JOURNAL OF VIROLOGY, Oct. 1985, p. 216–220
0022-538X/85/100216-05$02.00/0

Vol. 56, No. 1

Segregation of Restriction Fragment Length Polymorphism in an Interspecies Cross of Laboratory and Wild Mice Indicates Tight Linkage of the Murine IFN-β Gene to the Murine IFN-α Genes

FRANÇOISE DANDOY,[1] EDWARD DE MAEYER,[1]* FRANCOIS BONHOMME,[2] JEAN-LOUIS GUENET,[3] AND JAQUELINE DE MAEYER-GUIGNARD[1]

Institut Curie-Biologie, Campus d'Orsay, 91405 Orsay,[1] Université de Montpellier, 34060 Montpellier,[2] and Institut Pasteur, 75015 Paris[3], France

Received 1 April 1985/Accepted 24 June 1985

Southern blot analysis with murine (Mu) interferon (IFN)-α cDNA of restricted genomic DNA of three inbred strains of mice belonging to the species *Mus musculus domesticus* (BALB/c, C57BL/6, and DBA/2) revealed only a limited degree of polymorphism. For example, with *Hin*dIII there were only two polymorphic bands out of 14 hybridizing fragments. With Mu IFN-β cDNA there was no polymorphism at all between BALB/c and C57BL/6 in DNA restricted with seven different enzymes. In contrast, *Hin*dIII-restricted DNA of an inbred strain of wild mice (*M. spretus* Lataste) hybridized with the IFN-α probe displayed a high degree of polymorphism compared with the three strains of laboratory mice and was also polymorphic when probed with IFN-β cDNA. Although *M. musculus domesticus* and *M. spretus* Lataste represent different species, certain interspecies crosses are possible in the laboratory. This enabled us to follow segregation of restriction fragment length polymorphism in *Hin*dIII-restricted DNA obtained from 18 backcross progeny of a (DBA/2 × *M. spretus*)F1 × DBA/2 interspecies cross. There was complete coincidence between the segregation of parental (DBA/2) and (DBA/2 × *M. spretus*)F1-type IFN-β and IFN-α restriction fragment length polymorphism, indicating tight linkage of the IFN-β and IFN-α genes. In addition, in 15 of 18 progeny the segregation coincided with that of the *brown* locus on chromosome 4, in accord with previous results obtained with the IFN-α probe in strains derived from crosses between BALB/c and C57BL/6 mice. Thus, the Mu IFN-β gene is tightly linked to the Mu IFN-α gene cluster on chromosome 4 near the *brown* locus.

Three major interferon (IFN) families, IFN-α, IFN-β, and IFN-γ, can be distinguished by criteria of structure, antigenicity, and mode of induction. In humans, the IFN-α gene cluster and the IFN-β gene are linked on the short arm of chromosome 9, whereas the IFN-γ gene is on chromosome 12 (14, 15, 18, 20). Using segregation of restriction fragment length polymorphism (RFLP) between BALB/c and C57BL/6 genomic DNA probed with a murine (Mu) IFN-α2 cDNA, we recently mapped the Mu IFN-α gene cluster to chromosome 4 between the *brown* and *Mup-1* loci (5, 10). The localization of Mu IFN-α genes on chromosome 4 has been confirmed by others (12, 21), and the Mu IFN-γ gene has recently been located on chromosome 10 (13). The total number of Mu IFN-α genes and pseudogenes has not yet been established. Multiple restriction fragments are observed when mouse genomic DNA is cut with a series of restriction endonucleases that do not cut inside the coding regions of the seven known Mu IFN-α genes (6, 10, 11, 17, 23). For example, Southern blot analysis of BALB/c DNA reveals 13 hybridizing fragments when digested with *Eco*RI and 14 fragments when restricted with *Hin*dIII. Thus, as in humans, there are also many IFN-α genes in mice.

A cDNA (pMβ-3) with homology to the human IFN-β gene has been isolated by Higashi et al. (8) from mouse L cells. Screening by these authors of C3H mouse genomic DNA with the pMβ-3 probe revealed one single band after digestion with various restriction endonucleases, which indicated that the mouse genome has a single copy of IFN-β. Like the Mu IFN-α genes, the Mu IFN-β gene is on chromosome 4 (4, 21). The linkage of the Mu IFN-β gene to the Mu IFN-α genes, as measured by cosegregation in backcross progeny, has not yet been determined. For this reason, we looked for the existence of possible RFLP between BALB/c and C57BL/6 by using the pMβ-3 probe. Southern blot analysis of BALB/c and C57BL/6 DNA restricted with *Eco*RI, *Hin*dIII, *Xba*I, *Bam*HI, or *Pst*I, however, revealed the presence of single bands of identical size in both mouse strains. None of these five restriction endonucleases cut inside the cDNA, and therefore we tested two other enzymes having a restriction site inside the cDNA. With both *Bgl*II and *Kpn*I, two bands were generated, but again there was a lack of polymorphism between BALB/c and C57BL/6 mice. It therefore seemed unlikely that we would be able to exploit the recombinant inbred and bilineal congenic strains derived from BALB/c and C57BL/6 mice to study linkage of the Mu IFN-β gene, and we decided to examine mice genetically far more distant, such as *Mus spretus* mice, for further genetic analysis (1). The obtention of a system offering a higher degree of polymorphism than the one existing between BALB/c and C57BL/6 would indeed be desirable for the study of not only Mu IFN-β, but also of the Mu IFN-α gene cluster. In previous experiments, it was possible to map the IFN-α genes by analysis of RFLP after digestion of genomic DNA to both mouse strains with *Eco*RI or *Hin*dIII. The observed RFLP, however, was relatively limited, since only 1 of 13 *Eco*RI fragments and 2 of 14 *Hin*dIII fragments were different in BALB/c and C57BL/6 (5). In this paper, we present evidence for a high

* Corresponding author.

degree of RFLP observed with the Mu IFN-α probe when comparing DNA from *M. spretus* mice with DNA from BALB/c, C57BL/6, or DBA/2 mice after digestion with *Eco*RI or *Hin*dIII and for limited RFLP when *Hin*dIII-restricted DNA is probed with Mu IFN-β cDNA. Segregation of RFLP in backcross progeny of (DBA/2 × *M. spretus*)F1 × DBA/2 showed the existence of a tight linkage between the Mu IFN-β gene and the Mu IFN-α gene cluster.

MATERIALS AND METHODS

Mice. BALB/c and C57BL/6 mice were from the Orsay colony; they were originally obtained from D. W. Bailey, Jackson Laboratory, Bar Harbor, Maine. DBA/2 and inbred *M. spretus* mice (28 generations) were from the Pasteur Institute colony. Mice of the species *M. spretus* Lataste were originally captured from the wild in Granada, Spain (3); they do not belong to the species *M. musculus* but represent a different species of wild mice which in their natural habitat are reproductively isolated from *M. musculus* (2). In the laboratory, however, female *M. musculus* and male *M. spretus* breed to give birth to viable F1 hybrids, of which the females are fertile and the males are sterile. These F1 hybrids and their backcross progeny are very useful for segregation analysis, since *M. spretus* is polymorphic at most markers when compared with laboratory mice (1).

Extraction of DNA. Spleens were flash frozen in liquid nitrogen and ground in the presence of liquid nitrogen. The powder thus obtained was dissolved in sarcosyl (1%) in EDTA (0.1 M; pH 8) and left for 30 min at 55°C. After treatment with proteinase K (1 mg per spleen) and pancreatic RNase (0.5 mg per spleen) DNA was extracted three times with phenol-chloroform and once with chloroform. The DNA was then dialyzed to 10 mM Tris (pH 8)–10 mM NaCl–1 mM EDTA.

Southern blot analysis. High-molecular-weight DNA (20 μg per lane) digested for 4 h with a 10-fold excess of restriction endonuclease (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was electrophoresed through 0.8% agarose gels and transferred to Genescreen membranes (New England Nuclear Corp., Boston, Mass.) essentially as described by Southern (19). As a probe for Mu IFN-α sequences, we used the 820-base-pair insert from plasmid pMF1204 corresponding to Mu IFN-α2 (10), and for Mu IFN-β sequences, we used the 680-base-pair insert from plasmid pMβ-3 (8). Nick translation was done as described by Rigby et al. (16). Specific activities of 1×10^8 to 2×10^8 dpm/μg of fragment were routinely obtained. Hybridizations were carried out at 30°C in 50% formamide for 24 h. Membranes were washed twice with 0.3 M NaCl–0.06 M Tris hydrochloride (pH 8)–0.002 M EDTA–0.5% sodium dodecyl sulfate at 60°C for 40 min and twice with 0.003 M Tris base at room temperature for 30 min. Dried blots were exposed for 4 to 7 days to Cronex 4 films (DuPont Co., Wilmington, Del.).

RESULTS

Determination of the degree of polymorphism at Mu IFN-α and Mu IFN-β markers between *M. spretus* and *M. musculus*. Southern blot analysis with Mu IFN-α and Mu IFN-β probes was carried out with genomic DNA from *M. spretus*, BALB/c, C57BL/6, and DBA/2 mice after digestion with either *Eco*RI or *Hin*dIII. With the Mu IFN-α probe, there were 13 bands in BALB/c and *M. spretus* DNAs and 12 bands in *Eco*RI-digested C57BL/6 DNA (Fig. 1a). The RFLP

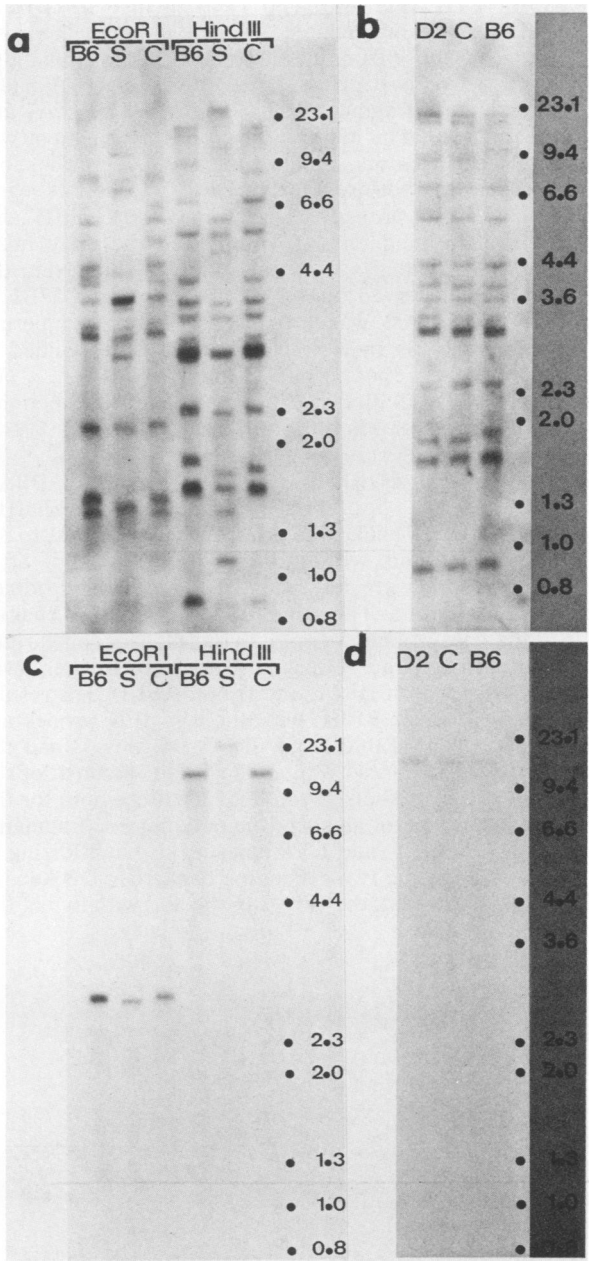

FIG. 1. RFLP with the Mu IFN-α and IFN-β probes in different inbred strains. (a) Southern blot analysis with the Mu IFN-α probe of C57BL/6 (B6), *M. spretus* (S), and BALB/c (C) DNAs. Lanes 1, 2, and 3, restriction with *Eco*RI; lanes 4, 5, and 6, restriction with *Hin*dIII. (b) Southern blot analysis of *Hin*dIII-restricted DBA/2 (D2), BALB/c (C), and C57BL/6 (B6) DNAs with the Mu IFN-α probe. (c) Southern blot analysis of C57BL/6 (B6), *M. spretus* (S), and BALB/c (C) DNAs with the Mu IFN-β probe. Lanes 1, 2, and 3, restriction with *Eco*RI; lanes 4, 5, and 6, restriction with *Hin*dIII. (d) Southern blot analysis of *Hin*dIII-restricted DBA/2 (D2), BALB/c (C), and C57BL/6 (B6) DNAs with the Mu IFN-β probe. Sizes of *Hin*dIII-restricted λ DNA and *Hae*III-digested φX174 DNA markers are indicated in kilobases on the right.

observed among DNA from *M. spretus* and those from the two other mouse strains was considerable, since seven bands migrated differently in *M. spretus* DNA compared with the DNAs from the two other strains. After restriction with *Hin*dIII, there were 14 fragments in C57BL/6, *M.*

spretus, BALB/c (Fig. 1a), and DBA/2 (Fig. 1b) DNAs. Again, there was a high degree of RFLP among DNA from *M. spretus* and those from the three other strains (6 bands).

With the Mu IFN-β probe, *Eco*RI-digested DNA had a single hybridizing fragment in C57BL/6, *M. spretus*, and BALB/c (Fig. 1c). The migration rate of this fragment was slightly faster in *M. spretus* than in the two other strains, but the difference was not pronounced enough to permit segregation analysis in progeny. Digestion with *Hin*dIII also yielded a single band for *M. spretus* and the three other strains (Fig. 1c and d). With this enzyme, however, the polymorphism observed among *M. spretus* and C57BL/6, BALB/c, and DBA/2 was pronounced enough to permit segregation analysis. In view of this result, we decided to probe DNA from backcross progeny (DBA/2 × *M. spretus*)F1 × DBA/2 after digestion with *Hin*dIII, since this would allow segregation of both the IFN-α and IFN-β patterns.

Segregation analysis (DBA/2 × *M. spretus*)F1 × DBA/2 backcross progeny. Southern blot analysis of *Hin*dIII-digested DNA of 18 backcross progeny (numbered 21 to 38) was first carried out with the IFN-β probe, and, after exposure, the blots were dehybridized and then rehybridized with the IFN-α probe. The same progeny were also characterized for alleles at a number of polymorphic loci on various chromosomes, and the segregation of these markers was compared with that of IFN-α and IFN-β RFLP. The results of the segregation of RFLP with the two IFN probes are given in Fig. 2 and Table 1. Of the 18 progeny, 8 had the parental DBA/2 IFN-α as well as the IFN-β pattern; of the remaining 10 DNAs, 8 displayed the F1 pattern both for the IFN-β fragment and for all six of the polymorphic fragments (28, 14.5, 6.3, 5.3, 1.5, and 1.1 kilobases [kb]) hybridizing to the IFN-α probe. The two remaining backcross DNAs also had the F1 pattern with the IFN-β probe and with five of the six IFN-α polymorphic fragments. In these two DNAs, the sixth polymorphic IFN-α fragment (5.3 kb) was replaced by another fragment of about 15.5 kb (Fig. 3); it cannot be decided at this stage whether this represents recombination inside the IFN-α locus or was due to a mutation in one of the male *M. spretus* mice used to obtain the F1 generation from which the backcross was derived. The segregation pattern of IFN RFLP was then compared with the segregation of the other markers for which the same backcross progeny had been typed (data not shown). The highest correlation (15 of

TABLE 1. Segregation of *brown* and IFN polymorphic sequences in (DBA/2 × *M. spretus*)F1 × DBA/2 backcross progeny

Backcross progeny no.	Segregation pattern with[a]:			
	brown	IFN-β[b]	IFN-α[b,c]	IFN-α (5.3 kb)[b]
21	+	+	+	+
22	+	+	+	R[d]
23	+	−	−	−
24	−	−	−	−
25	−	+	+	+
26	+	+	+	+
27	−	−	−	−
28	+	+	+	+
29	−	−	−	−
30	+	+	+	R[d]
31	+	−	−	−
32	−	−	−	−
33	+	+	+	+
34	−	−	−	−
35	−	−	−	−
36	+	+	+	+
37	+	+	+	+
38	+	+	+	+

[a] +, (DBA/2 × *M. spretus*)F1 pattern; −, DBA/2 pattern.
[b] *Hin*dIII restriction fragments.
[c] 1.1, 1.5, 6.3, 14.5, and 28 kb.
[d] R, The 5.3-kb fragment was replaced by a fragment of about 15.5 kb.

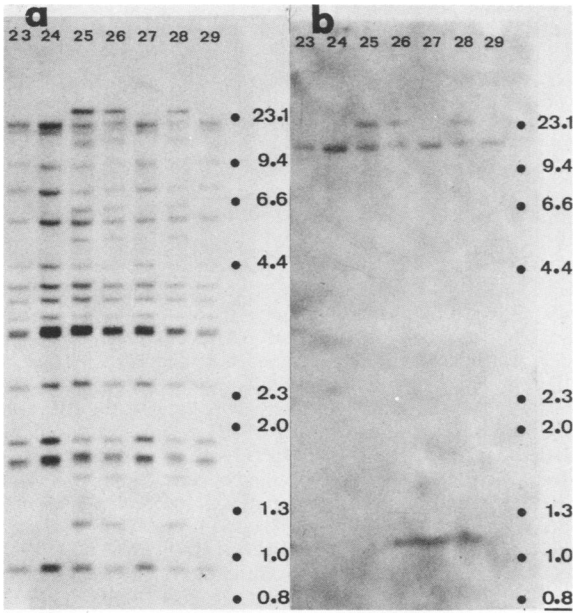

FIG. 2. Segregation of Mu IFN-α and IFN-β RFLP in backcross progeny. Southern blot analysis of *Hin*dIII-restricted DNAs of seven (DBA/2 × *M. spretus*)F1 × DBA/2 backcross progeny. (a) Mu IFN-α probe. (b) Mu IFN-β probe. Size markers as in Fig. 1. Lanes, backcross progeny no. 23 through 29.

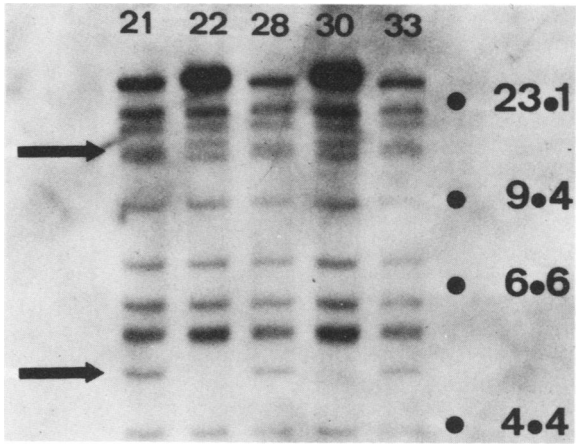

FIG. 3. Disappearance of the 5.3-kb Mu IFN-α restriction fragment and replacement by a 15.5-kb fragment in two backcross progeny. Southern blot analysis with the Mu IFN-α probes of *Hin*dIII-restricted DNAs of five backcross progeny. The upper arrow indicates the new IFN-α-hybridizing fragment of about 15.5 kb in backcross progeny 22 and 30; the lower arrow indicates the absence of the 5.3-kb fragment in the same backcross progeny. Sizes of *Hin*dIII-restricted λ DNAs are indicated (in kilobases) on the right.

18) was observed with the segregation of the *brown* locus (Table 1), which confirms the previous mapping of the IFN-α genes near the *brown* locus on chromosome 4 and extends this assignment to Mu IFN-β as well. From the segregation data, it can be deduced that the recombinant IFN-α sequence is more distal from the *brown* locus than the five other IFN-α polymorphic sequences and IFN-β. The relative positions of IFN-β and these five IFN-α bands cannot be deduced, since there was no segregation between them.

DISCUSSION

The total number of IFN-α and IFN-β genes in mice is not yet known; 13 different, nonallelic IFN-α structural genes have been described in humans (22), and there is evidence for the existence of at least 7 different IFN-α genes in mice as well (6, 11, 17, 23). In humans, so far only one IFN-β gene has been unambiguously identified, and it has been mapped on chromosome 9, closely linked to the IFN-α gene cluster (15, 18, 20). The Mu IFN-β probe used in our experiments shows 60% sequence homology with the human IFN-β gene and can therefore be considered to correspond to the Mu counterpart of the latter (8). The absence of recombination between the Mu IFN-β gene and the Mu IFN-α gene cluster on chromosome 4 suggests that the distance between both loci is less than 6 centimorgans. It is remarkable that, as in humans, the Mu IFN-β gene is linked to the IFN-α gene cluster. The conservation of such tight linkage during the 80,000,000 years since the divergence of the lineages that gave rise to the Mu and human genomes (see, for example, Jeffreys [9]) for an estimation of the evolutionary distance between mice and humans very likely has a functional implication, but one can only guess as to its meaning presently. Possibly it indicates that there is some advantage for the IFN-β and IFN-α genes to be induced simultaneously. In previous experiments, RFLP observed in BALB/c and C57BL/6 DNAs probed with Mu IFN-α cDNA was limited, consisting of 1 or 2 of 12 bands, depending on the enzyme used (5). This limited polymorphism is not surprising in view of the relatively small number of mice from which most strains of laboratory mice of the species *M. musculus* have been derived (7). This also explains the lack of polymorphism when restricted BALB/c and C57BL/6 DNAs were probed with Mu IFN-β cDNA. With this probe, only one band of equal size was observed in DNAs from both mouse strains after digestion with five different enzymes, and only two bands were observed in DNA restricted with an enzyme cutting inside the cDNA. This is in marked contrast to the multiple restriction fragments observed in DNA probed with Mu IFN-α cDNA and suggests that, contrary to the number of Mu IFN-α genes, there is only a single Mu IFN-β gene.

In contrast to the limited RFLP observed with the Mu IFN-α probe when mice of the BALB/c, C57BL/6, or DBA/2 strains were compared, 6 of 14 fragments were different when *Hin*dIII-restricted DNA from *M. spretus* mice were compared with DNA from either BALB/c or DBA/2 mice. Segregation analysis of five of six of these fragments revealed the highest coincidence (15 of 18) with segregation observed for the alleles at the *brown* locus on chromosome 4. This confirms our previous observation in the BALB/c-C57BL/6 system, in which segregation of one *Eco*RI and two *Hin*dIII restriction fragments showed linkage to *H-15* near the *brown* locus on chromosome 4. Taken together, these results in laboratory and wild mice confirm and reinforce the notion that most, if not all, IFN-α-hybridizable DNAs, or in other words the Mu IFN-α genes and possibly pseudogenes, are located on chromosome 4 near the *brown* locus. The 100% coincidence between the segregation of the IFN-α and IFN-β restriction patterns indicates tight linkage of the IFN-β gene and the IFN-α genes.

ACKNOWLEDGMENTS

We are indebted to Y. Kawade for providing us with the Mu IFN-β probe.

This work was supported by grant no. 920641, contrat programme: recherches fondamentales en amont des biotechnologies, to J.D.-G. from CNRS.

LITERATURE CITED

1. **Bonhomme, F., J. Catalan, J. Britton-Davidian, V. M. Chapman, K. Moriwaki, E. Nevo, and L. Thaler.** 1984. Biochemical diversity and evolution in the genus *Mus*. Biochem. Genet. **22**:275–303.
2. **Bonhomme, F., S. Martin, and L. Thaler.** 1978. Hybridization between *Mus musculus* L. and *Mus spretus* Lataste under laboratory conditions. Experientia **34**:1140–1141.
3. **Britton, J., and L. Thaler.** 1978. Evidence for the presence of two sympatric species of mice (genus *Mus* L.) in southern France based on biochemical genetics. Biochem. Genet. **16**:213–225.
4. **Dandoy, F., J. De Maeyer-Guignard, and E. De Maeyer.** 1985. Linkage of murine IFN-α and IFN-β genes on chromosome 4, p. 43–47. *In* H. Kirchner and H. Schellekens (ed.), The biology of the interferon system. Elsevier Science Publishers, Amsterdam.
5. **Dandoy, F., K. A. Kelley, J. De Maeyer-Guignard, E. De Maeyer, and P. M. Pitha.** 1984. Linkage analysis of the murine interferon-α locus on chromosome 4. J. Exp. Med. **160**:294–302.
6. **Daugherty, B., D. Martin-Zanca, B. Kelder, K. Collier, T. C. Seamans, K. Hotta, and S. Pestka.** 1984. Isolation and bacterial expression of a murine alpha leukocyte interferon gene. J. Interferon Res. **4**:635–643.
7. **Ferris, D. S., R. D. Sage, and A. C. Wilson.** 1982. Evidence from mtDNA sequences that common laboratory strains of inbred mice are descended from a single female. Nature (London) **295**:163–165.
8. **Higashi, Y., Y. Sokawa, Y. Watanabe, Y. Kawade, S. Ohno, C. Takaoka, and T. Taniguchi.** 1983. Structure and expression of a cloned cDNA for mouse interferon-β. J. Biol. Chem. **258**: 9522–9529.
9. **Jeffreys, A. J.** 1982. Evolution of globin genes, p. 157–176. *In* G. A. Dover and R. B. Flavell (ed.), Genome in evolution. Academic Press, Inc., New York.
10. **Kelley, K. A., C. A. Kozak, F. Dandoy, F. Sor, D. Skup, J. D. Windass, J. De Maeyer-Guignard, P. M. Pitha, and E. De Maeyer.** 1983. Mapping of murine interferon-α genes to chromosome 4. Gene **26**:181–188.
11. **Kelley, K. A., and P. M. Pitha.** 1985. Characterization of a mouse interferon gene locus. I. Isolation of a cluster of four α interferon genes. Nucleic Acids Res. **13**:805–823.
12. **Lovett, M., D. R. Cox, D. Yee, W. Boll, C. Weissmann, C. J. Epstein, and L. B. Epstein.** 1984. The chromosomal location of mouse interferon α genes. EMBO J. **3**:1643–1646.
13. **Naylor, S. L., P. W. Gray, and P. A. Lalley.** 1984. Mouse immune interferon (IFN-γ) gene is on chromosome 10. Somatic Cell Genet. **10**:531–534.
14. **Naylor, S. L., A. Y. Sakaguchi, T. B. Shows, M. L. Law, D. V. Goeddel, and P. W. Gray.** 1983. Human immune interferon gene is located on chromosome 12. J. Exp. Med. **57**:1020–1027.
15. **Owerbach, D., W. J. Rutter, T. B. Shows, P. Gray, D. V. Goeddel, and R. M. Lawn.** 1981. Leucocyte and fibroblast interferon genes are located on human chromosome 9. Proc. Natl. Acad. Sci. U.S.A. **78**:3123–3127.
16. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**: 237–251.
17. **Shaw, G. D., W. Boll, H. Taira, N. Mantei, P. Lengyel, and C.**

Weissmann. 1983. Structure and expression of cloned murine IFN-α genes. Nucleic Acids Res. **11**:555–573.

18. **Shows, T. B., A. Y. Sakaguchi, S. L. Naylor, D. G. Goeddel, and R. M. Lawn.** 1982. Clustering of leucocyte and fibroblast interferon genes on human chromosome 9. Science **218**:373–374.
19. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
20. **Trent, J. M., S. Olson, and R. M. Lawn.** 1982. Chromosomal localization of human leukocyte, fibroblast, and immune interferon genes by means of in situ hybridization. Proc. Natl. Acad. Sci. U.S.A. **79**:7809–7813.
21. **Van der Korput, J. A. G. M., J. Hilkens, V. Krozen, E. C. Zwarthoff, and J. Trapman.** 1985. Mouse interferon alpha and beta genes are linked at the centromere proximal region of chromosome 4. J. Gen. Virol. **66**:493–502.
22. **Weissmann, C., S. Nagata, W. Boll, M. Fountoulakis, A. Fujisawa, J. I. Fujisawa, J. Haynes, K. Henco, N. Mantei, H. Ragg, C. Schein, J. Schmid, G. Shaw, M. Streuli, H. Taira, K. Todokoro, and U. Weidle.** 1982. Structure and expression of human IFN-α genes. Philos. Trans. R. Soc. Lond. B. Biol. Sci. **299**:7–28.
23. **Zwarthoff, E. C., A. T. A. Mooren, and J. Trapman.** 1985. Organization, structure and expression of murine interferon alpha genes. Nucleic Acids. Res. **13**:791–804.