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Influenza Virus-Susceptible Mice Carry *Mx* Genes with a Large Deletion or a Nonsense Mutation

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The interferon-regulated mouse *Mx* gene encodes the 72-kilodalton nuclear Mx protein that selectively inhibits influenza virus replication. Mice carrying Mx^+ alleles synthesize Mx protein and resist influenza virus infection, whereas mice homozygous for Mx^- alleles fail to synthesize Mx protein and, as a consequence, are influenza virus susceptible. Southern blot analysis allowed us to define the following three distinct *Mx* restriction fragment length polymorphism (RFLP) types among classical inbred strains: RFLP type 1 in the Mx^+ strains A2G and SL/NiA, RFLP type 2 in BALB/c and 33 other Mx^- strains, and RFLP type 3 in CBA/J and 2 other Mx^- strains. cDNA clones of *Mx* mRNAs from BALB/c and CBA/J cells were isolated, and their sequences were compared with that of the wild-type *Mx* mRNA of strain A2G. *Mx* mRNA of BALB/c mice has 424 nucleotides absent from the coding region, resulting in a frame shift and premature termination of Mx protein. The missing sequences correspond exactly to *Mx* exons 9 through 11. These three exons, together with some flanking intron sequences, are deleted from the genomes of all *Mx* RFLP type 2 strains. The Mx^- phenotype of the *Mx* RFLP type 3 strain CBA/J is due to a point mutation that converts the lysine codon in position 389 to a termination codon. *Mx* RFLP type 3 strains have an extra *Hin*dIII site which maps to an intron and thus probably does not affect the coding capacity of *Mx* mRNA. We further show that the *Mx* mRNA levels in interferon-treated BALB/c and CBA/J cells are about 15-fold lower than in similarly treated Mx^+ cells. This is probably due to decreased metabolic stabilities of the mutant mRNAs.

Influenza virus resistance of mice is controlled by alleles at the *Mx* locus on chromosome 16 (for reviews, see references 8 and 25). The Mx^+ allele is present in influenza virus-resistant inbred and wild mice (9). It is transcriptionally activated by alpha and beta interferons (IFN-α and IFN-β) to produce a 3.3-kilobase (kb) mRNA encoding the nuclear 72-kilodalton Mx protein (23, 26). Cells of influenza virus-susceptible Mx^- mice fail to synthesize Mx protein (5, 10, 24, 27). A full-length cDNA encoding Mx protein was cloned and constitutively expressed in Mx^- cells. Such cells are resistant to infection with influenza virus but are still susceptible to infection with vesicular stomatitis virus, a rhabdovirus, demonstrating that Mx protein is the only IFN-induced product necessary for resistance to influenza virus (26). Transfected nonmurine cells that constitutively express *Mx* cDNA are also resistant to influenza virus (16).

Analysis of cloned genomic DNA from Mx^+ mice revealed that Mx protein is encoded by a gene consisting of 14 exons distributed over more than 55 kb of DNA (11). Probing of Southern blots of mouse genomic DNA with *Mx* cDNA yields a complex pattern of strong and weak signals, some of which are missing or have altered mobilities in Mx^- strains (26). Not all of these signals originate from a single gene; some are from hybridization of the *Mx* probe to one or more additional genes with closely related sequences. The structure of such an *Mx*-related gene, now designated *Mx2*, is presented elsewhere (29). To learn more about mutations which lead to influenza virus susceptibility, we initiated a detailed molecular analysis of Mx^- alleles. We report here that all 39 of the commonly used old inbred mouse strains that we tested can be grouped into three classes by virtue of the characteristic Southern blot patterns that their DNAs display upon being probed with *Mx* cDNA. Prototype strains of each of these restriction fragment length polymorphism (RFLP) types were analyzed more carefully.

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MATERIALS AND METHODS

Mice. Mouse strains A2G and BALB.A2G-*Mx* are kept in Zurich. Strain T9 was established from influenza virus-resistant Lake Casitas wild *Mus musculus domesticus* (9). T9 mice are kept in Zurich. All other mouse strains were from J. Hilgers, Amsterdam, The Netherlands, or were purchased from Jackson Laboratory, Bar Harbor, Maine.

DNAs. Genomic DNA samples were either purchased from the mouse DNA resource, Jackson Laboratory, or prepared from liver tissue by standard methods (12). Briefly, the tissue was homogenized, the cells were lysed with sodium dodecyl sulfate, and proteins were digested with proteinase K. After extraction with phenol and chloroform, the nucleic acids were precipitated with ethanol. RNA was digested with RNase A, and the DNA was recovered by ethanol precipitation.

Southern blot analysis. Samples (10 μg) of DNA were digested to completion with the restriction nuclease *Bam*HI, *Eco*RI, or *Hin*dIII. After electrophoresis through a 0.8% agarose gel, the DNA was transferred to GeneScreen nylon membranes by standard protocols (12, 19). Hybridization and washing of the blots were done by the method of Church and Gilbert (3). As probes, specific restriction fragments of the *Mx* cDNA clone pMx34 were used (26), labeled with ^{32}P-deoxynucleotides by either nick translation (21) or oligoprimer extension (6) to a specific activity of about 5×10^8 cpm/μg.

RNA isolation. Mouse embryo cells were prepared and grown as previously described (1). Confluent cell monolayers were treated for several hours with 500 U of recombinant

mouse IFN-α_2 per ml (22; a gift from C. Weissmann). In some experiments, the IFN treatment included 50 μg of cycloheximide per ml. Cell lysis with Nonidet P-40 and RNA extraction were as previously described (23). For the cloning experiments, mRNA was enriched by oligo(dT)-cellulose column chromatography.

S1 nuclease protection assay. The *Hin*dIII-*Bam*HI restriction fragment (2086 to 2314) of pMx34 (26) was subcloned into the corresponding sites of pSP65 (14). The resulting plasmid, pSPMxS1, was cut with *Bam*HI and 5′ end labeled with ^{32}P and polynucleotide kinase to a specific activity of 5×10^6 cpm/pmol. Following digestion with *Pvu*II, the 406-nucleotide (nt) fragment was isolated and purified from low-temperature agarose gels. Cytoplasmic RNA (35 μg) was hybridized overnight at 49°C to 0.01 pmol of denatured probe in 20 μl of buffer containing 80% formamide as described by Berk and Sharp (2). Nuclease S1 was added at 200 U/ml in a final volume of 220 μl, and the reaction was performed at 30°C for 90 min. The reaction products were analyzed by electrophoresis through an 8 M urea–6% polyacrylamide gel. Hybridization to *Mx1* (see below) mRNA results in protection of a radiolabeled 228-nt fragment. Quantification of the S1 signals was done by Cerenkov counting of the radioactivity in gel pieces after autoradiography.

cDNA cloning and sequencing. Vector-primed cDNA synthesis was essentially as described by Okayama and Berg (17) but with the cloning vector pHG327 (26). Libraries were prepared from poly(A)-enriched RNAs of BALB/c or CBA/J cells treated for 3 h with 50 μg of cycloheximide per ml and 500 U of mouse IFN-α_2 per ml. After ligation, the recombinant plasmids were transfected into *Escherichia coli*, and four pools of about 10^5 independent transfectants were grown in liquid cultures containing ampicillin. Plasmids were prepared from these pools, linearized by *Sal*I digestion, and size fractionated through agarose gels. Plasmids with inserts between 2 and 4 kb were religated and transfected into *E. coli*, which was plated on selective medium. Replicas were lifted from plates with nylon membranes (Biodyne; ICN Pharmaceuticals) and prepared for hybridization to radiolabeled probes as suggested by the membrane manufacturer. DNA sequencing was by the chemical degradation method (13).

RESULTS

Characterization of the wild-type *Mx* gene by Southern blot analysis. Southern blot analysis of chromosomal mouse DNA with *Mx* cDNA probes revealed a complex pattern of six or more bands with every restriction enzyme tested (Fig. 1). We had noticed earlier that small variations in the wash temperature between experiments resulted in reduced intensities of some signals but not others, suggesting the presence of a second gene related to *Mx* (26). The *Mx* gene of mouse strain A2G (the *Mx* gene encoding Mx protein), renamed here the *Mx1* gene, is now well characterized; genomic DNA containing all 14 exons of the *Mx1* gene was cloned and partially sequenced (11). A partial restriction map is shown in Fig. 2. Comparing this gene map with the corresponding Southern blot patterns permitted identification of signals which do not originate from the *Mx1* gene. The *Mx1* gene map predicts fragments of 8, 3.8, 2.5, 2.4, and 0.6 kb in the *Hin*dIII digest of chromosomal DNA of strain A2G and fragments of 5.6, 3.2, 2.5, 2, and 1 kb in the *Bam*HI digest. Our Southern blot analysis revealed genomic fragments of these predicted lengths (Fig. 1, asterisks); however, the

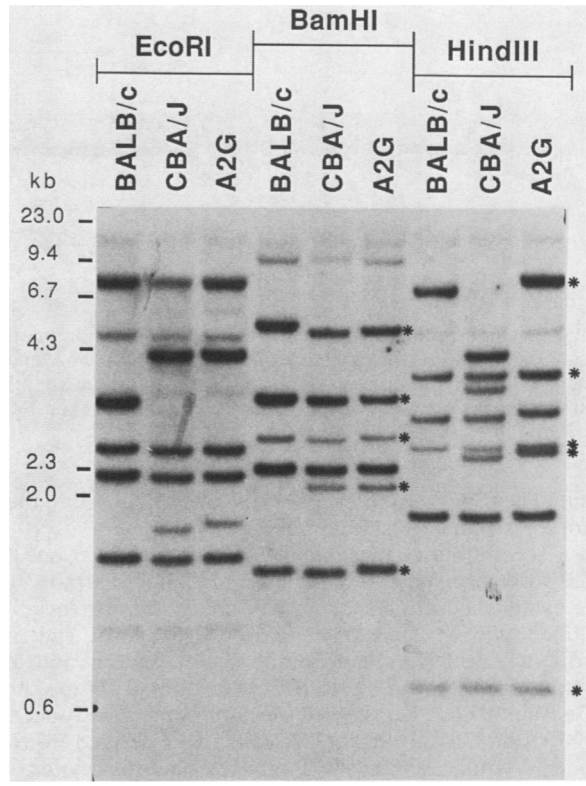

FIG. 1. Southern blot analysis of DNA from prototype mouse strains. The hybridization probe was the 2.3-kb *Bam*HI fragment of *Mx* cDNA clone pMx34 (26). The asterisks mark *Bam*HI and *Hin*dIII fragments of the *Mx1* gene that were predicted from analysis of cloned genomic DNA of strain A2G.

bands at about 5, 3, and 1.9 kb in the *Hin*dIII digest and the bands at about 12 and 2.3 kb and one band of the doublet at about 1 kb in the *Bam*HI digest must result from hybridization of the *Mx* probe to a gene(s) with a closely related sequence. The structure of this *Mx*-related gene, designated *Mx2*, is presented elsewhere (29).

***Mx* cDNA probe detects RFLPs and permits grouping of inbred mouse strains into three classes.** A preliminary survey of five inbred mouse strains showed that the mouse *Mx* locus is polymorphic (26). Three distinct Southern blot patterns were found; the pattern of the Mx^+ strain A2G differed from that of the Mx^- strains BALB/c, C57BL/6, and A/J. The Mx^- strain CBA/J gave a pattern that was distinct from both of these patterns, demonstrating that at least three *Mx* RFLP types exist in inbred mice.

To estimate the degree of variability at the *Mx* locus, we systematically studied *Mx* RFLPs in a total of 39 old inbred mouse strains. Genomic DNAs digested with the restriction endonuclease *Hin*dIII, *Bam*HI, or *Eco*RI were probed with a cDNA fragment which permits detection of all 14 exons of the *Mx* gene. Surprisingly, this screen did not yield any additional *Mx* RFLP types. That is, it was possible to group all 39 inbred strains into one of the three above-mentioned RFLP classes (Table 1). One RFLP class of inbred strains (now designated *Mx* RFLP type 1) consists of the Mx^+ strains A2G and SL/NiA. *Mx* RFLP type 2 consists of 34 Mx^- strains, including BALB/c. *Mx* RFLP type 3 consists of strains CBA/J, CE/J, and I/LnJ, which all show the Mx^- phenotype. One mouse strain of each RFLP class, namely, strains A2G, BALB/c, and CBA/J, was analyzed in more

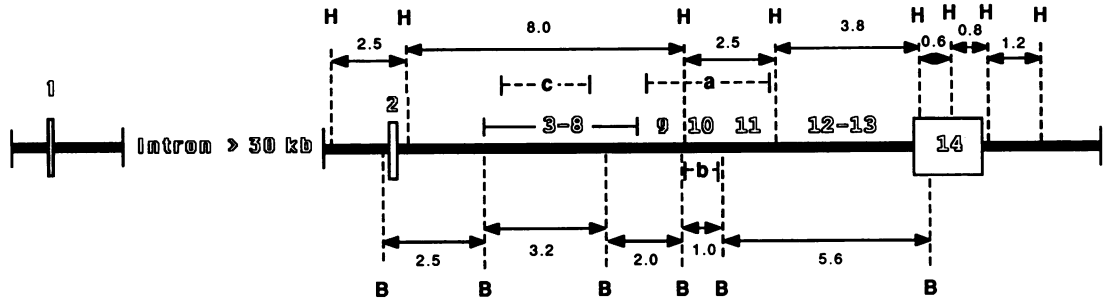

FIG. 2. Organization of the *Mx1* gene as deduced from partial sequencing of cloned genomic DNA of strain A2G (11). Boxed regions indicate the positions of exons 1, 2, and 14. The approximate positions of the other exons are indicated by outlined numbers. Only *Bam*HI (B) and *Hin*dIII (H) restriction sites are shown. Indicated are (a) the deletion of strain BALB/cJ that removes exons 9 through 11 and some flanking sequences, (b) the CBA/J nonsense mutation in exon 10, and (c) the approximate position of the extra *Hin*dIII site found in all RFLP type 3 strains.

detail. The Southern blot patterns of DNAs from these prototype strains are shown in Fig. 1.

In a second series of experiments, we extended our *Mx* RFLP screen to some new *M. musculus* inbred strains that were established directly from wild mice. Among these we identified two Mx^- strains, namely, SF/CamEi and SK/CamEi, whose DNAs had Southern blot patterns indistinguishable from that of strain BALB/c. DNA from the other Mx^- strain, PERA/Ei, showed the pattern previously seen in inbred strain CBA/J. Strain T9, which was derived from an influenza virus-resistant wild mouse, had the pattern of inbred strain A2G (Table 1). Strains MOLF/Ei and CAST/Ei, derived from wild *M. musculus molossinus* and *M. musculus castaneus*, respectively, showed distinct novel Southern blot patterns (data not shown). MOLF/Ei mice have the Mx^- phenotype, and CAST/Ei mice are Mx^+. It is noteworthy that although the *Mx1* probe showed some cross-reactivity to a second *Mx* gene, the above-described *Mx* RFLPs all appeared to result from strain-specific differences of the *Mx1* gene.

***Mx1* mRNA of BALB/c differs from wild-type *Mx* mRNA by a deletion in the coding region.** When treated with IFN-α or IFN-β, BALB/c embryo cells produce low amounts of an *Mx*-homologous mRNA that has a more rapid gel mobility than Mx^+ mRNA (26). To facilitate its cloning, we searched for culture conditions which would increase the concentration of this mRNA. We found that treatment of BALB/c cells for 3 h with 50 μg of cycloheximide per ml and 1,000 U of IFN-α_2 per ml yielded RNA preparations with satisfactorily high concentrations of *Mx* mRNA (Fig. 3).

Sequence analysis of corresponding cDNA clones revealed one major difference and several minor differences

TABLE 1. *Mx* RFLP distribution in inbred mouse strains

Mouse strain	Strain(s) in RFLP type:		
	1	2	3
Old	A2G, and SL/NiA	A/J, ABP/Le, AKR/J, AU/SsJ, BALB/cJ, BDP/J, BUB/BnJ, C3H/HeJ, C57BL/6J, C57BL/10J, C57BL/KsJ, C57L/J, C58/J, DA/HuSn, DBA/2J, FSB/GnEi, LIS/A, LP/J, MA/MyJ, MAS/A, NZB/BINJ, P/J, PL/J, RIIIS/J, RF/J, SEA/GnJ, SEC1/ReJ, SJL/J, ST/bJ, TSI/A, TW1/A, YBR/Ei, 020/A, and 129/J	CBA/J, CE/J, and I/LnJ
New	T9	SF/CamEi and SK/CamEi[a]	PERA/Ei

[a] Mouse strains SF/CamEi and SK/CamEi are probably not independent isolates (7).

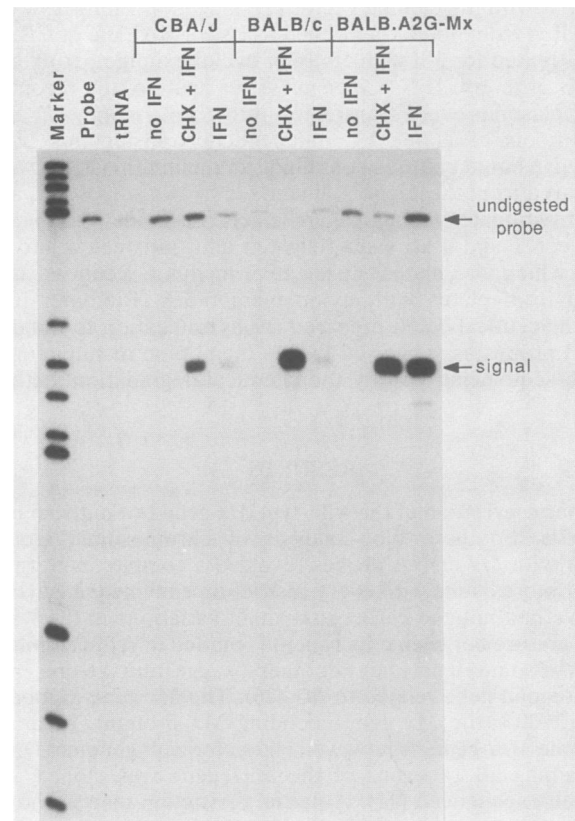

FIG. 3. S1 nuclease protection analysis of *Mx* mRNA in cells of prototype strains. Confluent cell monolayers were treated for 6 h with 500 U of IFN-α_2 (IFN) per ml or for 3 h with 50 μg of cycloheximide (CHX) per ml and 500 U of IFN-α_2 per ml or left untreated before cytoplasmic RNA was prepared and analyzed as described in Materials and Methods. The autoradiograph of the polyacrylamide gel is shown. The size marker was pBR322, digested with *Hae*III and 5′ labeled with ^{32}P.

between the structures of the *Mx1* mRNAs from Mx⁺ strain A2G and Mx⁻ strain BALB/c (Fig. 4). A block of 424 nt was missing from the coding region of the BALB/c mRNA. As a consequence, the open reading frame is shifted and out-of-frame translation presumably terminates only 8 triplets downstream, at a TAA stop codon. The missing sequences correspond exactly to *Mx1* gene exons 9, 10, and 11. These exons are actually deleted from the BALB/c genome; a probe derived from a part of the corresponding wild-type cDNA sequence (1184 to 1531), which hybridized to two *Hin*dIII and three *Bam*HI fragments of wild-type genomic DNA, failed to hybridize to genomic DNA of strain BALB/c (Fig. 5).

Besides this large deletion, *Mx1* mRNA of BALB/c differs from its counterpart of A2G cells by six 1-nt changes in the coding region, five of which are silent and one of which results in substitution of Met-584 by Thr (Fig. 4). The two sequences differ further by 1 nt in the 5′ noncoding region and by 8 nt in the 3′ noncoding region. In addition, BALB/c *Mx1* mRNA lacks 4 nt in the 3′ noncoding region, extending from position 2325 to position 2328 (Fig. 4). The two deletions, the nonsilent difference, and some of the silent differences were confirmed on a second independent cDNA clone, indicating that these changes are real differences between strains rather than cloning artifacts.

***Mx1* mRNA of CBA/J carries a nonsense mutation.** All known *Mx* RFLP type 3 strains fail to synthesize Mx protein and are influenza virus susceptible (9). Northern (RNA) blot analysis revealed low concentrations of an *Mx*-homologous mRNA of apparently normal size in RNA samples prepared from IFN-treated cells of the prototype RFLP type 3 strain CBA/J (data not shown). As in BALB/c cells, combined treatment of cultured CBA/J cells with cycloheximide and IFN yielded RNA preparations with increased *Mx* mRNA concentrations (Fig. 3).

Sequence analysis of appropriate cDNA clones showed that there are three relevant differences between the *Mx1* mRNAs of strains CBA/J and A2G (Fig. 4). (i) A point mutation converts the codon AAA for Lys-389 (exon 10) to a TAA termination codon. (ii) Glutamic acid residue 12 is converted to lysine. (iii) The 5′ noncoding region is 72 nt longer because of the inclusion of an extra exon between positions 25 and 26. We know from an earlier study that this exon, which is located in the first large intron of the *Mx1* gene, is present in the genome of strain A2G but is only rarely included in its mature *Mx1* mRNA (11). We found 12 more single-base changes; 6 of these are silent differences in the coding region, and the others are confined to the 3′ noncoding region. The in-frame stop codon, the amino acid difference at position 12, and the 5′ extra exon were also found in a second independent CBA-derived cDNA clone. In contrast, nt 1545 to nt 1546 were absent from only one of the two clones. This latter difference is therefore probably a cloning artifact.

The differences in *Mx1* mRNA structure between CBA/J and A2G cannot account for their RFLP difference. None of the base differences we detected would either create or destroy a *Hin*dIII site. The altered Southern blot pattern of

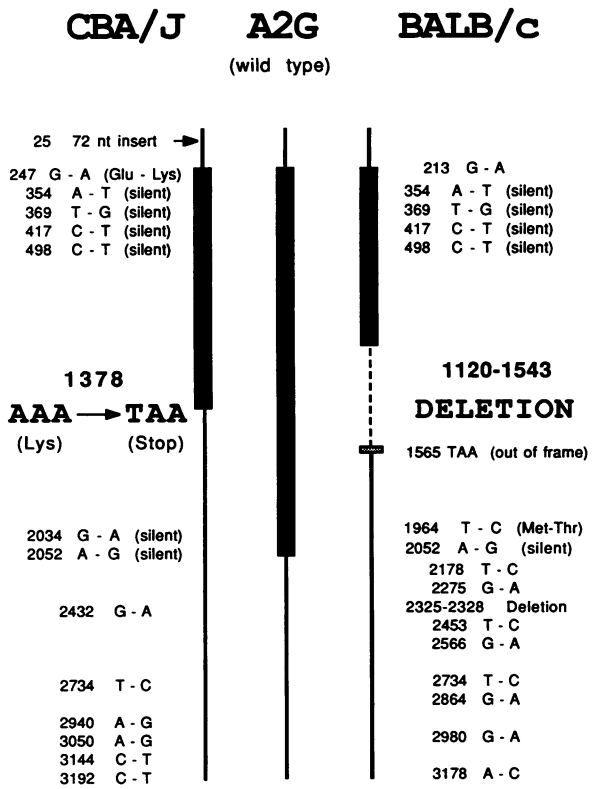

FIG. 4. Comparison of *Mx1* cDNAs of Mx⁺ strain A2G and Mx⁻ strains BALB/c and CBA/J. The A2G sequence is that of pMx34 (26). All differences found in *Mx1* cDNAs of BALB/c and CBA/J are indicated. The sequence of the 72-nt extra exon at position 25 of CBA *Mx1* mRNA was previously published (11). These sequence data have been deposited with GenBank, accession number JO 3369.

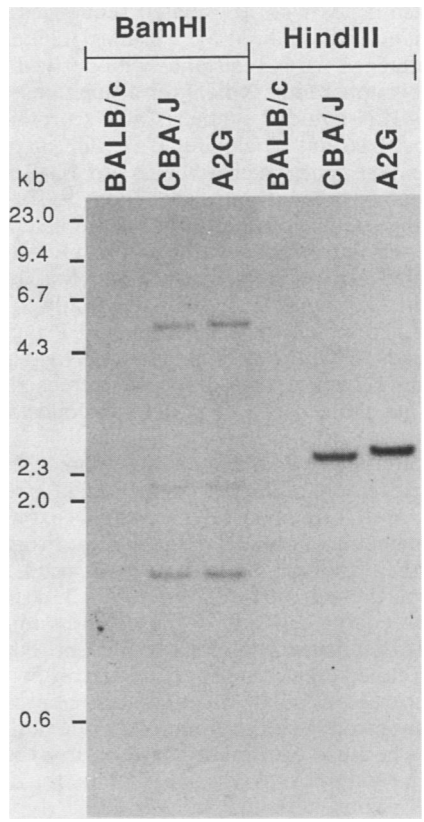

FIG. 5. Reprobing of the Southern blot shown in Fig. 1 with a probe specific for exons 9, 10, and 11 (*Pst*I-*Hha*I [1185 to 1532] fragment of pMx34).

CBA/J and probably all other *Mx* RFLP type 3 strains is apparently caused by an extra *Hin*dIII site located in an intron between exons 2 and 9 (Fig. 2).

Metabolic stability of mutant *Mx1* mRNAs is decreased. We performed quantitative S1 nuclease protection analyses on various RNA preparations from cells of CBA/J, BALB/c, and congenic Mx^+ BALB.A2G-*Mx* mice (Fig. 3). No *Mx1* mRNA was detectable in RNA samples from untreated quiescent cells of any of the three strains. Treatment with IFN-α_2 induced synthesis of *Mx1* mRNA. At 6 h after induction, the *Mx1* mRNA pool in BALB.A2G-*Mx* cells was about 15-fold higher than that in BALB/c or CBA/J cells. However, when the cells were subjected for 3 h to combined treatment with cycloheximide and IFN, the *Mx1* mRNA pools were high in both BALB.A2G-*Mx* and BALB/c cells. In CBA/J cells, combined treatment with cycloheximide and IFN led to *Mx1* mRNA levels about fivefold higher than those in cells treated with IFN alone. Thus, the *Mx* promoters of all three strains responded to IFN induction. However, the metabolic stabilities of the mutant *Mx* mRNAs were reduced.

DISCUSSION

A previous study had suggested that the mouse *Mx* locus is extremely polymorphic; Southern blot analysis of DNAs from five inbred strains yielded no less than three distinct RFLP classes (26). We show here, however, that all 39 of the commonly used inbred mouse strains tested fall into one of these three basic RFLP types. The observed *Mx* RFLPs all appear to result from strain-specific differences of the *Mx1* gene (the gene encoding Mx protein) rather than from differences in genes with *Mx*-related sequences. *Mx* RFLP type 1 is found in the inbred Mx^+ strains A2G and SL/NiA and the influenza virus-resistant, wild-derived strain T9. This RFLP is apparently typical for a functional *Mx1* locus and permits IFN-induced synthesis of Mx protein.

Most inbred strains, including BALB/c, carry a variant allele of the *Mx1* gene which gives rise to *Mx* RFLP type 2. The *Mx1* gene of these strains lacks exons 9, 10, and 11 and some flanking sequence because of a large deletion extending from within intron 8 to within intron 11. All *Mx* RFLP type 2 strains are phenotypically Mx^-; that is, they are influenza virus susceptible and lack the ability to synthesize Mx protein. Treatment of cells from RFLP type 2 strains with IFN leads to synthesis of an *Mx1* mRNA that lacks 424 nt of coding sequence and that has decreased metabolic stability. This shortened *Mx1* mRNA encodes a truncated Mx protein.

Mx RFLP type 3 is rare among inbred strains. The characteristic feature of this *Mx1* gene variant is replacement of the 8-kb *Hin*dIII fragment which contains exons 3 through 9 by two fragments of about 3.5 and 4.5 kb. From a detailed analysis of the prototype *Mx* RFLP type 3 strain, CBA/J, we conclude that the extra *Hin*dIII site in the *Mx1* gene has no effect on the coding capacity of the corresponding mRNA. Since cDNA sequence analysis revealed no base changes that would either create a new *Hin*dIII site or destroy an existing one, the *Hin*dIII site in question presumably is located in an intron. In CBA/J mice, *Mx* function appears to be impaired because of a point mutation in exon 10 which converts the codon AAA for Lys-389 to the termination codon TAA. Thus, although all *Mx* RFLP type 3 strains identified are Mx^-, this Southern blot pattern does not necessarily indicate that a mouse strain carries a nonfunctional *Mx1* gene. IFN-treated CBA/J cells express an *Mx1* mRNA of normal length but reduced metabolic stability. The mRNA encodes a truncated Mx protein.

With various preparations of potent antisera to Mx protein, it has not been possible to detect Mx protein or an immunologically related protein in BALB/c or CBA/J cells (unpublished data). Results presented in this paper show that the *Mx1* gene of these strains has mutations that abolish synthesis of the complete 72-kilodalton Mx polypeptide chain. From the cDNA sequences, one would predict that truncated forms of Mx protein may exist which lack roughly the carboxy-terminal 40% of the sequence of the wild-type Mx protein. Circumstantial evidence suggests that the abundance of the truncated proteins would be low; *Mx1* mRNA of BALB/c and CBA/J cells is metabolically unstable. At 6 h after induction with IFN, the cytoplasmic pools of mutated *Mx1* mRNAs are about 15-fold lower than the pools of wild-type *Mx1* mRNA in similarly treated Mx^+ cells. Only combined treatment of cells with IFN and cycloheximide, which is thought to increase the stability of otherwise rapidly degraded mRNAs, permits accumulation of mutated *Mx1* mRNAs to normal levels. It has been shown in other cases that abnormal mRNA may have a shorter half-life than its normal counterpart (4, 15, 30).

In the case of the truncated *Mx1* mRNA of BALB/c, instability might be explained by an unfavorable mRNA conformation. Alternatively, mutant mRNAs might not be translated efficiently, might not be covered with ribosomes, and thus might be more susceptible to the destructive action of nucleases (18). *Mx1* mRNA of CBA/J, which carries a point mutation that prevents its proper translation, could be metabolically unstable for the same reason. We do not know whether the extra 72 bases near the 5′ end of CBA *Mx1* mRNA, which presumably result from the use of an alternative noncoding exon, have a negative effect on mRNA stability. This extra exon was present in both clones of *Mx1* mRNA of CBA/J but present in less than 10% of wild-type *Mx1* mRNAs, as evidenced by primer extension analysis (11).

The mouse *Mx* locus maps to chromosome 16 (28). Since there are only a few known genetic markers on this chromosome, *Mx* can now be used for fine mapping of other genes. The *Mx* RFLPs described in this paper offer the basis for easy genetic analysis of large numbers of DNA samples. By using such methods, it was possible to map *Mx* to the immediate vicinity of the proto-oncogene *ets-2*, at the distal end of chromosome 16 (20).

Having established that three basic *Mx* RFLPs exist in inbred strains, the question about their origin arises. It was particularly interesting to ask whether *Mx* RFLP type 2 occurs in the wild, since this type is most abundant in inbred mice and its identification by Southern blot analysis is technically easy because the presence or absence of exons 9 to 11 can be assessed unambiguously. Unfortunately, this classification is more complicated with heterozygous animals. We have shown elsewhere that Mx^- alleles exist at high frequency in the wild and that heterozygosity at the *Mx* locus is quite common (9). To overcome the problem of heterozygosity, we analyzed some new inbred mouse strains rather than wild mice. These strains were established recently by inbreeding of wild mice, and thus they should carry *Mx* alleles of present-day wild mice. We found *Mx* RFLP type 2 in two such strains, SF/CamEi and SK/CamEi, suggesting that this Mx^- allele did not arise in captive mice. Similarly, *Mx* RFLP type 3 occurs in the wild, as was evident from the analysis of strain PERA/Ei.

Examination of the *Mx1* mRNA sequence differences

summarized in Fig. 4 revealed that *Mx1* mRNA of BALB/c differs from that of A2G in 17 positions, whereas *Mx1* mRNA of CBA/J differs in 15 positions. Six of the differences are shared between BALB/c and CBA, suggesting that these two strains are more closely related to each other than to strain A2G. It is conceivable that a single nonsense mutation in the *Mx* gene led to the establishment of Mx^+ and Mx^- mouse lines, represented in present-day mice by the prototype strains A2G and CBA/J. A subsequent deletion in the already nonfunctional *Mx* gene of the Mx^- line might eventually have led to establishment of the BALB/c Mx^- genotype. This scenario implies that the mutation which created the extra *Hin*dIII site in CBA/J and other *Mx* RFLP type 3 strains occurred after this event. However, our data do not formally exclude the possibility that the nonsense mutation and the *Mx* deletion represent independent mutations of distinct Mx^+ alleles.

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