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Somatic Cell Hybrid Mapping on Mouse Chromosome 11 (MMU11): Assignment of Markers Relative to Two Breakpoints in Band D

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Mouse × rat somatic cell hybrids were generated by fusing mouse cell lines that are heterozygous for reciprocal translocations involving the T42H and T9Ad breakpoints on mouse chromosome 11 (MMU11) to a thymidine kinase-negative (Tk^-) rat cell line, RT_2Tk^-. Selection in HAT medium with geneticin disulfate (G418) resulted in some hybrid clones retaining only one derivative translocation chromosome with that part of MMU11 carrying the *Tk-1* locus. Southern blot and PCR analyses of these hybrids were used to map the two breakpoints and 30 markers relative to them. The T42H breakpoint has been localized between *Mpo* and the *Cola-1/Hox-2* cluster of loci and is proximal to the T9Ad breakpoint. The T9Ad breakpoint is proximal to the distal loci *Tk-1, Gaa, D11Jkn1,* and *P4hb*. The positions of 14 loci (*Hox-2, Cola-1, Rara, Phb, Erba, Rnula-1, D11Pas1, Gfap, D11Mit13, D11Mit11, D11Mit12, Myla, Empb3* and *Gh*) have been further refined by their localization between the two breakpoints in band D. This study therefore improves the correlation of the genetic and physical maps of MMU11 and extends the known homology between MMU11 and human chromosome 17 (HSA17) by the assignment of three additional HSA17 markers, the profilin gene, *Pfn,* an anonymous marker, *D17s28h,* and the *Crk* oncogene, to above the T42H breakpoint; and the prohibitin gene, *Phb,* to between the T42H and T9Ad breakpoints in band D on MMU11.

INTRODUCTION

The use of interspecific somatic cell hybrids for human gene mapping has been extensive. To date, however, limited use has been made of somatic cell techniques for chromosomal assignments in species other than man. This is probably due to the paucity of selectable markers and, in the case of rodents, cytogenetic difficulties in distinguishing between the chromosomes of closely related species. However, as new markers and techniques that allow improved detection of interspecific variation are developed, these problems are being overcome.

The use of somatic cell hybridization for the mapping of mouse chromosome 11 (MMU11) is facilitated by the presence of the thymidine kinase gene on MMU11 (Kozak and Ruddle, 1977; Sawyer *et al.*, 1985). This allows hybrid selection in HAT medium following the fusion of two parent cell lines, one of which is thymidine kinase deficient. In most panels of mouse × Chinese hamster somatic cell hybrids that segregate mouse chromosomes and that have been used for gene assignments to mouse chromosomes (Kozak *et al.*, 1975; Kozak and Ruddle, 1977; D'Eustachio *et al.*, 1980), selection for the *Hgprt* locus has been used, and MMU11 has rarely been retained in these hybrids. In these and other studies, assignments to MMU11 have been made either by exclusion (e.g., *Rnula-1:* Michael *et al.*, 1986; *Sigje:* Smith *et al.*, 1989) or by using a panel including one hybrid with an entire chromosome complement (*Gh:* Jackson-Grusby *et al.*, 1988). In fact, Kozak and Ruddle (1977) suggested that MMU11 carries a locus/loci, the retention of which is detrimental to the survival of mouse × Chinese hamster hybrids. This does not appear to be the case for mouse × rat hybrids, although Killary and Fournier (1984) have identified a gene (*Tse*) on MMU11 responsible for the extinction of certain traits when MMU11 is present in mouse × rat somatic cell hybrids. Nevertheless, this has not precluded the successful generation of mouse × rat hybrids with a single MMU11 (Killary and Fournier, 1984) and a single (11;13) translocation chromosome (Joyner *et al.*, 1985).

Although the linkage map of MMU11 is well developed (Buchberg *et al.*, 1991), relatively few correlations of gene loci with chromosome bands have been made. The data so far accumulated have, nonetheless, revealed interesting homology with human chromosomes. Although at its proximal end MMU11 shows homology with at least six different human chromosomes, the distal half of this chromosome, from approximately band B1, reveals extensive homology with human chromosome 17 (Münke and Francke, 1987). So far, every marker on HSA17 known to have a mouse homologue

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has been mapped to MMU11. This homology forms the second largest segment of linkage conservation between mouse and man, with an empirical length of 25 cM, and is one of the five known conserved linkages that spans a human centromere (Nadeau and Reiner, 1990). Comparisons of gene order between HSA17 and MMU11 have revealed at least two rearrangements that have occurred in the evolution of these two chromosomes: one apparent from the inverted order of the *Myhs* and *Trp*53 loci, which might be expected during the evolution of a submetacentric chromosome (HSA17) from an acrocentric one (MMU11) or vice versa; and the other revealed by the location of the *Erba* and *Erbb* oncogenes proximal to *Mpo* on HSA17 as opposed to distal to *Mpo* on MMU11 (Buchberg *et al.*, 1989).

To define further the boundaries of synteny and the extent of homology between these two chromosomes, we have begun physical mapping of MMU11 within the region of linkage conservation. By fusing mouse cells derived from the heterozygous translocation stocks T42H[T(11;19)42H] and T9Ad[T(9;11)9Ad] to a thymidine kinase-deficient (Tk^-) rat cell line and subsequent hybrid selection in HAT medium, we have developed mouse × rat somatic cell hybrid lines that contain only a part of MMU11 for regional mapping on MMU11. Using these hybrids we have mapped 29 markers relative to the T42H and T9Ad breakpoints, both of which are characterized by cytogenetic breakpoints contained within band 11D.

MATERIALS AND METHODS

Cell lines. Fibroblast cultures were established from ear biopsies derived from the heterozygous mouse translocation stocks T42H [T(11;19)42H], purchased from The Jackson Laboratory (Bar Harbor, ME), and T9Ad[T(9;11)Ad] (Adler and Neuhäuser-Klaus, 1987). The chromosomes of cells harvested from primary cultures prior to fusion were G-banded to confirm the assignment of breakpoints to bands 11D and 19B for T42H and to 9B and 11D for T9Ad (Evans *et al.*, 1977; Morris and Robinson, 1991; Adler and Neuhäuser-Klaus, 1987; this report Fig. 1). RT_2Tk^- is a thymidine kinase-deficient rat cell line obtained from Dr. David Cox (UCSF) into which a neomycin-resistant marker has been inserted to allow selection against unfused mouse cells using geneticin disulfate (G418). FO(11)J18 is a mouse × rat somatic cell hybrid containing only MMU11 (Killary and Fournier, 1984).

Somatic cell hybridization. Somatic cell hybrids were generated by polyethylene glycol (PEG 1500, Boehringer Mannheim) fusions between the mouse T42H and T9Ad fibroblasts and RT_2Tk^-, using standard techniques. Subsequent selective propagation in Dulbecco's modified Eagle's medium (DMEM) with HAT (3×10^{-5} *M* hypoxanthine, 2×10^{-7} *M* aminopterin, 3×10^{-5} *M* thymidine) and geneticin disulfate salt (1 mg/ml final concentration, Sigma) ensured preferential loss of the mouse complement and selection for the mouse *Tk-1* locus. Eleven independent hybrid clones from 10 different petri plates were picked for the T42H series of hybrids, and 27 clones from 20 different plates were picked for the T9Ad series.

Characterization of somatic cell hybrids. Sequential G- and C-banding was used for the initial selection of somatic cell hybrids that had retained relatively few mouse chromosomes postfusion. Given the difficulties in identifying the derivative 19^{11} and 9^{11} chromosomes on a complex mouse/rat background, further characterization of the selected clones was done by a combination of DNA amplification using the polymerase chain reaction (PCR) and Southern blotting. Since the position of the *Tk-1* locus relative to the T9Ad breakpoint was previously unknown, the hybrids were initially characterized by hybridization to probes whose map positions on MMU11 were well established and which were known to map well above (e.g., *Hba* and *Il-3*) or well below (e.g., *D11Jkn1, P4hb*) the breakpoints in band D. A negative hybridization signal obtained with any of these probes in a particular hybrid would indicate that the whole of MMU11, either in the form of an intact chromosome or a combination of both derivative translocation chromosomes, had not been retained in that hybrid. A positive mouse-specific hybridization signal or PCR amplification with a MMU11 marker would indicate that part of MMU11 had been retained in HAT medium. Subsequent exhaustive Southern blotting with markers that span the length of MMU11 was used to determine which derivative translocation chromosomes had been retained in particular hybrids and to check for complex chromosomal rearrangements that may have occurred during cell fusion. Following the molecular screening of these clones, further cytogenetic analysis was performed on the three somatic cell hybrid lines, T42H4B, T42H5B, and T9Ad27B, which formed the basis for our regional mapping assignments.

PCR amplification. Species-specific PCR using mouse-specific primers was performed on either lysed cells or DNA extracted from cells harvested from tissue culture. Cells were washed once in phosphate-buffered saline (PBS) and pelleted by centrifugation. For cell lysis without subsequent DNA extraction, 10^5 cells were mixed with 20 μl of lysis buffer (10% 10× Promega PCR buffer, 0.45% NP-40, 0.45% Tween 20) and 1 μl of 1 mg/ml proteinase K per 20 μl of lysis buffer. Cells were lysed at 55°C for 1 h followed by boiling for 10 min, prior to PCR. Five microliters of the cell suspension or 1 μg of extracted DNA was used for the PCR. DNA amplification was performed for the markers *Trp53* (Abbott, 1992), *Sigje* and *Cola-1* (Aitman *et al.*, 1991), *Hox-2, Myla,* and *Empb3* (Love *et al.*, 1990), *Mpo, Gfap,* and *D11Pas1* (Hearne *et al.*, 1991), and *D11Mit13* (Dietrich *et al.*, 1992) using identical cycling parameters: initial denaturing for 5 min at 95°C, then 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C for 30 cycles. For the markers *D11Mit11* and *D11Mit12* (Dietrich *et al.*, 1992) the cycling parameters were initial denaturing for 3 min at 94°C, followed by 25 cycles of 30 s at 94°C, 2 min at 55°C, and 2 min at 72°C. Mouse and rat parent cell line DNAs were included in each experiment to check for specificity of the primers, and a negative control with no DNA accompanied each set of reactions. PCR products were electrophoresed through a 4% agarose gel (3% Nusieve, 1% HGT agarose).

DNA extraction and Southern blotting. Genomic DNA was extracted from fibroblast cell pellets (Miller *et al.*, 1988) and 10 μg of hybrid DNA or 5 μg of parent cell line DNA was digested with 2–5 U/μg DNA with *Bam*HI, *Eco*RI, or *Hin*dIII restriction enzymes (Promega). After electrophoresis through 0.8% agarose gels, the DNA was Southern-blotted to Hybond N nylon membrane (Amersham) according to the manufacturer's recommendations. DNA probes (either the whole plasmid or the insert isolated in low-melting agarose) were labeled with [^{32}P]dCTP by random priming (Feinberg and Vogelstein, 1983) and hybridized at 63°C according to the recommendations for Hybond N. Posthybridization washes rarely exceeded two washes at 50°C in 0.1× SSC, 0.1% SDS. Autoradiography was for 3–7 days at −70°C.

DNA probes. Each of the probes used for the characterization of somatic cell hybrids was tested with the three different restriction enzymes to determine which enzyme produced fragments of different sizes in mouse and rat DNA. The probes, their mouse locus designations, the restriction enzymes used to isolate fragments for hybridization, and the sizes of these fragments, as well as the enzyme used for Southern blotting, are listed in Table 1.

RESULTS

G-banded translocation chromosomes of the T(11;19)42H and T(9;11)9Ad parent cell lines are shown in Fig. 1. The cytogenetic ordering of the respective breakpoints in band 11D was not possible at the level of resolu-

TABLE 1

DNA Probes Used for Hybridization to Somatic Cell Hybrid Lines

Probe	Mouse locus	Hybridization fragment	Diagnostic enzyme	Probe Ref.
erbb	*Erbb*	1.8 kb *Eco*RI	*Eco*RI	(71)
Murine α-globin	*Hba*	2.1 kb *Eco*RI	*Hin*dIII	(42)
pILM21	*Il-3*	0.45 kb *Eco*RI/*Bam*HI	*Bam*HI	(18)
pC33.1	*Sparc*	1.1 kb *Eco*RI/*Hin*dIII	*Eco*RI	(46)
pCRK-1	*Crk*	0.3 kb *Bam*HI/*Hin*dIII	*Eco*RI	(49)
HP1.0	*Pfn*	1.0 kb *Hin*cII/*Pst*I	*Hin*dIII	(40, 41)
pTB7	*D17s28h*	1.6 kb *Hin*dIII/*Eco*RI	*Hin*dIII	(57)
phJHT-3	*Glut-4*	1.7 kb *Eco*RI	*Bam*HI	(43)
pHRpII5.5	*Rpo2-1*	2.4 kb *Eco*RI/*Pst*I	*Eco*RI	(60)
pSP65	*Sigje*	0.6 kb *Eco*RI	*Pst*I	(10)
pMPO62	*Mpo*	0.4 kb *Hin*dIII/*Eco*RI	*Bam*HI	(34)
MO-4.1	*Hox-2*	2.6 kb *Eco*RI/*Sac*I	*Eco*RI	(21)
pgIH-I	*Cola-1*	14 kb *Eco*RI	*Bam*HI	(20)
p63	*Rara*	1.6 kb *Kpn*I/*Eco*RI	*Hin*dIII	(59)
pHG7Bg4	*Phb*	1.0 kb *Apa*I	*Bam*HI	(70)
pE2A3D	*Erba*	2.5 kb *Eco*RI/*Hin*dIII	*Hin*dIII	(33)
pUE236	*Rnu1a-1*	0.5 kb *Eco*RI	*Eco*RI	(26)
pcH800	*Gh*	0.8 kb *Hin*dIII	*Hin*dIII	(45)
pIJ0.8	*D11Jkn1*	0.8 kb	*Bam*HI	(31)
pHAGII	*Gaa*	2 kb *Eco*RI	*Eco*RI	(25)
pHTB	*P4hb*	2.0 kb *Pst*I/*Hin*dIII	*Bam*HI	(9)

tion obtained in this study. For the T42H series of hybrids, seven hybrid cell lines showed positive hybridization or amplification to all the markers used, thus indicating the probable retention of the entire MMU11 as either an intact chromosome or a combination of both translocation chromosomes. Four clones, T42H3A, 3B, 4B, and 5B, gave positive signals only for those markers mapping to the distal part of MMU11. It could thus be deduced that the only part of MMU11 retained in these hybrids was that on the 19^{11} derivative chromosome. In this way we have previously mapped the *Tk-1* locus to below the T42H breakpoint in 11D–qter (Morris and Robinson, 1991). The hybrid clones T42H4B and T42H5B were chosen for subsequent analysis and mapping.

In the T9Ad series of hybrids, all of the cell lines except one, T9Ad27B, showed positive hybridization to all probes tested, thus indicating the retention of the entire chromosome 11 either by itself, as two translocation products, or as a combination of these. The hybrid T9Ad27B proved negative for all markers except *Gaa, D11Jkn1,* and *P4hb,* all of which are on distal MMU11. Implicit in this result is the fact that *only the* 9^{11} chromosome had been retained in this hybrid and that the *Tk-1* locus must be distal to the T9Ad breakpoint. Representative G- and C-banded metaphase spreads from the somatic cell hybrid T9Ad27B are shown in Figs. 2A and 2B, and G-bands of the RT_2Tk^- parent and somatic cell hybrid T42H4B are presented in Figs. 2C and 2D, respectively. Neither MMU11 nor the larger derivative translocation products 11^{19} and 11^{9} which theoretically could have been retained in T42H4B and T9Ad27B, respectively, are present in the hybrids, thereby confirming our mapping data. While the identification of the derivative 19^{11} translocation product in T42H4B is clear (Fig. 2D, double arrow), the presence of the 9^{11} translocation chromosome in the T9Ad27B hybrid is less secure (Fig. 2A, double arrow). Unequivocal cytogenetic identification of this chromosome was complicated by the poorer G-band resolution consistently obtained for this clone and the presence of several chromosome fragments (Fig. 2A, single arrow). These fragments, however, were determined to be entirely heterochromatic (Fig. 2B and insert, single arrows).

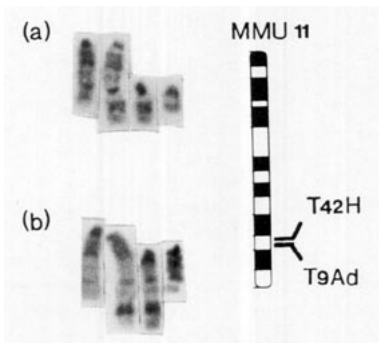

FIG. 1. G-banded translocation chromosomes of the T(11;19) *42H* and *T(9;11)9Ad* cell lines used in the construction of somatic cell hybrids. The chromosomes in order of presentation are (a) T42H: 11, 11^{19}, 19, 19^{11}; (b) T9Ad: 11, 11^{9}, 9, 9^{11}.

Negative mapping results were obtained for T9Ad27B with 14 of the loci that we had assigned to below the T42H breakpoint. These data indicated that these markers map to the region of 11D between the two breakpoints. Examples of hybridization of probes for *Erba, Cola-1, D11Jkn1,* and *P4hb* to Southern blots of DNA from the hybrids T42H4B, T42H5B, and T9Ad27B are shown in Fig. 3. Positive hybridization of

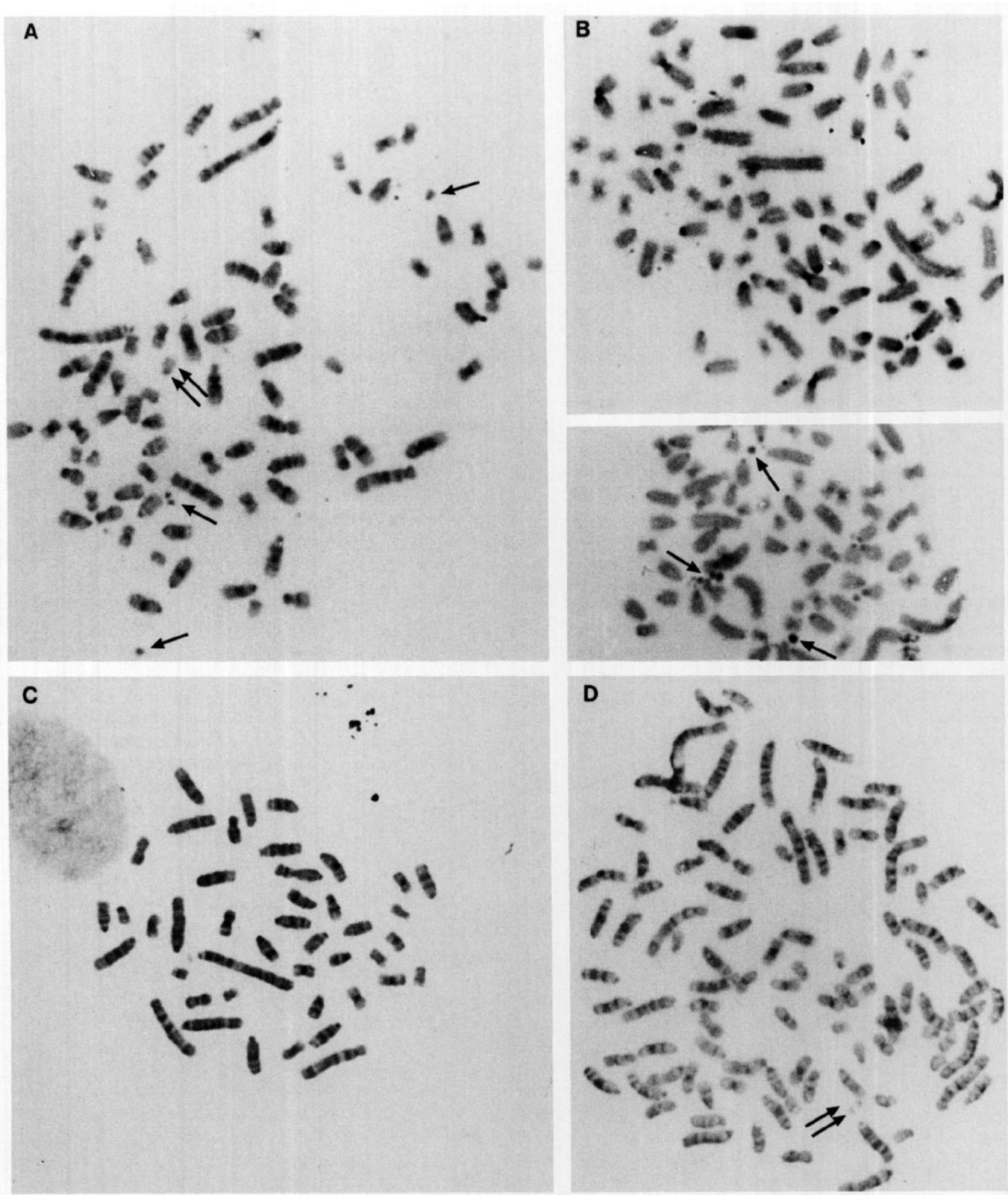

FIG. 2. Representative G-banded (**A**) and C-banded (**B**) metaphase cells of the somatic cell hybrid T9Ad27B. Chromosomal fragments (single arrows) and the 9^{11} translocation chromosome (double arrow) are indicated. G-banded chromosomes of the parent rat cell line, RT_2Tk^- (**C**); and a representative G-banded metaphase cell from hybrid T42H4B with the 19^{11} translocation chromosome (arrowed in **D**).

the probes for *D11Jkn1* and *Ph4b* to the T42H and T9Ad hybrids is shown in Figs. 3C and 3D, respectively, and the absence of hybridization of the *Erba* and *Cola-1* probes to T9Ad27B is shown in Figs. 3A and 3B, respectively. Our data therefore show that the T9Ad breakpoint is distal to that of T42H, and the loci *Hox-2, Cola-*

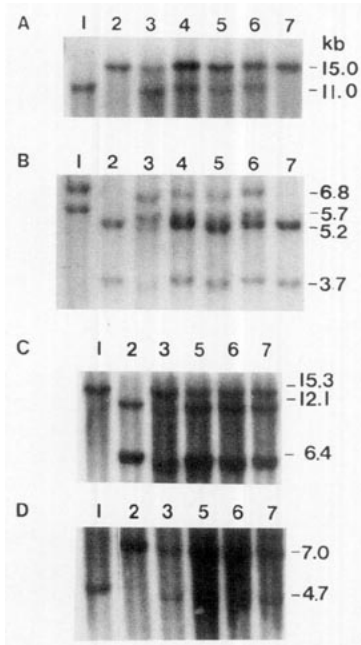

FIG. 3. Hybridization of DNA probes for *Erba* (**A**), *Cola-1* (**B**), *D11Jkn1* (**C**), and *P4hb* (**D**) to Southern blots of DNA digested with *Hin*dIII (**A**) and *Bam*HI (**B, C,** and **D**) from the following cell lines: **1,** mouse cell line C11D; **2,** rat cell line RT_2Tk^-; **3,** hybrid FO(11)J18 (whole MMU11); **4,** T42H1A (whole MMU11); **5,** T42H4B (11D-E); **6,** T42H5B (11D-E); **7,** T9Ad27B (11D-E).

1, Rara, Phb, Erba, Rnula-1, D11Pas1, Gfap, D11Mit13, D11Mit11, D11Mit12, Myla, Empb3, and *Gh* are between the two breakpoints. Similarly, the loci *Erbb, Hba, Il-3, Trp53, Crk, Pfn, D17s28h, Glut-4, Rpo2-1, Sigje,* and *Mpo,* which showed no hybridization to nor PCR amplification in the hybrids T42H4B (11D–E), T42H5B (11D–E), or T9Ad27B (11D–E), can be assigned to above the T42H and T9Ad breakpoints (11A–D) and *Tk-1, P4hb, Gaa,* and *D11Jkn1* to below the T9Ad breakpoint in bands D–E. For those of the above loci for which mouse-specific PCR primers were available (*Il-3, Sigje, Mpo, Cola-1,* and *Hox-2*), Southern blotting results were confirmed by PCR analysis.

Also using PCR amplification, the localizations of the microsatellite markers *D11Mit11, D11Mit12,* and *D11Mit13* were refined to between the two breakpoints in band D. PCR results for microsatellite markers at the loci *Sigje, D11Mit11,* and *D11Mit12* in all the T42H hybrids screened and T9Ad27B are shown in Fig. 4. The primers used for *Sigje* also amplified rat DNA, which is apparent as a smaller fragment than the mouse PCR product. Thus, hybrids that have the *Sigje* locus (T42H1A, 2A, 2B, 6A, 6B, 7A, and 7B) showed amplification of both fragments, whereas those lacking the murine *Sigje* locus (T42H3A, 3B, 4B, 5B, T9Ad27B, and all the neo clones which had been subjected to back-selection in BrdU) showed only the rat fragment. Positive mouse-specific amplification for *D11Mit11* and *D11Mit12* is apparent in all the hybrids, except the neo clones (which had lost any *Tk-1*-bearing part of MMU11) and T9Ad27B.

The markers used for the characterization of the T42H and T9Ad series of hybrids are listed in Table 2. The positions of these loci relative to the two breakpoints, their previous assignments to MMU11 and corresponding references, and the homologous human loci and chromosomal assignments are presented in Table 2. The markers in this table have been ordered within the groups above and below the breakpoints according to the most recent composite linkage map of MMU11 (Buchberg *et al.,* 1991). The linkage analysis of Dietrich *et al.* (1992) was used for the positioning of *D11Mit13, D11Mit11,* and *D11Mit12.* Markers mapped in this study have been placed arbitrarily within these groups. Following the linkage map, the T42H breakpoint can be localized between the markers *Mpo* and *Hox-2* and the T9Ad breakpoint to between *Gh* and *D11Jkn1.*

DISCUSSION

Using somatic cell hybrids containing different chromosome 11 translocation breakpoints, we have ordered T42H proximal to T9Ad in band D on MMU11 and have mapped markers relative to these breakpoints. These results provide a number of new assignments, as well as confirm and refine previous localizations to MMU11. The use of PCR amplification of microsatellite markers has proved to be a highly efficient means of characterizing new somatic cell hybrids (Abbott and Povey, 1991). As the technique is considerably more sensitive than Southern blotting, it has proved, in our hands, to be an efficient means of checking the clonality of hybrids. Weak PCR amplification of markers that showed no hybridization to Southern blots of hybrid DNA was determined to be due to a lack of clonality, and thus these hybrids could be excluded from further analysis. These

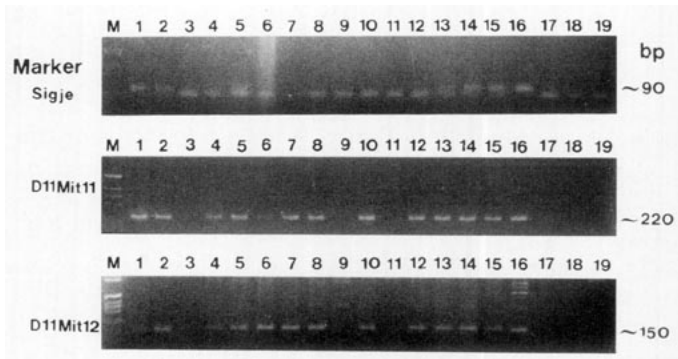

FIG. 4. PCR amplification of microsatellite markers at the loci *Sigje, D11Mit11,* and *D11Mit12* in all the T42H hybrids, T9Ad27B, and the neo clones (after back-selection with BrdU). Lanes: **M,** ϕX *Hae*III molecular weight marker; **1,** T(11;19)42H parent mouse cell line; **2,** T42H1A; **3,** T42H1Aneo2; **4,** T42H2A; **5,** T42H2B; **6,** T42H3A; **7,** T42H3B; **8,** T42H4B; **9,** T42H4Bneo2; **10,** T42H5B; **11,** T42H5Bneo2; **12,** T42H6A; **13,** T42H6B; **14,** T42H7A; **15,** T42H7B; **16,** T(9;11)9Ad parent mouse cell line; **17,** T9Ad27B; **18,** T9Ad27Bneo5; **19,** RT_2Tk^- rat parent cell line. The approximate sizes (in bp) of mouse-specific PCR products are shown on the right. Mouse-specific amplification with *Sigje* in T42H2A (lane **4**) and T42H6A (lane **12**) was very weak and the PCR reaction for T42H3B (lane **7**) was unsuccessful.

TABLE 2

Markers Mapped Relative to the T42H and T9Ad Breakpoints in This Study, Together with Previous Assignments to MMU11, Accompanying References, and Their Human Localization (Human Gene Mapping Workshop 11, 1991)

Mouse locus	MMU11 assignment	References to MMU11 assignments	Human locus	Human assignment
Erbb	11A1–A4	(71, 67)	EGFR	7p13–p12
Hba	11A	(42)	HBA1	16p13
Il-3	11A5–B1	(30, 69)	IL3	5q23–q31
Sparc	11B1	(47)	SPARC	5q31–q33
Rpo2-1	11B1–C	(60)	POLR2	17p13.1
Pfn	11	(40)	PFN1	17p13.3
D17s28h	11pter–D	This study	D17S28	17p13.3
Trp53	11B4–B5	(64)	TP53	17p13.1
Crk	11pter–D	This study	CRK-1	17p
Glut-4	11	(28)	GLUT4	17p13
Sigje	11	(68)	JE	17q11–q21
Mpo	11C–E1	(63)	MPO	17q21–q23
		T42H breakpoint		
Hox-2	11D–E	(61, 53, 22)	HOX2	17q21–q22
Cola-1	11D	(55)	COL1A1	17q21–q22
Rara	11D	(48)	RARA	17q21.1
Phb	11D	This study	PHB	17q21
Erba	11D–E	(71)	THRA1	17q11–q12
Rnula-1	11D–E	(50)	RNU2	17q12–q21
D11Pas1	11	(14)	UA[a]	—
Gfap	11	(6)	GFAP	17q21
Myla	11E	(62)	MYL4	17q21–qter
Empb3	11	(44)	EPB3	17q21–qter
Gh	11distal	(32, 15)	GH	17q24–q25
D11Mit13	11distal	(13)	DCP1	17q23
D11Mit11	11distal	(13)	UA	—
D11Mit12	11distal	(13)	UA	—
		T9AD breakpoint		
Gaa	11	(24)	GAA	17q23
Tk-1	11D-E	(27, 52)	TK1	17q23–q25
D11Jkn1	11	(31)	—	UA
P4hb	11	(8)	P4HB	17q25

[a] UA, unassigned.

data led us to conclude that most of the hybrids of the T9Ad series that were found to have retained all of MMU11 lacked clonality rather than having selectively retained an intact MMU11 or a combination of derivative chromosomes in each cell.

New assignments to MMU11 resulting from this study are those of the human homologue of the CRK-1 oncogene (Mayer *et al.*, 1988), the anonymous human DNA marker D17S28 to MMU11A–D, and the prohibitin gene, PHB, to between the two breakpoints in band D. CRK-1 and D17S28 are reported to map to HSA17p (D. H. Ledbetter, personal communication; Nakamura *et al.*, 1988, respectively) and PHB to 17q (White *et al.*, 1991) and thus these assignments strengthen the known homology between MMU11 and HSA17. The mapping of the prohibitin gene to 11D is of particular interest since this gene has recently been found to be altered in sporadic breast cancer (Sato *et al.*, 1992). Prohibitin is an intracellular protein with antiproliferative activity (Nuell *et al.*, 1991) and its locus was initially mapped to the region 17q21–q22 (White *et al.*, 1991), close to a locus linked to early-onset human breast cancer (Hall *et al.*, 1990). The finding of somatic mutations in tumor tissue of some breast cancer patients suggests that this gene may be a tumor suppressor gene associated with at least some breast cancers. The identification of the homologous mouse locus will be instrumental in the development of an animal model for breast cancer.

Further, our investigation has provided regional localization of the profilin (PFN1), insulin-responsive glucose transporter (*Glut-4*), and myeloperoxidase (*Mpo*) genes to above the T42H breakpoint (11A–D). The positions of all the markers mapped between the T42H and T9Ad breakpoints in this study, whose previous positions on MMU11 are given in Table 2, can now be refined/confirmed to be in band D. In addition, the mapping of *Tk-1* distal to the T9Ad breakpoint is supportive

of its localization to bands E1–E2 as reported by the *in situ* hybridization and chromosome aberration analyses of Hozier *et al.* (1991).

The number of important genes that have been mapped to band D is interesting and seems to confirm the hypothesis that more active genes will be found in G-negative than in G-positive chromosome bands (Korenberg *et al.*, 1988). G-negative bands presumably have a high GC content and many HTF islands. The genetic distance between the most proximal (*Hox-2*) and the most distal (*Gh*) markers mapped between the T42H and T9Ad breakpoints in this study is ±10 cM (Buchberg *et al.*, 1991). If 1 cM is equivalent to 2 Mb in the mouse genome (Davisson and Roderick, 1981), this would represent 20 Mb of DNA. This is an extremely large segment for DNA analyses; nevertheless, since it is one of the more densely mapped regions of MMU11, it would be amenable to pulsed-field gel electrophoresis (PFGE). PFGE on HSA17 in this region has physically linked HOX-2 and the nerve growth factor receptor gene locus (NGFR) on a 590-kb *MluI* fragment (Bentley *et al.*, 1989) and similar data on MMU11 would provide the ultimate comparison between these two chromosomes at the molecular level.

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