

The chromosomal location of mouse interferon α genes

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The chromosomal location of mouse leukocyte-interferon (IFN- α) genes was determined by Southern blot analysis of DNA from a panel of Chinese hamster x mouse somatic cell hybrids using a mouse IFN- α cDNA as a hybridization probe. All resolvable mouse genes are located on mouse chromosome 4. In addition, two common restriction site polymorphisms within these genes were identified in several mouse strains.

Key words: interferon genes/mouse chromosome 4/restriction fragment length polymorphisms/somatic cell hybrids

Introduction

Interferons (IFNs) are proteins secreted by a variety of vertebrate cells in response to viral infection or other stimuli, including double-stranded RNA and mitogens. IFNs confer resistance to infection by a broad range of viruses upon target cells, inhibit cell proliferation and affect the immune response (for reviews, see Epstein, 1979, 1981; Lengyel, 1982). Human IFNs have been classified into three antigenically distinct groups, IFN- α , IFN- β , and IFN- γ . DNA sequences encoding all three types of human IFNs (Nagata *et al.*, 1980a; Derynck *et al.*, 1980; Taniguchi *et al.*, 1980a; Goeddel *et al.*, 1980a, 1980b, 1981; Streuli *et al.*, 1980; Gray *et al.*, 1982; Devos *et al.*, 1982; Taya *et al.*, 1982), mouse IFN- β (Higashi *et al.*, 1983) and mouse IFN- α (Shaw *et al.*, 1983) have recently been isolated by recombinant DNA techniques. Sequence determination has revealed more than a dozen members of the human IFN- α gene family, all of which show 80% or more homology (Nagata *et al.*, 1980b; Goeddel *et al.*, 1981; Brack *et al.*, 1981; Weissmann, 1981, 1983), one structurally related IFN- β gene (Derynck *et al.*, 1980; Taniguchi *et al.*, 1980a, 1980b; Houghton *et al.*, 1981; Tavernier *et al.*, 1981; Degraeve *et al.*, 1981; Lawn *et al.*, 1981b) and a purported second IFN- β gene (Weissenbach *et al.*, 1980; Sagar *et al.*, 1982). All IFN- α genes and at least one IFN- β gene are located on human chromosome 9 (Owerbach *et al.*, 1981; Shows *et al.*, 1982; Trent *et al.*, 1982; Slate *et al.*, 1982) and do not contain introns (Nagata *et al.*, 1980b; Lawn *et al.*, 1981a; Tavernier *et al.*, 1981; Degraeve *et al.*, 1981; Houghton *et al.*, 1981). Likewise, the IFN- α genes of mouse investigated to date do not contain introns (Shaw *et al.*, 1983). In contrast, the single cloned human IFN- γ gene contains three introns and is located on chromosome 12 (Gray *et al.*, 1982; Taya *et al.*, 1982; Trent *et al.*, 1982). The mouse IFN- γ gene has been reported to be located on chromosome 10 (Naylor *et al.*,

1984).

In view of the importance of the mouse as a model for the investigation of the potential anti-viral, anti-tumour and other bioregulatory effects of IFNs, it is important to obtain information on the structure, number and location of the mouse interferon genes. Here we report the chromosomal location of the mouse IFN- α structural genes by hybridization of DNA from Chinese hamster x mouse somatic cell hybrids with a radiolabelled mouse IFN- α cDNA.

Results

Detection of mouse interferon α sequences in high molecular weight DNA

Genomic DNA isolated from a panel of 10 Chinese hamster x mouse somatic cell lines was digested with the restriction enzyme *Hind*III, electrophoresed in an agarose gel and transferred to a nitrocellulose filter. This filter was hybridized with a ³²P-labelled mouse IFN- α cDNA [MuIFN- α 2 (Shaw *et al.*, 1983)]; the resulting autoradiograph is shown in Figure 1. The mouse chromosomes present in the panel of hybrid cell lines are shown in Table I.

Digestion of DNAs with *Hind*III was found to result in maximum resolution of the mouse *versus* the hamster pattern of MuIFN- α 2-related restriction fragments. Other enzyme digests such as *Eco*RI or *Bam*HI produced very few resolvable mouse-specific fragments. Mouse (A9) DNA (Figure 1, track 13) contains 12 *Hind*III fragments hybridizing to ³²P-labelled MuIFN- α 2 DNA, ranging in length from >10-kb to 1 kb (numbered 1–12 in Figure 1). Seven mouse DNA fragments (numbers 1, 2, 3, 6, 7, 9 and 11) are clearly distinguishable from the hamster background fragment pattern by size differences and one fragment (number 8) by intensity difference (cf. Figure 1, tracks 11, 12 and 13). High stringency washes (0.1 x SSC, 0.1% SDS at 68°C) of the filter shown in Figure 1 did not eliminate the hamster cross-hybridization. We were therefore unable to map the four mouse *Hind*III fragments (numbers 4, 5, 10 and 12) which co-migrate with hamster fragments.

Tracks 1–10 in Figure 1 are *Hind*III digested cell hybrid DNAs. All resolvable MuIFN- α 2-related mouse fragments segregate together and are present in tracks 1, 2, 6, 7 and 9. One anomalous 1.2-kb DNA fragment related to MuIFN- α 2 is present just above fragment 12 in three out of the five positive hybrid cell lines (tracks 1, 2 and 9). As is demonstrated below, this represents a common mouse IFN- α polymorphism. Only mouse chromosomes 4 and 15 are common to all of the positive hybrids (Table I). Chromosome 4 is present only in hybrids positive for MuIFN- α 2-related mouse DNA fragments and is absent from all other hybrids in the panel. However, chromosome 15 is present in one other cell hybrid (I-7B-4) (Figure 1, track 3) which does not contain the MuIFN- α 2-related mouse DNA sequences. The pattern of hybridization is thus completely concordant for the presence of mouse chromosome 4 and discordant in one case for the

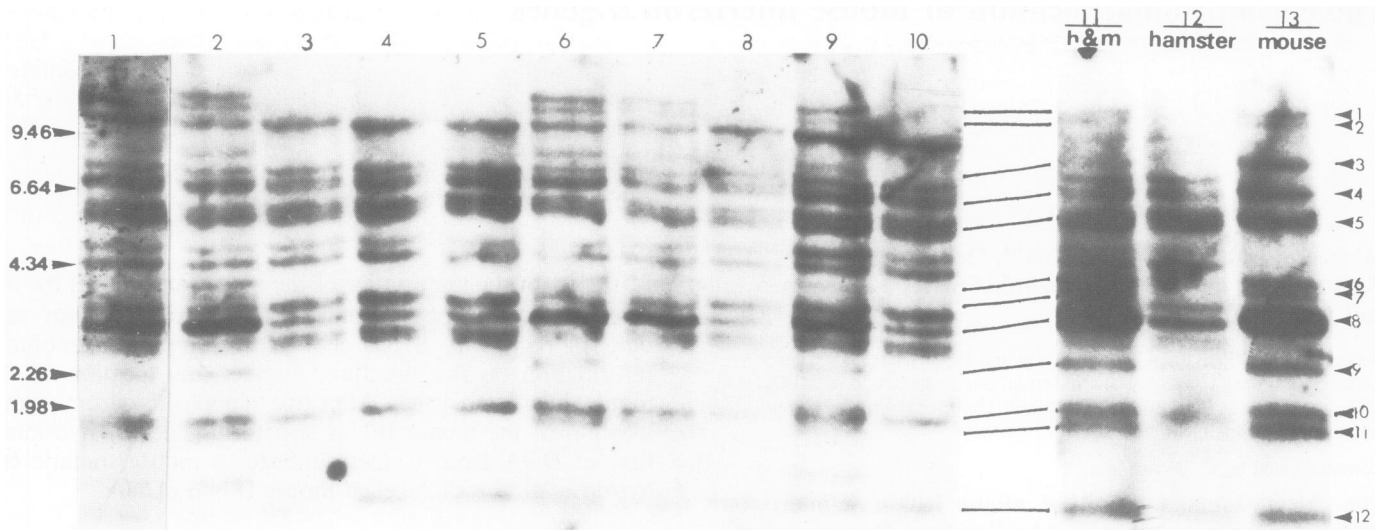


Fig. 1. Detection of mouse-specific IFN- α DNA sequences in somatic cell hybrids. 10 μ g samples of DNAs extracted from each cell hybrid were digested with *Hind*III, electrophoresed in a 0.8% agarose gel, transferred to nitrocellulose and hybridized with radiolabelled MuIFN- α 2 DNA as described in Materials and methods. **Tracks 1–10** refer to somatic cell hybrids, the mouse chromosomal contents of which are listed in Table I. **Track 1** is a longer exposure than **tracks 2–10**. **Tracks 11, 12 and 13** are a mixture of mouse and hamster DNAs, hamster (380-6) DNA and mouse (A9) DNA respectively. Numbers at the left side refer to the lengths in kilobases of size markers electrophoresed in parallel. Numbers at the right side show the positions of mouse fragments referred to in the text. Connecting lines show the alignments of these mouse-specific fragments between the two parts of the figure.

Table I. Mouse chromosome distribution in cell hybrids

Track in Figure 1	Clone	Mouse chromosome																			Hyb ^a		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		X	
1	I-3A-2		+		+			+	+					+	+		+	+		+	+		+
2	I-3A-3	+	+		+												+	+		+	+		+
3	I-7B-4	+	+							+										+	+		
4	III-12					+	+							+	+	+			+				+
5	III-13								+									+					+
6	III-14	+			+			+									+	+				+	+
7	III-19				+				+								+					+	+
8 ^b	III-23		+	+					+					+	+					+		+	+
9	II-3A		+	+	+		+								+	+	+	+	+	+	+	+	+
10	III-16		+	+		+	+	+	+	+				+	+	+		+	+	+	+	+	+

^aHyb indicates those hybrid cell lines scored positive for mouse IFN- α from Figure 1.

^bThis cell line contains translocation chromosomes as described by Cox *et al.* (1982).

presence of mouse chromosome 15. The positive hybridization signals are discordant for all other mouse chromosome assignments. In addition, this assignment was confirmed in a parallel experiment using a cell hybrid which contained mouse chromosome 4 but not chromosome 15 (data not shown).

Polymorphisms in the IFN- α genes

As has been noted above, Figure 1 shows an anomalous MuIFN- α 2-related *Hind*III restriction fragment in tracks 1, 2 and 9 with a length of 1.2 kb which is not detectable in A9 DNA.

The cell hybrids used in this study were derived from a variety of parental mouse strains (Cox *et al.*, 1982). Clones I-3A-2 and I-3A-3 (tracks 1 and 2 in Figure 1) were derived from the same fusion of cells from one ICR mouse but are in-

dependent clonal isolates, whereas clone II-3A (track 9, Figure 1) was derived from a separate fusion. The presence of the novel 1.2-kb fragment within these cell lines reflects a polymorphism in mouse IFN- α sequences which occurs in ICR mice as well as in several inbred mouse strains. This was determined by extracting DNA from the spleens of individual animals of various mouse strains, digesting the DNAs with *Hind*III, electrophoresis, Southern blotting, and hybridization of the resulting filter with ³²P-labelled MuIFN- α 2 DNA (Figure 2). Mice of ICR, SWR and AKR origin contain the 1.2-kb *Hind*III polymorphism while A9 DNA (C3H), DBA, and C57Bl/6 mice do not. One other polymorphism is detectable. A fragment with a slightly lower mobility than mouse fragment 5 is present in the DBA, AKR and ICR tracks (fragment 5'). In addition, the signal intensity from this fragment is greater in the DBA and AKR tracks relative to ICR DNA.

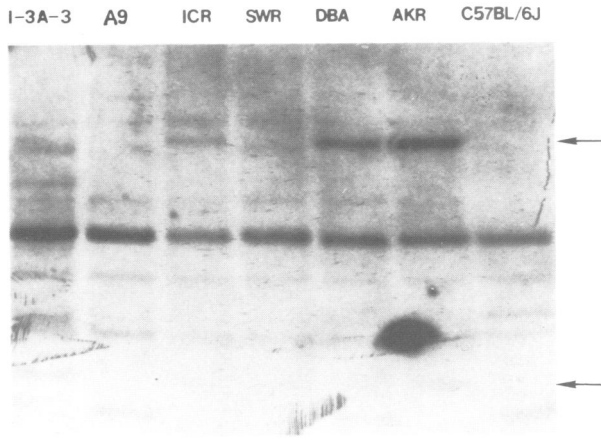


Fig. 2. Detection of polymorphic IFN- α -related DNA in different inbred and outbred mouse strains. 10 μ g samples of DNAs extracted from the cell hybrid I-3A-3, the cell line A9 (C3H) and five mouse strains were digested with *Hind*III, electrophoresed in a 0.8% agarose gel, transferred to a nitrocellulose filter and hybridized with radiolabelled MuIFN- α 2 DNA as described in Materials and methods. Arrows at the right side show the positions of the polymorphic fragments referred to in the text (upper arrow, polymorphic fragment 5'; lower arrow, 1.2-kb polymorphic fragment).

Discussion

We have mapped the majority of mouse DNA sequences homologous to an IFN- α cDNA to chromosome 4 and have identified two common *Hind*III restriction site polymorphisms within these genes. One of these polymorphisms is detectable by difference in mobility and the other by difference in mobility and intensity. This alteration in intensity may be caused by several co-migrating restriction fragments, possibly reflecting an increase in the number of IFN- α genes yielding homologous restriction fragments. An alternative explanation is that the intensity difference is due to fragments which are very closely related to MuIFN- α 2, while the weaker bands represent more distantly related sequences. The strong additional bands in DBA and AKR, and perhaps ICR, may be due to conversion of some IFN- α gene to IFN- α 2. A number of studies have shown that a member of a gene family may acquire part or all of the nucleotide sequence of another member in one genetic event (Roberts and Axel, 1982; Weiss *et al.*, 1983; Mellor *et al.*, 1983; Liskay and Stachelak, 1983). This interpretation is supported by evidence that gene conversion has occurred within the human IFN- α gene family (Todokoro and Weissmann, unpublished data).

Since the three mouse IFN- α genes investigated to date do not contain introns or *Hind*III sites within their coding regions (Shaw *et al.*, 1983), it is possible that each detected *Hind*III fragment represents one or more IFN- α gene. We therefore tentatively conclude that the mouse has 12 or more IFN- α genes and that at least eight of these are located on chromosome 4. The number of these which are functional transcription units is unknown.

Several gene families having a high degree of sequence homology have been shown to be tightly linked [for a review, see D'Eustachio and Ruddle (1983)]. Those human IFN- α genes which readily cross-hybridize with each other are clustered together on chromosome 9 and are linked to the related gene for IFN- β (Owerbach *et al.*, 1981; Shows *et al.*, 1982; Trent *et al.*, 1982; Slate *et al.*, 1982). There are not less than 13 closely related human IFN- α genes (and six or more

pseudogenes), and several of these are found in the same or overlapping genomic DNA segments (Brack *et al.*, 1981; Weissmann, 1983). The genes for human soluble aconitase (*ACO1*) (Mohandas *et al.*, 1982), galactose-1-phosphate uridyl transferase (*GALT*) (Konds and Nakamura, 1984), IFN- α and IFN- β (Trent *et al.*, 1982) have all been mapped to the short arm of human chromosome 9. The centromere proximal region of mouse chromosome 4 contains the mouse *Aco-1* and *Galt* genes (Nadeau and Eicher, 1982), whereas the distal region of mouse chromosome 4 contains a block of genes which are homologous with genes on the short arm of human chromosome 1 (Pearson and Roderick, 1982). It is therefore quite probable that all 12 detected mouse IFN- α *Hind*III restriction fragments are linked to *Aco-1* and *Galt* on the centromere proximal region of chromosome 4 and that a mouse IFN- β gene will be found to be linked to these sequences. The future sublocalization of these genes on chromosome 4 may be more precisely defined by investigating somatic cell hybrids which contain different translocated segments of chromosome 4 or by *in situ* hybridization to specific chromosomes.

Materials and methods

Parental and hybrid cells

Chinese hamster x mouse somatic cell hybrids segregating mouse chromosomes were made by polyethylene glycol fusion of mouse spleen cells or peritoneal macrophages with an established Chinese hamster cell line (380-6) deficient for hypoxanthine phosphoribosyltransferase (EC 2.4.2.8). The derivation and characterization of these hybrid clones have been previously described (Cox *et al.*, 1982). Ten hybrid cell lines were used, which together contain all the mouse chromosomes except for chromosome 11 and the Y chromosome. The genetic constitution of the hybrid cells was verified by electrophoretic analysis of mouse chromosome-specific gene products and/or karyotype analysis at the time the DNA was prepared.

Preparation and hybridization of DNA

High mol. wt. DNA isolated from cell hybrids and mouse spleens was digested with a 5-fold excess of the restriction endonuclease *Hind*III (New England Biolabs). Samples of 10 μ g were separated by electrophoresis on 0.8% agarose gels. Agarose gels were cast and run in 40 mM Tris-50 mM sodium acetate-1 mM EDTA adjusted to pH 7.8 with acetic acid. DNA was transferred to nitrocellulose filters (Southern, 1975), which were prehybridized, hybridized and washed as described by Wahl *et al.* (1979). Labelled DNA fragments were detected by autoradiography (Laskey and Mills, 1977).

DNA probes

The α -interferon probe used was the mouse cDNA clone (MuIFN- α 2) described by Shaw *et al.* (1983). It was constructed by insertion of a mouse α -interferon cDNA into the *Pst*I site of pBR322 by oligodeoxyguanylate:oligodeoxycytidylate homopolymeric tailing. DNAs were 32 P-radiolabelled to specific activities of $10^7 - 2 \times 10^8$ c.p.m./ μ g by nick translation (Rigby *et al.*, 1977).

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Note added in proof

While this paper was in preparation, Kelley *et al.* (1983) reported that one mouse IFN- α BamHI restriction fragment is located on mouse chromosome 4. Kelley, K.A., Kozak, C.A., Dandoy, F., Sor, F., Skup, D., Windass, J.D., De Maeyer-Guignare, J., Pitha, P.M. and De Maeyer, E. (1983). *Gene*, **26**, 181-188.