease, fractionated by electrophoresis on 0.8% agarose gels, transferred to nitrocellulose filters, and hybridized with (*A*) p*fos*-1 (13) or (*B*) pCl.2 plasmid DNA, radiolabeled by nick translation (12, 16). Both somatic cell hybrids contain complete sets of E36 Chinese hamster chromosomes and the mouse X chromosome. MAE28 also contains mouse chromosome 12, and MAE32 contains mouse chromosome 16 (9). The sizes of mouse-specific DNA fragments, in kb, are shown.

specific recombinant phage, DNA from phage plaques was transferred onto nitrocellulose filters and probed with radiolabeled total mouse genomic DNA in the presence of a 2,000-fold excess of unlabeled E36 genomic DNA. Under these conditions, no members of an E36 library hybridized with the probe, while 3% of the members of the MAE28 library did so. The fraction of positive phage approximately equaled the fraction of the MAE28 genome that is of mouse origin.

Unique-sequence subclones of these phage were generated by ligating random fragments of the insert from each phage into the M13mp7 vector and screening for M13 recombinants that failed to hybridize with radiolabeled total mouse genomic DNA. Originally developed for shotgun cloning of small DNA fragments (17), this procedure allowed identification of subclones of each recombinant phage suitable for use as probes of mouse genomic DNA, without knowledge of either the restriction map of the original cloned DNA segment or the positions of reiterated sequences within it.

Southern blotting experiments were carried out to confirm that each cloned DNA fragment was of murine origin, and to distinguish chromosome 12–specific and X chromosome–specific fragments. Samples of genomic DNA from mouse, Chinese hamster, and somatic cell hybrids MAE28 (mouse chromosomes 12 + X) and MAE32 (mouse chromosomes 16 + X) digested with EcoRI were analyzed with unique-sequence subclones of each DNA fragment (Fig. 2). In all cases, a genomic DNA fragment was visualized in mouse and somatic cell hybrid DNA of the same size as the cloned fragment. At this level of resolution, these mouse DNA sequences appear not to have been altered either in their passage through the somatic cell hybrid or in the cloning process. Five cloned fragments were

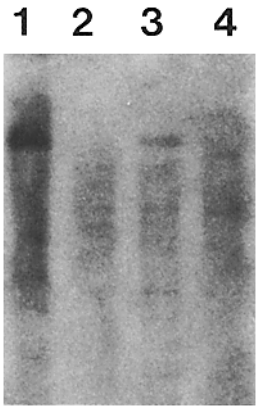

FIGURE 2. Assignment of recombinant phage 19 (*D12-2*) to mouse chromosome 12. MethA (*1*), E36 (*2*), MAE28 (*3*), and MAE32 (*4*) DNA samples were analyzed exactly as in Fig. 1. The hybridization probe was the unique sequence subclone M13ϕ19-25.

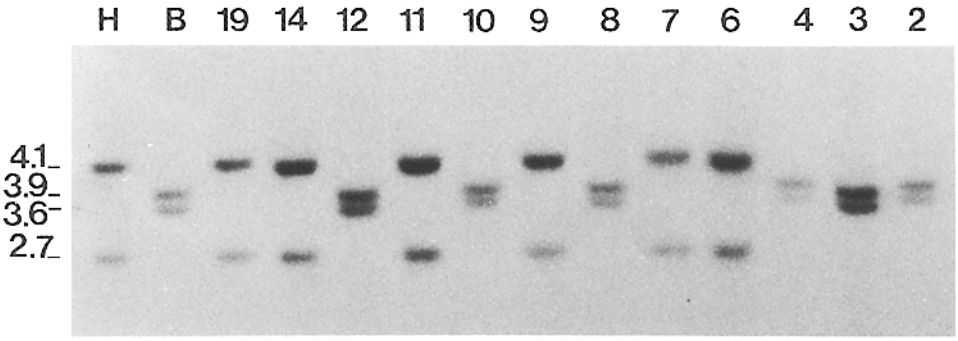

FIGURE 3. DNA polymorphism at *D12-2*. 10-μg samples of liver DNA were digested with TaqI endonuclease, then fractionated and hybridized with radiolabeled M13ϕ19-25 DNA as described in Fig. 1. DNA samples shown here were from the inbred strains C3H/HeJ (H) and C57BL6/J(B), and from the members of the BXH recombinant inbred strain set (BXH2, . . . , 19—see Table II). DNA fragment sizes, in kb, are shown.

detected in mouse and MAE28 DNA only, and thus were derived from chromosome 12. 11, detectable in mouse and both somatic cell hybrids, were derived from the X chromosome. These have not been characterized further.

Polymorphisms Associated with Cloned DNA Fragments. Liver DNA samples from inbred strains of mice were digested to completion with various restriction endonucleases, and analyzed by Southern blotting. A typical result is shown in Fig. 3, and the results are summarized in Table I. Restriction fragment-length polymorphisms were associated with all five DNA fragments derived from chromosome 12. In most cases, the variant patterns could not be explained by single point mutations, but only by multiple point mutations affecting recognition sites for more than one enzyme, or by the insertion, deletion, or rearrangement of DNA sequences.

The name *D12-n* is proposed for this group of loci, *D*NA markers of chromosome *12*, with each locus assigned an arbitrary serial number *n*. This nomencla-

TABLE I
Polymorphic Loci Defined by Cloned DNA Fragments

Locus	Probe	Alleles	Strains
D12-1	M13ϕ7-97	*a* (R:7.9; T:3.4, 1.8; M:3.7)	BALB/cJ, C3H/HeJ, DBA/2J, A/J, AKR/J, DBA/1J
		b (R:12.0; T:3.8, 1.8; M:3.7)	C57BL/6J, NZB/B1NJ, 129/SvJ, PL/J, C57L/J, SM/J, LP/J
		c (R:12.0; T:3.8, 1.8; M:3.9, 3.6)	SJL/J, SWR/J
D12-2	M13ϕ19-25	*a* (T:4.1, 2.7)	BALB/cJ, NZB/B1NJ, SJL/J, 129/SvJ, C3H/HeJ, DBA/2J, A/J, SWR/J, AKR/J, DBA/1J, PL/J, C57L/J, SM/J, LP/J
		b (T:3.9, 3.6)	C57BL/6J
D12-3	M13ϕ20-1	*a* (R:6.9; T:3.8, 2.0)	SWR/J, SJL/J, SM/J
		b (R:7.9; T:4.2, 3.6)	C57BL/6J, NZB/B1NJ, 129/SvJ, C3H/HeJ, DBA/2J, A/J, AKR/J, DBA/1J, BALB/cJ, PL/J, C57L/J, LP/J
D12-4	M13ϕ26-15	*a* (T:7.7, 1.8)	SWR/J
		b (T:9.2)	C57BL/6J, NZB/B1NJ, SJL/J, 129/SvJ, C3H/HeJ, DBA/2J, A/J, AKR/J, DBA/1J, BALB/cJ, PL/J, C57L/J, SM/J, LP/J
D12-5	M13ϕ30-3	*a* (M:4.4, 3.7)	BALB/cJ, AKR/J
		b (M:3.8)	C57BL/6J, SWR/J, C57L/J
c-fos	p*fos*-1	*a* (R:4.0; M:8.5)	SWR/J
		b (R:5.7; M:3.2)	C57BL/6J, NZB/B1NJ, 129/SvJ, C3H/HeJ, DBA/2J, A/J, AKR/J, DBA/1J, BALB/cJ, PL/J, C57L/J, SM/J
		c (R:5.7; M:8.5)	SJL/J
—	pC1.2*	*a* (R:8.9, 7.9, 3.8, 2.5)	BALB/cJ, NZB/B1NJ, SJL/J, 129/SvJ, DBA/2J, SWR/J, AKR/J, DBA/1J, BALB/cJ, PL/J, C57L/J, LP/J
		b (R:7.9, 7.5, 5.9, 5.2, 3.8)	C57BL/6J, C3H/HeJ, A/J, SM/J

Mouse liver DNA was analyzed as shown in Fig. 2, and variant alleles defined according to the patterns of DNA fragments reactive with unique sequence, subclone probes specific for each locus. Fragment sizes for each enzyme are in kb. R, EcoRI; T, TaqI; M, MspI. C57BL/6J is the prototype strain for the *b* allele at each locus; for other alleles the first strain listed is the prototype.

* Polymorphisms were also observed for DNA digested with BamHI, HindIII, MspI, and TaqI. Each of these showed the same strain distribution as the EcoRI polymorphism tabulated here.

ture conforms to that proposed for mouse genes (19) and to that for human DNA fragments (20).

Three allelic forms of *c-fos* and two forms of the DNA sequences reactive with the pCl.2 clone were found. Again, these polymorphisms appeared to be due to DNA rearrangements or multiple point mutations (Table I).

Linkage Map of Chromosome 12. To work out the linkage relationships of these DNA markers to one another and to the gene cluster encoding immunoglobulin heavy chains (*Igh*), DNA samples from RI mouse strain sets were typed (Fig. 3; Table II). RI mice were typed as well for the switch region length polymorphism associated with the germline α and μ heavy chain constant region genes (4). Every mouse tested was homozygous for one of the progenitor strain forms of each DNA marker.

TABLE II

Strain Distribution of DNA Polymorphisms in RI Strain Sets

	AKXL																
	5	6	7	8	9	12	13	14	17	19	21	24	25	28	29	37	38
Locus																	
D12-5	L	A	L	L	A	A	L	L	L	L	L	A	A	L	A	L	A
D12-1	A	A	L	L	A	A	L	A	A	L	L	L	A	L	A	L	A
*Igh-Cα**	A	L	L	A	A	A	L	L	A	L	L	A	A	A	L	A	A

	BXD																								
	1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
Locus																										
D12-2	B	D	B	B	B	D	B	D	D	B	B	B	B	B	—	—	D	D	D	B	D	B	B	B	B	D
D12-1	D	B	B	B	D	D	B	B	D	B	D	B	D	D	—	—	D	D	D	D	D	D	D	B	D	D
*Igh-Cα**	B	B	B	D	B	D	D	D	B	B	D	D	D	B	—	—	B	B	D	B	D	D	B	D	D	D
Env-34‡	B	B	B	D	B	D	D	D	D	—	D	D	B	B	D	B	B	B	D	D	D	D	B	D	D	D
pC1.2	B	B	B	D	D	D	D	B	B	B	D	D	B	D	—	—	B	B	D	D	D	D	B	D	B	D
Env-21‡	B	B	B	—	D	B	D	B	B	B	D	D	B	D	D	B	B	B	D	D	D	B	D	D	B	D
Env-25‡	B	B	B	—	D	B	D	B	B	B	D	D	B	D	D	B	B	B	B	B	B	B	B	B	D	D

	BXH							BXJ						
	2	3	4	6	7	8	9	10	11	12	14	19	1	2
Locus														
D12-2	B	B	B	H	H	B	H	B	H	B	H	H	J	B
D12-1	H	B	B	B	H	H	H	H	H	H	H	H	J	B
*Igh-Cα**	H	B	H	H	H	B	H	B	B	H	B	H	B	B

	SWXL						
	4	7	12	14	15	16	17
Locus							
D12-3	S	S	S	L	S	S	L
D12-1	S	S	S	L	S	S	L
D12-4	L	L	L	L	S	S	L
c-fos	L	L	L	L	S	S	L
*Igh-Cμ**	L	L	L	L	L	L	L

	CXJ							
	1	3	6	8	9	10	11	15
Locus								
D12-5	C	C	J	C	J	C	J	C
D12-3	C	C	J	C	J	J	J	C
D12-1	C	C	J	C	J	J	J	C
c-fos	C	C	C	C	J	J	J	J
*Igh-Cα**	C	J	C	J	J	J	J	J

	CXB						
	D	E	G	H	I	J	K
Locus							
D12-2	B	C	C	C	C	B	B
D12-1	C	C	C	C	C	B	C
*Igh-Cα**	B	B	C	B	B	C	B
pC1.2	B	B	C	C	B	C	C

RI mice were typed for DNA polymorphisms by Southern blotting, as shown in Fig. 2. All strains were homozygous for one of the progenitor forms of every locus tested, as indicated by the letters: A, AKR; B, C57BL/6; C, BALB/c; D, DBA/2; H, C3H/He; J, SJL; L, C57L; S, SWR; —, not tested.

* To detect polymorphism at *Igh-Cα*, HindIII-cut DNA was hybridized with radiolabeled pα(J558)[15]DNA, and to detect polymorphism at *Igh-Cμ*, EcoRI cut DNA was hybridized with radiolabeled pμ(3741)[9]DNA (4). All *Igh-C* polymorphisms detected in this way were concordant with those previously detected using anti-allotype antisera (1, 38, 51).

‡ Data of Blatt et al. (25).

Assuming that the organization of genetic loci on chromosome 12 is the same in all of the progenitor mouse strains used to form these RI strain sets, data for all of the sets can be summed (Table III). For each pair of loci, the ratio of the number of RI strains carrying nonparental combinations of alleles to the total number of RI strains scored was used to estimate the recombination frequency between the two loci. Genes more than ~15 cM apart appear unlinked in sets of RI strains of the size typically examined here, and the accuracy of estimates of recombination frequency falls as this limit is approached (21). Nevertheless, using

TABLE III

Recombination Frequencies between Loci on Chromosome 12, Measured in RI Strains

	D12-2	*D12-5*	*D12-3*	*D12-1*	*D12-4*	*c-fos*	*Igh-C*	*Pre-1*	*pC1.2*
D12-2		—	—	—	—	—	—	—	—
D12-5	0/0		3.8 ± 4.4*	7.1 ± 4.1	—	—	—	—	—
D12-3	0/2	1/8		<6.2	—	16.7 ± 12.2	—	—	—
D12-1	18/43	5/25	0/15		—	16.7 ± 12.2	—	—	—
D12-4	0/0	0/0	3/7	3/7		<18.2	—	—	—
c-fos	0/0	3/8	5/15	5/15	0/7		11.1 ± 7.9	—	—
Igh-C	21/43	12/25	9/15	41/75	2/7	4/15		10.7 ± 4.4	10.5 ± 5.2
Pre-1‡	18/29	8/14	0/0	19/43	0/0	0/0	12/46		<2.9
pC1.2	19/33	0/0	1/2	15/31	0/0	0/0	8/31	0/29	

For each pair of loci, the total number of RI strains carrying nonparental combinations of alleles is shown as a fraction of the total number of RI strains typed at these loci (Table II). From these numbers, estimated recombination frequencies and their standard errors were calculated, or, where no nonparental allele combinations were found, 95% upper confidence limits for the recombination frequency were calculated (21). Recombination frequencies are shown only for loci that appear linked in this survey.

* Recombination frequency, in cM, ± standard error.

‡ Numbers of recombinant strains and estimated recombination frequencies between *Pre-1* and other loci were calculated using published *Pre-1* strain distribution patterns (1, 38, 51).

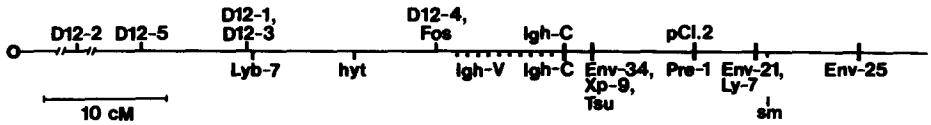

FIGURE 4. Linkage map of mouse chromosome 12, composed of polymorphic DNA fragments. The centromere is to the left. The total length of the chromosome is set at 75 cM. Loci previously mapped are shown below the line. Five of these, *Igh-C*, *Env-34*, *Pre-1*, *Env-21*, and *Env-25*, can be directly incorporated into the DNA fragment map. Relative positions of other loci (*Lyb-7*, *hyt*, *Igh-V* genes, and *sm*) can be deduced, but have not been directly tested in breeding experiments (1, 2, 7, 22–25, 50).

these recombination frequencies the markers can be ordered: *D12-5*—(*D12-1,3*)—(*c-fos*, *D12-4*)—(*Igh-C*, pCl.2/*Pre-1*). The recombination data do not allow this map to be oriented with respect to the centromere. The order, centromere—*Igh-C*—*Pre-1*, was established previously, however (2), and assuming a length of ~75 cM for chromosome 12, *Pre-1* should lie ~20 cM from the telomere (22–24). The marker *Env-25* has been placed near the center of the *Pre-1*—telomere interval by analysis of BXD RI strains (25). If *D12-1* were in this interval, it would fall within 10 cM ($r = 0.1$) of *Env-25* and the expected fraction of *D12-1*—*Env-25* recombinants among BXD RI strains would be no greater than $R = 4r/(1 + 6r) = 0.25$ (21). 13 of 23 strains were recombinant (Table II), a discrepancy that is unlikely to be due to sampling fluctuation alone ($\chi^2_1 = 12.12$, $p < 0.001$). *D12-1* can therefore be placed proximal to *Pre-1*, orienting the map with respect to the centromere. The same argument excludes *D12-2* from the *Pre-1*—telomere interval (14/23 recombinant; $\chi^2_1 = 15.66$, $p < 0.001$), placing it by default in the centromere—*D12-5* interval (Fig. 4).

Discussion

The use of random DNA fragments as genetic markers is well established (20, 26), as is the use of interspecies somatic cell hybrids as a source of DNA fragments specifically derived from a single chromosome (27). Previous work has been focused on the human genome, however. Here, methods are described to recover mouse-specific DNA fragments from mouse × Chinese hamster somatic cell hybrids. These methods were used to isolate five such fragments from mouse chromosome 12. All five were polymorphic among inbred strains of mice. The genetic loci that they define have been ordered into a tentative linkage map, together with *Igh*, *Pre-1*, the *c-fos* oncogene, and the locus defined by cDNA clone pCl.2.

Mouse chromosome 12–specific DNA fragments were identified on the basis of their content of mouse-specific reiterated DNA sequences. The frequency with which these fragments were identified roughly equaled the portion of the hybrid cell genome that is of mouse origin. The screening procedure thus appears to be efficient and specific for the identification of arbitrary mouse genomic DNA fragments, despite the close relationship between the mouse and the Chinese hamster. Somatic cell hybrids carrying various single mouse chromosomes or chromosome pairs on a Chinese hamster background exist (e.g., 9, 28, 29) as do procedures for systematically producing such hybrids (30). It should therefore be possible to obtain random DNA fragment markers for virtually any mouse chromosomal region of interest.

All five of the chromosome 12–specific DNA fragments isolated in this way were polymorphic (Table I). The ancestry of inbred mouse strains is only partly known, but the 15 strains examined here certainly have ancestors in common, including possibly a single maternal ancestor (31, 32). The extent of polymorphism among inbred mouse strains at these loci should therefore tend to underrepresent the extent of polymorphism in an outbred mouse population. Most of the polymorphisms detected in this survey could not be explained by single point mutations. Rather, point mutations affecting multiple restriction enzyme recognition sites, or DNA rearrangements—insertions, deletions, or inversions—appear to be necessary to account for the variants observed (Table I).

These loci differ from the polymorphic human DNA fragments described by Barker et al. (33) in both respects. Only 9 of 31 human genomic DNA fragments were polymorphic in a group of 9 unrelated individuals, and all of the polymorphic variants found could be explained by single point mutations. While the numbers of human and mouse DNA fragments characterized are small, the differences are unlikely to reflect only sampling fluctuation. They may be due to the sources of the genomic DNA fragments. All of the mouse fragments contain reiterated sequences, while the human fragments were selected to be free of them. The role of transposable reiterated sequences as insertional mutagens is well established in yeast, *Drosophila*, and mice (34–37). It seems unlikely that radically different mutational mechanisms operate, or operate at radically different rates, in rodents and primates. It is therefore attractive to speculate that, in both humans and mice, regions of the genome rich in reiterated sequence will prove rich in polymorphic DNA rearrangements as well. More detailed characterization of genomic DNA fragments from both species will be required

to test this notion, and to work out possible relationships between specific reiterated sequences and specific mutational events.

RI strain sets, derived by inbreeding pairs of F_2 hybrid mice from a cross between two inbred progenitor strains, have been widely used to establish gene linkage relationships (21, 38). Here, it has been possible to construct a tentative map of chromosome 12 incorporating the five DNA markers, the cDNA clone pCl.2, *Igh-C*, and *c-fos* (Table III, Fig. 4). At the same time, given the uncertainties in the estimated recombination frequencies, this map must be regarded as a working model, to be refined by three-point crosses and incorporation of additional markers.

The map contains three pairs of loci that are not resolved by recombination events. Two of these pairs—*D12-1*, *D12-3* and *D12-4*, *c-fos*—nevertheless appear to consist of distinct loci. Polymorphisms associated with the two members of each pair show different strain distribution patterns (Table I), and unique DNA sequences from one member of a pair do not cross-hybridize with unique sequences from the other. The third pair, *Pre-1* and pCl.2, however, are not readily distinguished. *Pre-1* is a serum protease inhibitor synthesized in the liver (F. Kueppers and R. Riblet, personal communication). pCl.2 is a cDNA corresponding to a liver mRNA species. The predicted amino acid sequence of the pCl.2 product is homologous to that of human α_1 antitrypsin, a serum protease inhibitor encoded at a locus on human chromosome 14 closely linked to *Igh* (K. Krauter, personal communication; Krauter and D'Eustachio, unpublished observations) (39). pCl.2 therefore appears to be one of the members of the *Pre-1* gene family. Its extensive polymorphism makes it a convenient marker for genetic analyses of the locus.

The location of *c-fos* near *Igh* is intriguing. In the human, *c-fos* has been localized to chromosome 14, close to the human *Igh* gene complex, by in situ hybridization and analysis of somatic cell hybrids (40). In the mouse, reciprocal translocations involving chromosomes 12 and 15 occur frequently in plasmacytomas, and activate the *c-myc* gene (normally on chromosome 15) in juxtaposing it with *Igh-C* (41–46). Both *c-fos* and *c-myc* encode proteins found in normal cells, although neither has a known function. Nevertheless, the proteins are similar in two respects. Both are found predominantly in the nucleus (but not in nucleoli), giving grainy staining patterns with fluorescent antibodies. Analysis of tumors and of tissue culture model systems suggests that overproduction of either protein is in itself sufficient to cause cellular transformation (47–49). Together, these data raise the possibility of a role for *c-fos* in lymphoid tumors, a possibility that, with appropriate antisera and recombinant DNA probes, can now be investigated directly.

Summary

Mouse chromosome 12 encodes the heavy chains of immunoglobulins (*Igh*), a family of T cell surface molecules, and a tumor antigen that may be homologous to immunoglobulins. To refine and extend the genetic map of this chromosome, a procedure has been developed to isolate chromosome 12–specific DNA fragments from a somatic cell hybrid carrying the chromosome on a Chinese hamster background. Five fragments have been isolated and characterized in detail. All

are polymorphic, defining loci *D12-1*, *2*, *3*, *4*, and *5*. Using recombinant inbred mouse strains, a tentative linkage map of chromosome 12 has been worked out that incorporates these markers, the *c-fos* oncogene, *Igh*, and *Pre-1*/α_1 antitrypsin.

This strategy should be applicable to any mouse chromosome or chromosomal region that can be isolated in a somatic cell hybrid.

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