Genetic Mapping of the *Mx* Influenza Virus Resistance Gene within the Region of Mouse Chromosome 16 That Is Homologous to Human Chromosome 21

ROGER H. REEVES,^{1*} BRUCE F. O'HARA,¹ WILLIAM J. PAVAN,¹ JOHN D. GEARHART,^{1,2}
AND OTTO HALLER³

Departments of Physiology¹ and Cell Biology and Anatomy,² Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, and Institute for Immunology and Virology, University of Zurich, Zurich, Switzerland³

Received 11 March 1988/Accepted 12 July 1988

A total of 318 progeny from four backcrosses involving different laboratory strains and subspecies of *Mus musculus* were analyzed to map the *Mx* gene to the region of mouse chromosome 16 (MMU 16) which is homologous to human chromosome 21 (HSA 21). This result suggests that *Mx* will be found in the region of HSA 21 which has been implicated in Down syndrome when inherited in three copies.

The *Mx* gene is responsible for the resistance of some strains of mice to orthomyxovirus infection (9, 11). The expression of *Mx* is tightly regulated by alpha and beta interferons (19), the receptor for which is encoded by a gene located on mouse chromosome 16 (MMU 16) and on human chromosome 21 (HSA 21) (mouse, *Ifr*; human, *IFREC*) (2, 10). Three transcriptionally active alleles of *Mx* have been reported. The *Mx*⁺ allele encodes a nuclear protein of approximately 72 kilodaltons which is responsible for the viral resistance phenotype (8). Chromosomal DNA analysis has shown that the *Mx*⁺ allele consists of 14 exons distributed over at least 55 kilobases of DNA (9a). Two *Mx* mutant alleles produce nonfunctional transcripts, and mice homozygous for these alleles are susceptible to influenza virus infection. The *Mx*⁻ phenotype of strain BALB/cJ is due to a deletion of three exons from the genome, while that of CBA/J probably arises from a nonsense mutation in the *Mx* coding region (19a). The majority of laboratory strains carry the BALB/cJ-type *Mx* mutant allele, which can be distinguished from *Mx*⁺ and the CBA/J *Mx* mutation on the basis of restriction fragment length polymorphisms (RFLPs) (21). The genetics, physiology, and cellular and molecular biology of the *Mx* gene and its products have been studied in mice, rats, and humans (reviewed in reference 20).

Mx has been mapped to MMU 16 (22) and, recently, to HSA 21 (M. Horisberger, M. Wathelot, J. Szpirer, C. Szpirer, Q. Islam, G. Lavan, G. Huez, and J. Content, Somatic Cell Mol. Genet., in press). Six genes and three anonymous DNA segments which map to HSA 21 have been mapped previously to MMU 16, and eight of them have been localized genetically or cytologically to the distal end of the mouse chromosome (1, 15, 17; S. V. Cheng, J. H. Nadeau, R. E. Tanzi, P. C. Watkins, J. Jagadeesh, B. A. Taylor, J. L. Haines, N. Sacchi, and J. F. Gusella, Proc. Natl. Acad. Sci. USA, in press). We report here that *Mx* is tightly linked to the proto-oncogene *Ets-2* within the cluster of genes common to the human and mouse chromosomes. Since this region is highly conserved between the two species, it is likely that the human *Mx* gene will be found in the corresponding location on HSA 21.

Molecular probes (6) recognizing the *Mx* gene were synthesized from a 2.3-kilobase *Bam*HI fragment derived from

cDNA clone pMx34 (21) or from a 1.7-kilobase fragment derived by *Taq*I digestion of the 2.3-kilobase segment. These probes were used to visualize segregation of *Mx* RFLPs in four backcrosses, two of which have been used previously to localize nine genes on MMU 16. The backcross (Czech II × BALB/cPt) × Czech II is designated CZCxC (15). Czech II is an inbred strain derived from *Mus musculus musculus*. The backcross (CBA/J × BALB/cJ) × BALB/cJ is designated CBCxC (16).

Two additional backcrosses were produced with mice segregating the dwarf (*dw*) gene, (CBA/J × DW/J) × DW/J and (MOLD/Rk × DW/J) × DW/J. These crosses are designated CBDWxDW and MODWxDW, respectively. MOLD/Rk is an inbred strain derived from *Mus musculus molossinus* by Thomas Roderick at the Jackson Laboratory. Male *dw/dw* animals were made fertile by administration of ovine growth hormone (50 µg/day; National Hormone and Pituitary Program, University of Maryland School of Medicine) and thyroxine (2 µg three to five times per week) (Andrej Bartke, personal communication).

DNA was extracted from organs of backcross progeny at 3 weeks of age, when *dw/dw* animals were easily distinguished from *dw/+* littermates by their retarded growth (7). Restriction analysis of *Ets-2* and of the gene encoding the cytoplasmic form of superoxide dismutase, *Sod-1*, was accomplished with molecular probes as described previously (14). Backcross data were compiled in a database with Lotus 1-2-3 software, and a program written in the simple programming language of Lotus 1-2-3 was used to extract different classes of backcross progeny, to determine gene order, and to calculate recombination frequency (14).

RFLPs were used to distinguish *Mx* alleles among the four strains analyzed in this study (Fig. 1). DW/J, BALB/cJ, and BALB/cPt DNAs were identical at all loci. Additional RFLP differences were detected after digestion with *Xba*I, *Hinc*II, *Bam*HI, *Sst*I, and *Taq*I (data not shown). Staehli et al. (21) have shown previously that particular RFLPs are characteristic of strains which are *Mx*⁺ or *Mx*⁻. The restriction patterns obtained from Czech II and MOLD/Rk DNAs with the enzymes *Eco*RI, *Hind*III, and *Pst*I are identical to those from DNA of BALB.A2G-Mx mice, which are *Mx*⁺, suggesting that Czech II and MOLD/Rk mice are *Mx*⁺ as well. However, the animals must be challenged with virus to determine with certainty the nature of their *Mx* phenotypes,

* Corresponding author.

FIG. 1. Restriction analysis of DNAs from strains CBA/J, Czech II, BALB/cJ, and MOLD/Rk with the *Mx* probe. DW/J and BALB/cJ DNAs gave identical patterns with all restriction endonucleases. The Czech II and MOLD/Rk patterns are identical to those reported for *Mx*⁺ mice (18). (a) *Bgl*II; (b) *Eco*RI; (c) *Hind*III; (d) *Pst*I. (e) DNAs from the progeny of the CBCxC backcross analyzed after digestion with *Bgl*II. Positions of molecular weight markers are indicated. kb, Kilobases.

since restriction patterns of DNA from the *Mx*⁻ strain, CBA/J, are the same as those seen in DNA from *Mx*⁺ animals with several restriction endonucleases (19a).

In a previous analysis of the CZCxCZ backcross, the *Sod-1* locus was reported to be proximal to *Ets-2* (15). This conclusion was based on a single recombination between these genes among 86 progeny in a three-point analysis with *Mtv-6*, an endogenous retroviral sequence which maps 25 centimorgans (cM) proximal to *Sod-1*. This gene order was confirmed by the analysis of *Sod-1* and *Ets-2* relative to *dw* in the MODWxDW backcross (Table 1). Four recombinations occurred between *Sod-1* and *Ets-2*. In each case, the *Sod-1* allele was of the same parental type as *dw*. An additional recombinant in the CBDWxDW cross was also consistent with this gene order. The map distances calculated on MODWxDW place *Sod-1* 4 cM proximal to *Ets-2* and 15 cM distal to *dw*.

Analysis of *Mx* in 318 animals from the four backcrosses identified only two recombinations between *Mx* and the closest marker, *Ets-2* (Table 2). Thus, these genes are tightly linked, and *Mx* must be near the distal end of MMU 16. While *Mx* is clearly distal to *Sod-1*, its position relative to *Ets-2* cannot be ascertained from these data. In the

TABLE 1. Three-point analysis of *dw*, *Sod-1*, and *Ets-2* on the MODWxDW backcross^a

<i>dw</i>	<i>Sod-1</i>	<i>Ets-2</i>	Frequency
M ^b	M	M	33
T ^c	T	T	41
M	M	T	2
T	T	M	2
M	T	T	7
T	M	M	7
M	T	M	0
T	M	T	0

^a Recombination frequencies: *dw* to *Sod-1*, 0.152 ± 0.037 ; *dw* to *Ets-2*, 0.196 ± 0.041 ; *Sod-1* to *Ets-2*, 0.043 ± 0.021 . Pairwise recombination frequencies are listed with standard errors. Recombination frequency is the number of recombinations between a gene pair divided by the total number of animals tested (*n*). Standard error is calculated as $\pm p \times [(1 - p)/n]$, where *p* is the recombination frequency.

^b M, Homozygous for the DW/J type allele.

^c T, Heterozygous for the DW/J type allele.

TABLE 2. Pairwise recombination fractions determined on four backcrosses

Backcross	Gene	<i>dw</i>	<i>Sod-1</i>	<i>Mx</i>
CBDWxDW	<i>Sod-1</i>	10/64		
	<i>Mx</i>	12/69	2/64	
	<i>Ets-2</i>	11/69	2/64	1/64
MODWxDW	<i>Sod-1</i>	14/92		
	<i>Mx</i>	18/92	4/92	
	<i>Ets-2</i>	18/92	4/92	0/92
CZCxCZ	<i>Mx</i>		1/87	
	<i>Ets-2</i>		1/93	0/87
CBCxC	<i>Mx</i>		5/71	
	<i>Ets-2</i>		6/71	1/71

single crossover between these genes in the CBDWxDW cross, *Ets-2* and *Sod-1* alleles from the F₁ parent were of the DW/J type, while *Mx* was of the CBA/J type, suggesting that *Ets-2* is proximal to *Mx*. Analysis of the CBCxC cross, however, indicated the opposite order for these genes. In the single CBCxC animal in which recombination occurred between *Ets-2* and *Mx*, *Sod-1* and *Mx* were of the same type, CBA/J, while the relevant *Ets-2* allele was from BALB/cJ. Each of these animals was typed three or more times with each of the three probes with the same result. Thus, it appears that one of the recombinant chromosomes represents a double crossover within the 4-cM region in which these genes are located. Four additional recombinants between *Mx* and *Ets-2* detected in a large backcross with the BALB/cJ and MOLD/Rk strains are consistent with a gene order in which *Mx* is proximal to *Ets-2* and distal to the gene encoding the amyloid precursor protein, *App* (B. F. O'Hara et al., manuscript in preparation).

The proximal-to-distal gene order determined in this study was *dw-Sod-1-(Mx-Ets-2)* (Fig. 2). The *Mx* gene was reported previously to be unlinked to the proximal MMU 16 marker, *md* (55% recombination, *n* = 108) (22), consistent with the results presented here. *Mx* is near several genes which, in humans, are found in the region of HSA 21 associated with Down syndrome when present in three copies. *Sod-1*, *Ets-2*, *App*, and the gene *Prgs*, which encodes the purine synthesis enzyme phosphoribosyl glycinamide synthetase, are located in the region C3 → ter on MMU 16 (1, 12, 15) and on HSA 21 (13, 18, 24). Thus, it is likely that the human *Mx* homolog will be found in the analogous region of HSA 21.

Gene dosage imbalance occasioned by trisomy generally results in an increase in the amount of gene product in the cell. In the case of *IFREC*, the 50% increase in receptor seen in fibroblasts from individuals with Down syndrome results in a three- to eightfold increase in sensitivity to interferon, as measured in a vesicular stomatitis virus protection assay (23). Since *Mx* expression is tightly regulated by interferon, it will be of interest to study *Mx* expression in individuals with Down syndrome, in whom both *IFREC* and *Mx* are present in three copies. Individuals with Down syndrome exhibit impaired immune function including increased susceptibility to opportunistic infections (5).

This work was supported by Public Health Service grants R01 HD 22262 (R.R.) and P01 HD 19920 (R.R. and J.G.) from the National Institutes of Health and by Swiss National Science Foundation grant 3.507-0.86 (O.H.).

FIG. 2. Derived genetic map of MMU 16. Genes mapped in this study are positioned by averaging data from different crosses. This type of map serves as a basis for comparison of actual distances determined on each cross, which can be determined from Table 2. Positions of markers not examined in this cross are summarized elsewhere (3, 4, 15; Cheng et al., in press; R. H. Reeves, R. A. Morgan, C. Bendotti, M. L. Oster-Granite, J. T. Coyle, and J. D. Gearhart, in P. Davies, and C. Finch, ed., *The Molecular Biology of Alzheimer's Disease*, in press; O'Hara et al., in preparation).

LITERATURE CITED

1. Cox, D. R., and C. J. Epstein. 1985. Comparative gene mapping of human chromosome 21 and mouse chromosome 16. *Ann. N.Y. Acad. Sci.* 450:169-178.
2. Cox, D. R., L. B. Epstein, and C. J. Epstein. 1980. Genes coding for sensitivity to interferon (IFREC) and soluble superoxide dismutase (SOD-1) are linked in mouse and man and map to mouse chromosome 16. *Proc. Natl. Acad. Sci. USA* 77:2168-2172.
3. Davisson, M. T., and T. H. Roderick. 1981. Recombination percentages, p. 283-313. In M. C. Green (ed.), *Genetic variants and strains of the laboratory mouse*. Gustav Fischer Verlag, New York.
4. Davisson, M. T., and T. H. Roderick. 1987. Genetic map of the mouse. *Mouse News Letter* 79:5-9.
5. Epstein, C. J. 1986. The consequences of chromosomal imbalance, p. 283-297. Cambridge University Press, London.
6. Feinberg, A., and B. Vogelstein. 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 137:266-267.
7. Fuhrmann, G., D. Di Scala-Guenot, and A. Ebel. 1985. Somatostatin levels in the central nervous system of the Snell dwarf

- mouse; is somatostatin excess the primary molecular defect in the dw/dw dwarfism? *Brain Res.* **328**:161-164.
8. **Haller, O.** 1981. Inborn resistance of mice to orthomyxoviruses. *Curr. Top. Microbiol. Immunol.* **92**:25-52.
 9. **Haller, O., M. Acklin, and P. Staehli.** 1987. Influenza virus resistance of wild mice; wild-type and mutant *Mx* alleles occur at comparable frequency. *J. Interferon. Res.* **7**:647-656.
 - 9a. **Hug, H., M. Coastas, P. Staehli, M. Aebi, and C. Weissmann.** 1988. Organization of the murine *Mx* gene and characterization of its interferon- and virus-inducible promoter. *Mol. Cell. Biol.* **8**:3065-3079.
 10. **Lin, P.-F., D. L. Slate, F. C. Lawyer, and F. H. Ruddle.** 1980. Assignment of the murine interferon sensitivity and cytoplasmic superoxide dismutase genes to chromosome 16. *Science* **209**:285-287.
 11. **Lindenmann, J.** 1964. Inheritance of resistance to influenza virus in mice. *Proc. Soc. Exp. Biol. Med.* **116**:505-509.
 12. **Lovett, M., D. Goldgaber, P. Ashley, D. R. Cox, D. C. Gajdusek, and C. J. Epstein.** 1987. The mouse homolog of the human amyloid B protein gene is located in the distal end of mouse chromosome 16: further extension of the homology between human chromosome 21 and mouse chromosome 16. *Biochem. Biophys. Res. Commun.* **144**:1069-1075.
 13. **Moore, E. E., C. Jones, F.-T. Kao, and D. C. Oates.** 1977. Synteny between glycineamide ribonucleotide synthetase and superoxide dismutase (soluble). *Am. J. Hum. Genet.* **29**:389-396.
 14. **Reeves, R. H.** 1988. Use of Lotus 1-2-3 spreadsheet software for managing and analyzing genetic backcross data. *Biotechniques* **6**:12-14.
 15. **Reeves, R. H., D. Gallahan, B. F. O'Hara, R. Callahan, and J. D. Gearhart.** 1987. Genetic mapping of *Prm-1*, *Ig1-1*, *Smst*, *Mtv-6*, *Sod-1*, and *Ets-2* and localization of the Down syndrome region on mouse chromosome 16. *Cytogenet. Cell Genet.* **44**:76-81.
 16. **Reeves, R. H., J. D. Gearhart, and J. W. Littlefield.** 1986. Genetic basis for a mouse model of Down syndrome. *Brain Res. Bull.* **16**:803-814.
 17. **Reeves, R. H., N. K. Robakis, M. L. Oster-Granite, H. M. Wisniewski, J. T. Coyle, and J. D. Gearhart.** 1987. Genetic linkage in the mouse of genes involved in Down syndrome and Alzheimer's disease in man. *Mol. Brain. Res.* **2**:215-221.
 18. **Robakis, N. K., H. M. Wisniewski, E. C. Jenkins, E. A. Devine-Gage, G. E. Houck, W. P. Silverman, and W. T. Brown.** 1987. Chromosome 21q21 sublocalization of the gene encoding the beta-amyloid peptide present in cerebral vessels and neuritic (senile) plaques of people with Alzheimer disease and Down syndrome. *Lancet* **i**:384-385.
 19. **Staehli, P., P. Danielson, O. Haller, and J. G. Sutcliffe.** 1986. Transcriptional activation of the mouse *Mx* gene by type I interferon. *Mol. Cell. Biol.* **6**:4770-4774.
 - 19a. **Staehli, P., R. Grob, E. Meier, J. G. Sutcliffe, and O. Haller.** 1988. Influenza virus-susceptible mice carry *Mx* genes with a large deletion or a nonsense mutation. *Mol. Cell. Biol.* **8**:4518-4523.
 20. **Staehli, P., and O. Haller.** 1987. Interferon-induced *Mx* protein: a mediator of cellular resistance to influenza virus. *Interferon* **8**:1-23.
 21. **Staehli, P., O. Haller, W. Boll, J. Lindenmann, and C. Weissmann.** 1986. *Mx* protein: constitutive expression in 3T3 cells transformed with cloned *Mx* cDNA confers selective resistance to influenza virus. *Cell* **44**:147-158.
 22. **Staehli, P., D. Pravtcheva, L.-G. Lundin, M. Acklin, F. Ruddle, J. Lindenmann, and O. Haller.** 1986. Interferon-regulated influenza virus resistance gene *Mx* is localized on mouse chromosome 16. *J. Virol.* **58**:967-969.
 23. **Tan, Y. H., E. L. Schneider, J. Tischfield, C. J. Epstein, and F. H. Ruddle.** 1974. Human chromosome 21 dosage: effect on the expression of the interferon-induced antiviral state. *Science* **185**:61-63.
 24. **Tanzi, R. E., J. F. Gusella, P. C. Watkins, G. A. P. Bruns, P. St. George-Hyslop, M. L. VanKeuren, D. Patterson, S. Pagan, D. M. Kurnit, and R. L. Neve.** 1987. Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science* **235**:880-884.