The EMBO Journal vol.6 no.11 pp.3291–3297, 1987

Construction of a detailed molecular map of the mouse X chromosome by microcloning and interspecific crosses

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Communicated by M.F.Lyon

A large number of microclones obtained by microdissection of the mouse X chromosome have been mapped using an interspecific *Mus domesticus/Mus spretus* cross. Clones displaying close linkage to a number of loci of known phenotype but unknown gene product, such as *mdx* (X-linked muscular dystrophy), have been obtained. Over a central 30 cM span of the mouse X chromosome, 17 clones have been mapped and ordered at a sufficient density to contemplate the complete physical mapping of this region that will aid in the isolation of a number of unidentified genes. Some of the mapped microclones detect moderately repetitive sequences that were clustered in several discrete regions of the mouse X chromosome.

Key words: X chromosome/microdissection/interspecific cross

Introduction

New techniques in molecular genetics have in recent years provided an opportunity to study the molecular basis of phenotypically defined genetic loci and the long-range structure and organization of mammalian chromosomes (Brown, 1985). The use of restriction fragment length polymorphism (RFLP) analysis (White, 1985) has allowed the mapping of random genomic probes to the human genome. The resultant molecular map has provided starting points that have assisted in the eventual cloning of exonic sequences from disease loci for retinoblastoma (Friend *et al.*, 1986) and Duchenne muscular dystrophy (Monaco *et al.*, 1986).

Interspecific *Mus domesticus/M. spretus* crosses have recently been developed for the genetic or molecular mapping of mouse chromosomes (Roberts *et al.*, 1985). The divergence of the *M. domesticus* and *M. spretus* genomes markedly increases the frequency of detectable restriction fragment length variants (RFLV) enabling rapid analysis of backcross progeny for molecular probes. This approach has now been used to map a number of random genomic probes and known gene sequences to various mouse chromosomes (Amar *et al.*, 1985; Avner *et al.*, 1987; Barlow *et al.*, 1987). As in humans, expansion of the present maps should provide a number of start points for the molecular analysis of interesting genetic loci. The advantages of carrying out these studies in the mouse are however 2-fold: firstly the genetic analysis is facilitated by the lack of restraint on breeding experiments and the variety of genetic techniques available. Secondly the eventual analysis of gene products encoded at loci of interest will be easier to perform on an animal model.

The techniques for microdissection and microcloning were originally developed as a means of obtaining sequences from regions of interest in *Drosophila* polytene chromosomes (Scalenghe *et al.*, 1981). Since that time microdissection has been successfully applied to the analysis of mammalian chromosomes (Rohme *et al.*, 1984; Fisher *et al.*, 1985; Bates *et al.*, 1986). We have previously reported the microdissection of a proximal region of the mouse X chromosome (Fisher *et al.*, 1985). Here we describe the mapping of a number of the resultant microclones, utilizing an interspecific *M. domesticus/M. spretus* cross, to create a detailed molecular map of a region of the mouse X chromosome. The resultant map has a high density of molecular markers — on average, one every 2 cM — that should allow the creation of a complete physical map of a large region of the mouse X chromosome.

Results

Microclone analysis

Mouse X-chromosome specific DNA clones were obtained by microdissection of the proximal region of the mouse X chromosome (clone bank pmc16) or alternatively by dissection of the whole X chromosome (clone bank pmc14, see Materials and methods). Individual microclones were analysed for the presence

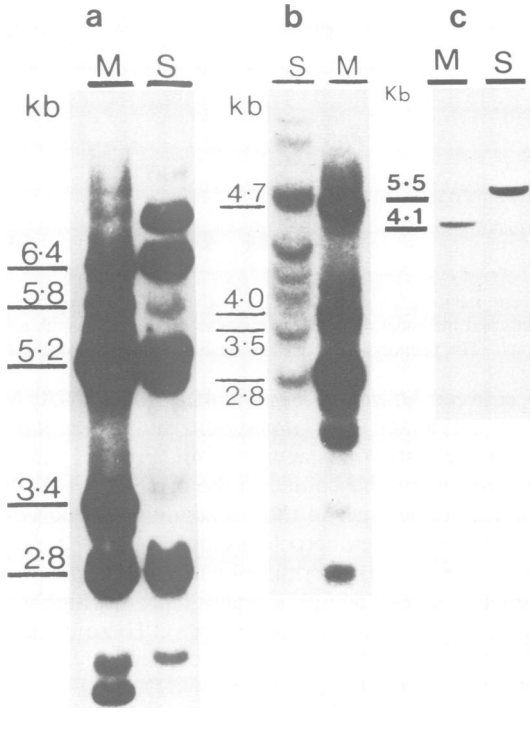

Fig. 1. Detection of restriction fragment length variants between *M. domesticus* and *M. spretus* DNA. Microclones 36 (**a**), 222 (**b**) and 66 (**c**) were hybridized to *Taq*I-digested *M. domesticus* (M) and *M. spretus* (S) DNA as described in Materials and methods.

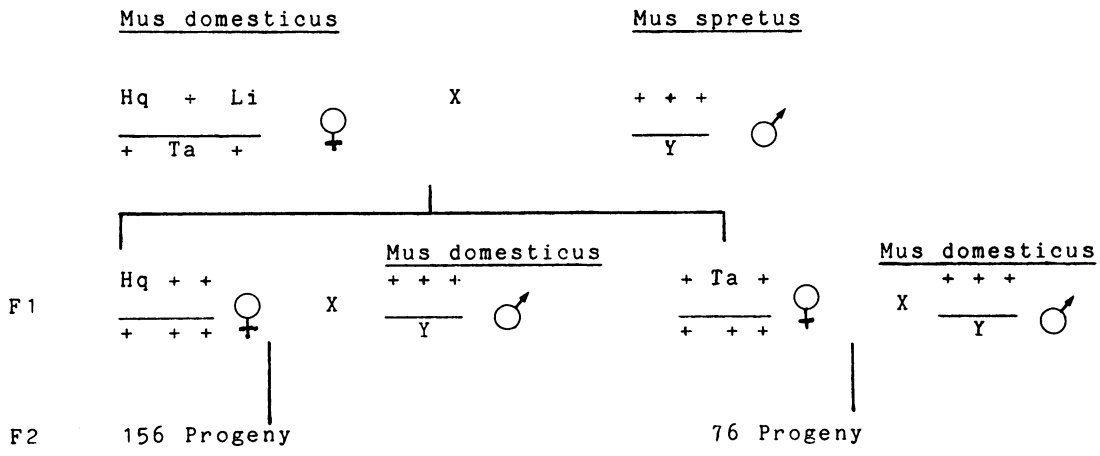

Fig. 2. Details of the interspecific *M. domesticus/M. spretus* cross. A female *M. domesticus* carrying the coat texture mutations *Hq*, *Ta* and *Li* was crossed with a *M. spretus* male. Of the resultant female progeny two were found to carry only the *Hq* mutation and another two carried only the *Ta* mutation. These mice were backcrossed to inbred 129 *M. domesticus* mice. The resultant backcross progeny were scored for coat texture and sex prior to use in genetic mapping experiments.

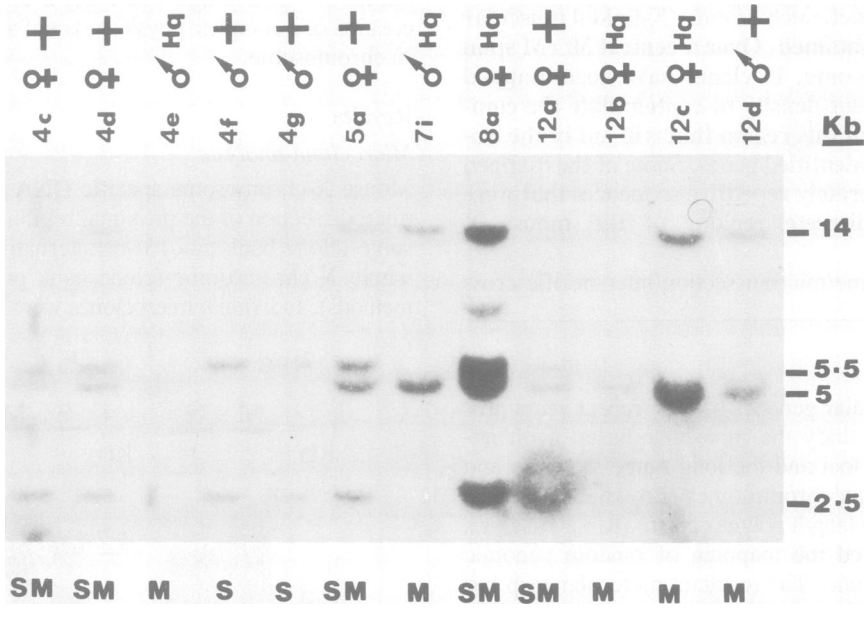

Fig. 3. Genetic mapping of microclone 10. Radiolabelled microclone 10 probe was hybridized to *Msp*I-digested DNA from a number of backcross progeny (see Materials and methods). Two *M. domesticus* (M) bands (14 kb and 5 kb), and two *M. spretus* (S) bands (5.5 kb and 2.5 kb) were detected in the resultant autoradiograph. The phenotype of each mouse is indicated above the figure and the genotype for microclone 10 below.

of RFLV between *M. domesticus* and *M. spretus* DNA. Microclones were hybridized to *M. domesticus* and *M. spretus* DNA digested with the restriction enzymes *Taq*I (Figure 1) and *Msp*I. These enzymes were chosen due to the presence of the hypermutable dinucleotide CpG in their recognition sequences.

A small number of the microclones analysed give dense autoradiographic smears after hybridization to *M. domesticus* and *M. spretus* genomic digests and presumably represent highly repetitive dispersed sequences (data not shown). These clones were missed in the original attempt to screen out repetitive sequences by hybridization with labelled total mouse DNA (Fisher *et al.*, 1985), possibly due to the small size of the microclones involved. A larger number of microclones were found to detect moderately repetitive sequences (e.g. clone 36, Figure 1a). The complexity of the banding pattern detected by these clones and the possibility that these sequences were dispersed throughout the mouse genome indicate that many of these microclones may not be amenable to genetic mapping studies. The remainder of the microclones analysed were apparently single-copy (e.g. clone 66, Figure 1c). A small proportion (3 out of 39) of these microclones did not detect RFLV in either *Taq*I or *Msp*I digests. These clones were not analysed further.

Microclone mapping

Interspecific crosses segregating for the coat texture mutations Harlequin (*Hq*) and Tabby (*Ta*) were set up as described in Materials and methods. The details of the genetic cross are shown in Figure 2. *Hq* and *Ta* were utilized in the interspecific cross, as their genetic position on the mouse X chromosome indicates that they lie around the likely physical limits of the proximal microdissection (see Figure 5).

Microclones detecting RFLV were hybridized to filters of *Taq*I- or *Msp*I-digested backcross progeny DNAs. Figure 3 illustrates a typical example (microclone 10). Male mice displayed either

Table I. Recombinational analysis of X chromosome clones in the *M. domesticus/M. spretus* interspecific cross

	222	10	*Hq*	191	172	66	67	219	59	36	91	255	G28	64	23	pCA1	120	*Ta*	43	225
141	0/46 0	7/164 4 ± 2	13/142 9 ± 2	0/24 0	1/46 2 ± 2	1/50 2 ± 2	0/16 0	11/91 12 ± 3	7/61 12 ± 4	4/53 8 ± 4	6/29 21 ± 8	6/35 17 ± 6	20/86 23 ± 5	11/44 25 ± 6	14/43 33 ± 7	21/73 29 ± 5	22/66 33 ± 6	19/62 31 ± 6	30/81 37 ± 5	24/45 53 ± 7
222		0/33 0	1/26 4 ± 4	0/9 0	nt	0/8 0	nt	2/44 5 ± 3	0/8 0	4/44 9 ± 4	3/13 23 ± 11	5/15 33 ± 12	7/28 25 ± 8	6/19 32 ± 11	7/18 39 ± 12	8/25 32 ± 9	11/27 41 ± 10	7/20 35 ± 11	5/12 42 ± 14	24/45 53 ± 7
10			3/127 2 ± 1	0/22 0	0/43 0	0/43 0	0/14 0	2/73 3 ± 2	1/58 2 ± 2	1/41 2 ± 2	1/21 5 ± 5	3/29 10 ± 6	11/77 14 ± 4	7/35 20 ± 7	8/35 23 ± 7	12/65 19 ± 5	15/49 31 ± 7	14/46 30 ± 7	22/69 32 ± 6	14/33 42 ± 9
Hq				0/15 0	0/47 0	0/25 0	0/16 0	1/50 2 ± 2	1/56 2 ± 2	2/26 8 ± 5	2/18 11 ± 7	3/18 17 ± 9	9/65 14 ± 4	4/26 15 ± 7	4/27 15 ± 7	8/41 20 ± 6	5/23 22 ± 9	nt	16/46 35 ± 7	12/26 46 ± 10
191					0/7 0	0/8 0	0/7 0	0/9 0	nt	1/15 7 ± 6	nt	nt	3/13 23 ± 12	nt	nt	nt	nt	4/9 44 ± 17	5/10 50 ± 16	4/9 44 ± 17
172						0/23 0	0/14 0	nt	0/22 0	nt	nt	nt	2/18 11 ± 7	nt	nt	nt	nt	nt	16/44 36 ± 7	nt
66							0/7 0	0/18 0	0/29 0	0/14 0	nt	2/10 20 ± 13	3/14 21 ± 11	4/12 33 ± 14	4/10 40 ± 15	6/22 27 ± 9	6/18 33 ± 11	8/27 30 ± 9	17/43 39 ± 8	nt
67								nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	7/14 50 ± 14	nt
219									1/30 3 ± 3	2/44 5 ± 3	4/30 13 ± 6	6/35 17 ± 6	12/60 20 ± 5	11/42 26 ± 7	12/42 29 ± 7	15/57 26 ± 6	21/69 30 ± 6	15/46 33 ± 7	11/32 34 ± 8	21/44 48 ± 8
59										0/9 0	0/18 0	2/23 9 ± 6	4/31 13 ± 6	4/29 14 ± 6	4/28 14 ± 7	4/28 14 ± 7	4/20 20 ± 9	4/11 36 ± 15	11/28 40 ± 9	nt
36											0/14 0	4/16 25 ± 11	5/29 17 ± 7	5/18 28 ± 11	6/19 32 ± 11	7/33 21 ± 7	8/27 30 ± 9	7/28 25 ± 8	5/16 31 ± 12	19/44 43 ± 8
91												4/27 15 ± 7	4/31 13 ± 6	4/29 14 ± 6	4/30 13 ± 6	4/31 13 ± 6	4/25 16 ± 7	3/14 21 ± 11	nt	8/13 61 ± 13
255													2/37 5 ± 4	2/36 6 ± 4	2/34 6 ± 4	2/33 6 ± 4	5/31 16 ± 7	5/20 25 ± 10	nt	6/15 40 ± 13
G28														1/46 2 ± 2	1/46 2 ± 2	3/61 5 ± 3	5/39 13 ± 5	4/25 16 ± 7	7/29 24 ± 8	9/28 32 ± 9
64															0/41 0	0/42 0	4/34 12 ± 5	3/21 14 ± 8	1/12 8 ± 8	6/17 35 ± 12
23																0/43 0	3/32 9 ± 5	1/19 5 ± 5	nt	6/18 33 ± 11
pCA1																	4/39 10 ± 5	2/35 6 ± 4	1/16 6 ± 6	6/25 24 ± 9
120																		2/48 4 ± 3	4/32 12 ± 6	5/27 19 ± 8
Ta																			6/39 15 ± 6	4/19 21 ± 9
43																				1/12 8 ± 8

The number of recombinants out of the total number of mice analysed for each two-point analysis (upper figure) and the calculated genetic distance in cM with standard error (lower figure) are given. Two-point analyses where little or no data were obtained are designated nt (not tested).

the *M. domesticus* (M) allele or *M. spretus* (S) allele, while female mice had the genotype SM or M alone. For three probes 36 (Figure 1a), 222 (Figure 1b) and 225 (not shown) showing complex but different banding patterns, often also of widely differing intensity in *M. domesticus* and *M. spretus* DNA, it was difficult to score reliably the SM genotype from the M genotype in females. These probes were scored only in males where the separate M and S genotypes were easily observed. Two further clones, 191 and 67, were also only scored in males where there was an absence of hybridizable signals to *M. spretus* DNA. Only two clones (141 and 10) were analysed through the bulk of progeny. A variety of sets of the total 232 progeny were utilized for the analysis of the remaining clones. The number of recombinants out of the total number of mice analysed for each two-point analysis is given in Table I. The order of clones was determined by a simple multipoint analysis taking account of the minimum required number of crossovers and allowed the accurate positioning of microclones on the X chromosome genetic map (see Table I and Figure 5). Only four backcross progeny inherited an X chromosome demonstrating a double crossover (Table II).

In some cases for the common progeny analysed closely linked groups of clones failed to show recombination. Where recombination between two or more neighbouring clones was not observed they are bracketed in Figure 5, and in these cases their order on the chromosome is estimated from calculations of genetic distance in further separate sets of progeny mice with other clones or genetic loci (see Table I). In particular four clones, 191, 172, 67 and 66, all fail to show recombination events between themselves or with *Hq*. In addition 191, 172, 67 and 66 all fail to show crossovers with microclone 10; 191 and 66 fail to show crossovers with locus 219, and no crossovers are detected between 172, 66 and 59. Taking all the evidence together these four clones all appear to be closely linked to *Hq* but their exact position with respect to other clones cannot be ascertained at this stage utilizing the numbers of progeny examined. In support of this it can however be established by simple multipoint analysis

Table II. Genotypes of the four female backcross progeny demonstrating double crossovers on the mouse X chromosome

Clone	Mouse number			
	1722 5d	1722 8b	1724 6b	1725 3c
141	M	M	S	S
222	nt	nt	nt	nt
10	S	M	S	S
191	nt	nt	nt	nt
172	nt	nt	nt	S
66	nt	nt	nt	nt
67	nt	nt	nt	nt
219	S	S	S	nt
59	S	nt	nt	nt
36	nt	nt	nt	nt
91	nt	M	nt	nt
255	nt	nt	nt	nt
G28	M	M	nt	M
64	M	M	nt	nt
23	M	M	nt	nt
DMD	M	M	nt	nt
120	nt	M	M	nt
43	nt	nt	S	S
225	nt	nt	nt	nt

The genotype of the X chromosome derived from the female parent is indicated. The intervals for each crossover are indicated by brackets; M, *M. domesticus* allele; S, *M. spretus* allele; nt, not tested.

utilizing crossovers with more distal markers that 172 lies in the interval 141−G28 and that 66 lies proximal to 255. Similarly at this stage there are no informative crossovers to indicate whether microclone 120 maps just proximal or just distal to *Ta*.

As the complexity of the map grew it was possible to detect individual mice in which a recombination event had occurred between two of the mapped microclones. By selecting specific recombinant mice it was possible to construct a localization panel on which subsequent microclones could be positioned quite readily. An example of this type of pedigree analysis is shown in Figure 4. In this manner it was possible to localize clone 91 to the interval between G28 and 219. Subsequent recombinational analysis on a large number of progeny confirmed this localization. Pedigree analysis in a few mice was also used to indicate that two clones, 191 and 67, map proximal to 36 and G28 respectively, in agreement with their close linkage to *Hq* (Table I and see above). This type of analysis is formally the same as that used in RI (recombinant inbred) strains (Green, 1981).

Seventeen microclones were positioned on the basis of the multipoint analysis and genetic distances (Table I) determined from a large number of progeny. In addition to the mapping of microclones, two other clones were analysed through large numbers of progeny in the backcrosses. G28 is a mouse genomic probe homologous to the human pGD3 clone mapping to the 3′ side of the human G6PD gene (Avner *et al.*, 1987). pCA1 is a cDNA probe encoding part of the human Duchenne muscular dystrophy gene (G.Cross, Y.Edwards and K.Davies, in preparation). The remaining microclones (14, 44, 93 and 117) were positioned only by pedigree analysis in a few progeny. For the 19 clones positioned on the basis of multipoint analysis and genetic distances, in all 1309 pieces of data were acquired representing the inheritance of some 1309 *M. domesticus* or *M. spretus* alleles from the F1 female progeny. A full computer printout of the data is available on request.

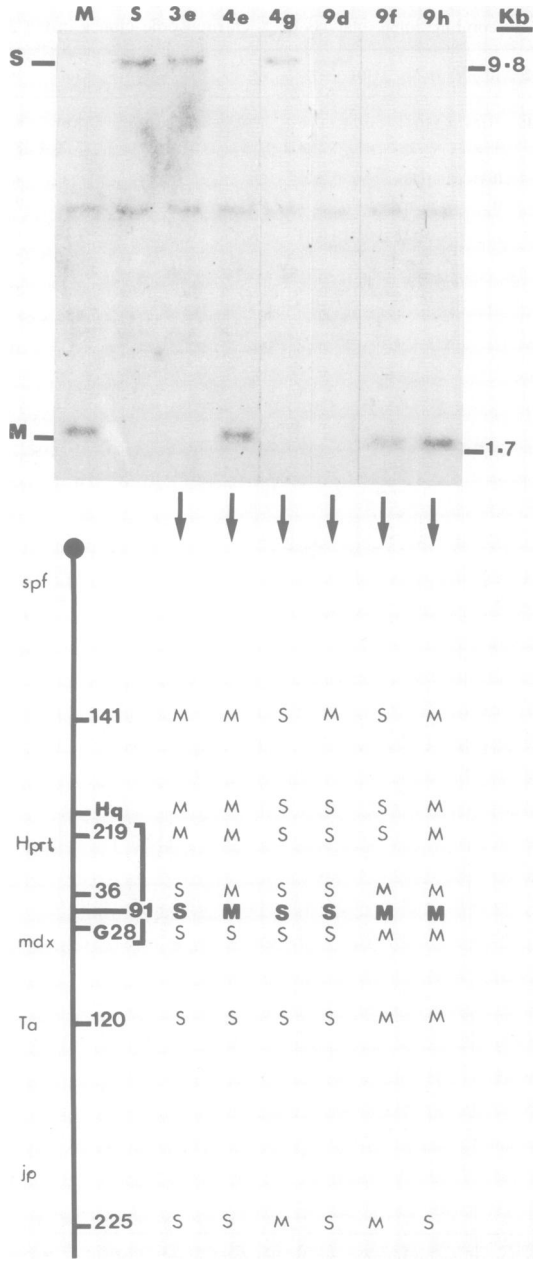

Fig. 4. Mapping of microclone 91 by pedigree analysis. Radiolabelled microclone 91 probe was hybridized to *Taq*I-digested DNA of *M. domesticus* (M), *M. spretus* (S) and a selection of male backcross progeny. The *M. domesticus* (M) and *M. spretus* (S) alleles of 1.7 kb and 9.8 kb respectively are indicated. The map position of microclone 91 was determined by comparing the genotypes with those obtained with a number of other clones for which the X-chromosome location was already determined (shown on right hand side of the X-chromosome diagram). The position of several other loci not segregating in the analysis is indicated to the left of the diagram. The bracketed region between G28 and 219 indicates the interval defined as containing 91 on the basis of this pedigree analysis alone.

The majority of clones mapped within a region comprising approximately 50% (50 cM) of the mouse X chromosome. However 17 clones were mapped within a 30 cM region extending from approximately 9 cM proximal to *Hq* (the 141 locus) to the *Ta* locus. Nine out of the 15 clones analysed from the proximal dissection definitely map in this genetic region. Of the six remaining clones, four clones definitely map distal to *Ta*. Comparison of the physical and genetic maps of the mouse X chromosome

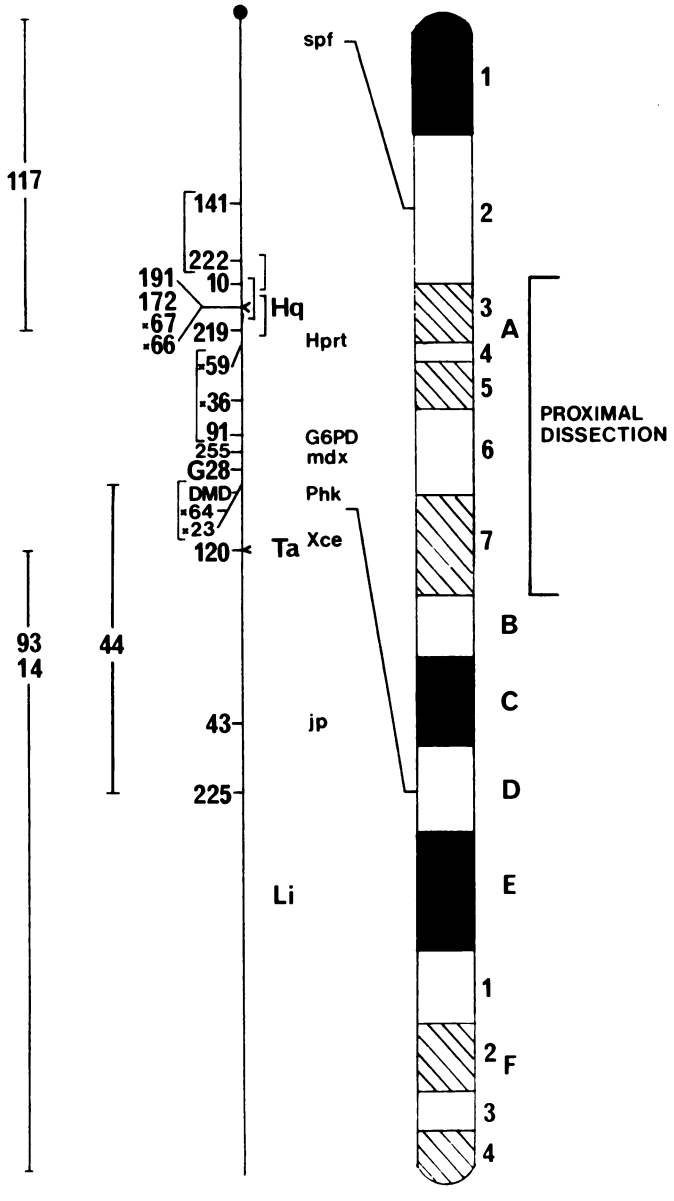

Fig. 5. Molecular map of the mouse X chromosome. The genetic map positions of microclones and gene probes on the mouse X chromosome are illustrated to the left of the genetic map. Brackets indicate neighbouring clones that fail to show recombination events. Clones prefixed by an x arise from a whole mouse X-chromosome microdissection (pmc14, see text). The remaining mapped clones indicated, except G28 and DMD (pCA1) — the human Duchenne muscular dystrophy gene exonic probe, arise from a proximal microdissection of the mouse X chromosome (pmc16, Fisher *et al.*, 1985). Four clones, 117, 93, 14 and 44, have been mapped only to defined genetic intervals which are indicated. To the right of the genetic map are shown the relative positions of the *Hq* and *Ta* loci used in the interspecific cross. In addition, to the right of the genetic map, approximate relative positions of a number of other genetic loci on the mouse X chromosome are indicated. The concordance between the physical and genetic maps of the X chromosome is indicated in two instances: the gene coding for ornithine carbamoyltransferase at the sparse fur locus (*spf*) is located in **band A2** (Lyon *et al.*, 1986) and Searle's translocation (Green, 1981) is located at **band D**. The estimated physical limits of the proximal microdissection (Fisher *et al.*, 1985) are shown. Scale of genetic map: 10−*Hq* interval is 2 cM; total genetic length of X chromosome map is 100 cM.

(Figure 5) indicates that these originate from a physically distal region of the X chromosome. In addition 120, close to *Ta*, must also be located distal to the dissected region. While the derivation of proximal clones from the proximal regional dissection is better than 50%, the reasons for the recovery of distally located clones is not clear but may possibly reflect the inaccuracies of microdissection of this scale.

Table III. Characteristics of the mapped microclones derived from the proximal X chromosome dissection (pmc16) and the total X chromosome dissection (pmc14)

	Microclone	Size (kb)	Enzyme used for mapping	Comments
	141	1.2	*TaqI*	Localized repeat (50 copies)
	225	0.18	*TaqI*	Localized repeat (10 copies)
	222	0.14	*TaqI*	Moderately repeated — homologous Y chromosome sequence and possible autosomal homologues (see text)
	117	0.12	*TaqI*	Moderately repeated
	191	0.19	*TaqI*	Low copy
	172	0.25	*TaqI*	Low copy
	255	0.95	*TaqI*	Low copy
pmc16	219	0.65	*TaqI*	Low copy
	120	0.19	*TaqI*	Low copy
	93	0.18	*TaqI*	Low copy
	91	0.12	*TaqI*	Low copy
	44	0.8	*MspI*	Low copy
	43	0.28	*TaqI*	Low copy — homologous autosomal sequence
	14	0.14	*TaqI*	Low copy
	10	0.55	*MspI*	Low copy
	36	0.4	*TaqI*	Moderately repeated
	67	0.25	*TaqI*	Low copy
pmc14	66	0.35	*TaqI*	Low copy
	64	0.23	*TaqI*	Low copy
	59	0.28	*TaqI*	Low copy
	23	0.26	*TaqI*	Low copy

Copy number estimates refer to the *M. domesticus* genome and will be described in detail elsewhere (E.Fisher, N.Brockdorff, V.Chapman, M.Lyon and S.Brown, in preparation).

Characteristics of mapped microclones

Table III details some of the characteristics of the mapped microclones. One of the most striking aspects of this study was the finding that some moderately repeated sequences were X-chromosome specific and behaved as single loci in the interspecific crosses. This has been demonstrated with clones 141 and 225 which are present in *M. domesticus* at approximately 50 copies and 10 copies respectively (data not shown). All copies map to the described loci (Figure 5). The organization and inheritance of 141 and 225 sequences are dealt with in more detail elsewhere (E.Fisher, N.Brockdorff, V.Chapman, M.Lyon and S.Brown, in preparation). Two other clones, 36 (Figure 1a) and 222 (Figure 1b), also detect multiple copies within the genome. In the case of 36 segregation in male backcross progeny of the 3.4 kb *M. domesticus* band (which has no *M. spretus* counterpart) localizes at least these 36 sequences to the mouse X chromosome (Figure 5). In the case of 222 segregation in male backcross progeny of the major 2.8 kb *M. domesticus* band (which in *M. spretus* has only a very faint counterpart) localizes at least these sequences to the X chromosome (Figure 5). However in the case of 222, bands at 3.5 kb and 4.7 kb appeared to show variable intensity in male backcross progeny, indicating possible autosomal linkage of some of the 222 sequences (data not shown). Clone 117 also

showed multiple bands on genomic digests of *M. domesticus* and *M. spretus* DNA (data not shown). A number of bands were held constant in these two genomes; however segregation of variant bands in pedigree analysis (see above) localized at least these sequences to a proximal region of the mouse X chromosome (see Figure 5). In addition clone 222 detected one band displaying Y-chromosome specific segregation, and clone 43 detected one band displaying autosomal segregation.

Discussion

Microcloning of the mouse X chromosome coupled with genetic mapping utilizing an interspecific *M. domesticus/M. spretus* cross has provided a high-resolution molecular map of the mouse X chromosome. Some 23 clones have been mapped to differing extents on the mouse X chromosome. Of these, 17 clones have been mapped to a central region of the mouse X chromosome spanning some 30 cM and extending from approximately 9 cM proximal to *Hq* to the *Ta* locus. On average these clones would be spaced less than 2 cM apart. However taking all the genetic evidence together (see Table I), it is clear that the density of clones is relatively high around the *Hq* locus and around the pCA1 (DMD) and G28 loci.

Recently pulse field gel electrophoresis (Schwartz and Cantor, 1984; Carle *et al.*, 1986) has been utilized for the construction of long-range physical restriction maps over several megabases of mammalian genomes (Burmeister and Lehrach, 1986). In the mouse on average 1 cM of genetic distance is equivalent to approximately 1.7 megabases, though this relationship must be fallible given the recent evidence of recombination hotspots in the mouse major histocompatibility complex (Steinmetz *et al.*, 1987). However given that our mapped probes are linked at distances of the order of 2 cM, it must be reasonable to assess their physical linkage using pulse field mapping. In this way it is possible to contemplate the construction of a complete physical map extending over a central 30 cM region of the mouse X chromosome. Any microclones that fail to show physical linkage with their neighbours could be incorporated in the map by chromosome jumping to adjacent chromosome regions (Poustka *et al.*, 1987).

The provision of a large number of mapped clones over a 30 cM region of the mouse X chromosome provides a number of molecular markers to define the molecular map limits of a number of interesting loci in this region and thus provide start-points for their isolation. Such loci include *mdx* (X-linked muscular dystrophy), which has been considered as a possible homologue to the human X-linked Duchenne muscular dystrophy locus (Bulfield *et al.*, 1984). The DMD exonic probe we have characterized maps provocatively close to *mdx* (see Figure 5), and the implications of this result are discussed elsewhere (Brockdorff *et al.*, 1987). Other clones we have mapped in the region of *mdx* may also help define in further genetic tests the genetic limits of the *mdx* locus and later, after pulse field mapping, the physical limits as well. In a similar fashion the large number of microclones mapped around the *Hq* locus (see Figure 5) should enable us to define the molecular limits of this coat-texture mutation.

The long-range molecular mapping of mammalian chromosomes is an essential prerequisite, not only for the isolation and characterization of mutant loci, but also for any study of the long-range sequence composition of mammalian chromosomes and its relationship to chromosome structure and genetic behaviour. In addition it is probably an essential prerequisite of any attempt at the acquisition of the complete DNA sequence of a mammalian chromosome. The data presented here are an intermediate step towards this goal on the mouse X chromosome.

Materials and methods

Microdissection and microcloning

The preparation and characterization of the clone bank obtained from regional microdissection of the X chromosome (pmc16) was described previously (Fisher *et al.*, 1985). In addition a second clone bank (pmc14) obtained by dissection of the whole X chromosome was utilized in this study. The library was constructed from 100 dissected chromosomes, yielding approximately 2000 recombinant clones. Sixty-nine clones were analysed for insert size and repeat content as was described for the regional dissection (Fisher *et al.*, 1985). The small size of many of the microclones meant that it was necessary to size inserts on polyacrylamide gels after end-labelling (Bates *et al.*, 1986). Most of the inserts used in subsequent analysis were sub-cloned into the plasmid vectors pGEM4 (Promega), pSP64 or pUC13 using standard methods.

Mouse crosses

An interspecific *M. domesticus/M. spretus* cross segregating for the X-linked coat texture mutations *Hq* and *Ta* was set up as follows: a female *M. domesticus* mouse carrying the X-linked mutations *Hq*, *Ta* and Lined (*Li*) (Green, 1981) was crossed to a male *M. spretus*. Four female progeny, two carrying *Hq* and two carrying *Ta*, were backcrossed to inbred 129 mice, and a total of 232 progeny were produced, 156 segregating for *Hq* and 76 segregating for *Ta* (see Figure 2).

Progeny DNA analysis

After scoring progeny for coat mutation and sex, animals were killed and tail and liver tissue was immediately frozen in liquid nitrogen prior to preparation of high mol. wt DNA (Grosschedl *et al.*, 1984). Progeny DNAs were digested with the restriction enzymes *Taq*I or *Msp*I, electrophoresed on agarose gels and transferred to Hybond-N (Amersham) nylon membranes by Southern blotting (Southern, 1975). Microclone inserts were cut out from low melting point agarose gels and oligo-labelled according to a published method (Feinberg and Vogelstein, 1985). Hybridization was performed at 65°C in 3× SSC, 1× Denhardt's solution, 10% dextran sulphate, 100 mg/ml denatured salmon sperm DNA, 0.1% $NaDodSO_4$ at 65°C. Filters were washed in 1× SSC, 0.5% $NaDodSO_4$ at 65°C and exposed on Kodak XAR-5 film with intensification for 1–14 days.

Acknowledgements

We would like to thank Andy Greenfield and Jamal Nasir for constructive comment; Phil Avner for the G28 probe and Kay Davies for the pCA1 probe. This work was supported by grants from the Medical Research Council (G8223373CB) and the Nuffield Foundation to S.D.M.B. E.M.C.F. was in receipt of a SERC Case Studentship.

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Received on May 12, 1987; revised on July 27, 1987