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Interferon-Regulated Influenza Virus Resistance Gene *Mx* Is Localized on Mouse Chromosome 16

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Genomic Southern blots of mouse-hamster somatic cell hybrids were analyzed with a probe prepared from a cDNA encoding murine Mx protein, the product of the interferon-regulated influenza virus resistance allele *Mx*$^+$. Results of this analysis indicate that the *Mx* gene is located on mouse chromosome 16. In appropriate backcross mice, no linkage was observed between *Mx* and *md*, a marker previously mapped close to the centromere of chromosome 16, suggesting a more distal localization of *Mx*.

Inbred mouse strains carrying different alleles at the influenza virus resistance locus *Mx* (15) differ from one another in relative susceptibility to infection with influenza viruses (7). Resistance selectively affects influenza viruses; susceptibility of mice to other viruses is not influenced by the *Mx* gene (7, 14, 15). In mouse cells carrying the resistance allele *Mx*$^+$, alpha/beta interferon induces the synthesis of a unique 75,000-dalton protein, termed Mx protein, that is not detectable in interferon-treated cells from *Mx*$^-$ mice lacking the influenza virus resistance allele (10, 21, 24). The Mx protein accumulates in the nucleus of *Mx*$^+$ cells treated with alpha/beta interferon (4). Gamma interferon does not efficiently induce synthesis of the Mx protein (4, 21) and, concomitantly, does not efficiently protect *Mx*$^+$ cells against influenza virus infection (24). Expression in *Mx*$^-$ 3T3 mouse cells of cDNA encoding the Mx protein led to the accumulation of recombinant Mx protein in the nuclei of transfected cells and, at the same time, conferred to these cells resistance against infection with influenza virus (23). The Mx protein inhibits influenza virus replication at an early step (9) presumably by affecting primary transcription (12) or else mainly translation of influenza virus-specific mRNAs (16).

Classical genetic analysis did not reveal the chromosomal localization of the *Mx* gene. Forty-eight genetic markers have been tested over the years for possible linkage to *Mx*. Although the markers involved were distributed widely over all of the chromosomes and thus covered almost the entire mouse genome apart from chromosome 16, no linkage to *Mx* was found (J. Lindenmann, L.-G. Lundin, and O. Haller, unpublished observations). An alternative approach to map the *Mx* gene became feasible with the availability of an appropriate cDNA probe (23). Here, we report that genomic Southern analysis of mouse-hamster somatic cell hybrids with a probe derived from the *Mx*$^+$ cDNA maps the *Mx* gene to mouse chromosome 16.

We have shown previously that Southern blots of *Eco*RI-digested liver DNA from BALB/c mice (homozygous for an *Mx*$^-$ allele) give three major bands at 2.5, 4, and 10 kilobases (kb) when probed with the radiolabeled 1.65-kb *Bam*HI fragment of plasmid pMx41 (23). The same restriction pattern was observed with *Eco*RI-digested DNA from the BALB/c cell line MethA, the parental mouse cell line used to generate hamster-mouse cell hybrids. At low washing stringency, *Eco*RI-digested MethA mouse DNA gave strong hybridization signals at 4 and 10 kb, a signal of moderate intensity at 2.5 kb, and signals of low intensities at 4.2 and 6 kb (Fig. 1). *Eco*RI digestion of DNA from the Chinese hamster cell line E36 generated several fragments ranging from about 2.5 to 15 kb which showed weak cross-hybridization to the mouse probe. Under the experimental conditions used, hybridizing *Eco*RI fragments of mouse-hamster hybrid cell lines could easily be classified by their mobilities relative to marker DNAs as either mouse DNA-derived or hamster DNA-derived fragments (Fig. 1). DNA from a set of nine mouse-hamster hybrid cell lines was analyzed. Table 1 characterizes these cell lines with respect to their contents of mouse genetic material, which varies from 2 to 17 mouse chromosomes retained in individual hybrid cell lines. Three of the hybrid cell lines, namely, 4B31Az3, TUCE12G/7, and mAE32, gave both mouse-specific and hamster-specific hybridization signals when probed with *Mx* cDNA, while the other six hybrid cell lines gave the hamster-specific signals only (Fig. 1; Table 1). Hybrid cell line mFE2/3 carries all mouse chromosomes except 5, 9, and 16. Yet, we were unable to detect mouse-specific hybridization signals in mFE2/3 DNA. On the other hand, the microcell hybrid mAE32 contains only mouse chromosomes 16 and X. Nevertheless, we observed strong mouse-specific hybridization signals in *Eco*RI-digested mAE32 DNA, suggesting that the *Mx* gene is localized on chromosome 16. A complete concordance study is presented in Table 2. These data unambiguously assign the *Mx* gene to mouse chromosome 16.

Chromosome 16 is presently one of the least well-characterized of the autosomal chromosomes of the mouse. The relative positions of only a few genetic markers are known. Markers *md* (6), *Akv-2* (11), *Mtv-6* (1), and *Igl-1* (3) are located on the proximal part of chromosome 16; *dw* (6) maps to the middle part of the chromosome, and *wv* (6) is on the distal part of chromosome 16. We performed a classical backcross linkage analysis with the coat color marker *md*. A2G females (+/+; *Mx*$^+$/*Mx*$^+$) were mated with C57BL/6J-*md* males (*md*/*md*; *Mx*$^-$/*Mx*$^-$), and the resulting heterozygous F_1 females were backcrossed to their parental

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TABLE 1. Mouse chromosomes retained in hamster-mouse hybrid cell lines and segregation of *Mx*

Hybrid	Mouse chromosomes retained																				*Mx*
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	
mFE2/3	+	+	+	+		+	+	+		+	+	+	+	+	+		+	+	+	+	−
mFE2/1/7	+	+	+			+	+	+	+			+	+		+		+		+	+	−
4B31Az3		+					+					+			+	+			+		+
mc8	+	+		+			+		+			+	+	+					+	+	−
TUCE12G/7		+			+	+[a]		+[a]		+		+	+	+	+	+	+	+	+	+	+
ma8c												+								+	−
CEC						+[b]														+[b]	−
R44																	+[c]	+[c]			−
mAE32																+				+	+

[a] The presence of portions of these chromosomes was demonstrated by isozyme analysis (18); the segments retained by chromosomes 6 and 8 could not be identified cytologically.

[b] Chromosomes joined in an X/6 translocation; the rearranged chromosome contains the entire X chromosome and the portion of chromosome 6 distal to bands B1/B2 (17, 25).

[c] Cytologically normal chromosomes 17 and 18 included in a large rearranged chromosome which contains some additional material of unknown chromosomal origin.

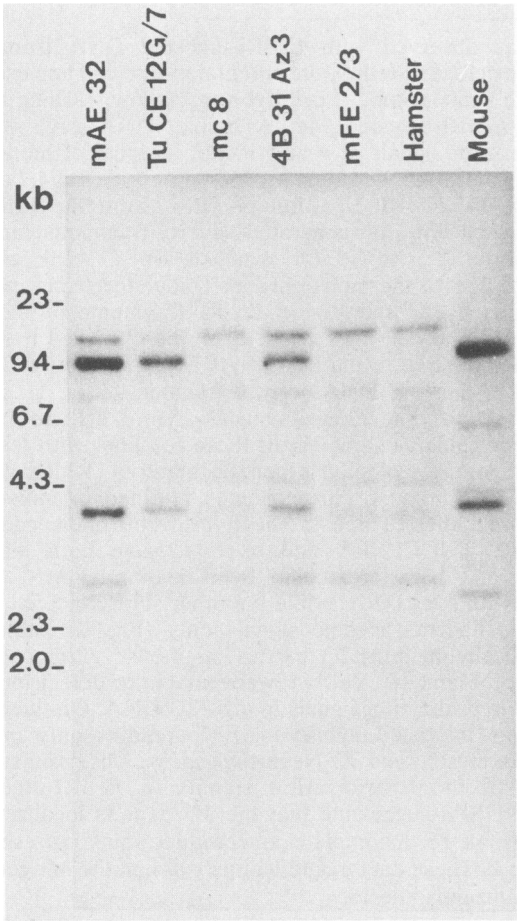

FIG. 1. Genomic Southern analysis of mouse-hamster cell hybrids. A 15-μg portion of high-molecular-weight DNA from parental hamster E36 or mouse MethA cell lines, or from the mouse-hamster hybrid cell lines mAE32, TUCE12G/7, mc8, 4B31Az3, and mFE2/3, respectively, was completely digested with restriction endonuclease *Eco*RI, electrophoresed through 0.8% agarose gels, transferred to a nitrocellulose membrane, and hybridized with the ^{32}P-radiolabeled 1.65-kb *Bam*HI fragment of pMx41 as described previously (23). The membrane was washed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 45°C before exposure to X-ray films at −70°C with an intensifier screen. Size markers were generated by digesting phage λ DNA with *Hin*dIII.

C57BL/6J-*md* fathers. Fifty-eight of 108 backcross animals analyzed showed recombination between the two loci *Mx* and *md*, giving a recombination frequency ± standard error of 55.2 ± 4.8% (Table 3). We conclude from these data that *Mx* most likely maps to the distal portion of chromosome 16.

Assignment of the *Mx* gene to mouse chromosome 16 has both theoretical and practical implications. Among the genes shown by somatic cell hybrid analysis to be on mouse chromosome 16 is the gene *Ifrc* (2, 13), which encodes the mouse interferon type I cell surface receptor. At present, we do not know whether *Mx* and *Ifrc* are closely linked. The human gene encoding interferon type I receptor has been assigned to chromosome 21 (5). It will be of interest to learn whether in humans, in analogy to the mouse, chromosome

TABLE 2. Cosegregation of *Mx* and chromosome 16 in hamster-mouse hybrid cell lines

Mouse chromosome	Concordance[a]		Disconcordance[a]		% Concordance
	+/+	−/−	+/−	−/+	
1	0	3	3	3	33
2	2	3	3	1	55
3	0	4	2	3	44
4	0	4	2	3	44
5	1	6	0	2	77
6	0	3	2	2	43[b]
7	1	3	3	2	44
8	0	4	2	2	50[b]
9	0	4	2	3	44
10	1	5	1	2	66
11	0	5	1	3	55
12	2	2	4	1	44
13	1	3	3	2	44
14	1	4	2	2	55
15	2	4	2	1	66
16	3	6	0	0	100
17	1	3	3	2	44
18	1	4	2	2	55
19	2	3	3	1	55
X	2	1	5	1	33

[a] Read as follows: e.g., two hybrids contain chromosome 2 and *Mx* (+/+), three hybrids lack chromosome 2 and also lack *Mx* (−/−), three hybrids contain chromosome 2 but lack *Mx* (+/−), and one hybrid lacks chromosome 2 but contains *Mx* (−/+).

[b] The rearranged chromosomes 6 and 8 were not included in the calculations.

TABLE 3. Recombination frequencies of *Mx* and *md* in backcross mice[a]

Genotype		No.	Recombinants (*Mx*—*md*)[b]
Mx	*md*		
+/−	*md*/+	28	
−/−	*md*/*md*	22	
+/−	*md*/*md*	29	29
−/−	*md*/+	29	29

[a] Mice (genotype) and crosses were as follows: C57BL/6J-*md* (*Mx*$^-$/*Mx*$^-$,*md*/*md*); A2G (*Mx*$^+$/*Mx*$^+$, +/+); F_1 = (A2G × C57BL/6J-*md*); (F_1 × C57BL/6J-*md*)BC were analyzed. To assess the *Mx* genotype, animals were inoculated intracerebrally with 100 50% lethal doses of neurotropic influenza A virus NWS as described before (15). Surviving animals were classified as *Mx*$^+$/*Mx*$^-$.

[b] Recombination frequency was 55.2 ± 4.8%.

21 carries also the gene encoding the human Mx protein homolog (22).

Two other mouse genes whose activities are stringently regulated by interferons, *202* (20) and *Gbp-1* (19), have previously been mapped to chromosomes 1 and 3, respectively. Our finding that *Mx* maps to chromosome 16 is further evidence that interferon-regulated genes are not clustered but rather are randomly distributed over the mouse genome.

Genetic analysis of mouse chromosome 16 suffers from lack of sufficient markers. Precise mapping of *Mx* might help to accelerate the fine mapping of other genes that have been tentatively assigned to chromosome 16. *Mx* is a convenient marker: the presence of the dominant allele *Mx*$^+$ can rapidly be assessed in large numbers of offspring by testing them for resistance to influenza viruses (7). Alternatively, interferon-induced resistance against influenza virus (8) and induced synthesis of the Mx protein (10, 21) can be detected in cells in tissue culture. Finally, restriction fragment length polymorphisms allow us to distinguish not only between *Mx*$^+$ and *Mx*$^-$ but also between different *Mx*$^-$ alleles (23).

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