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Structure of Mouse Interferon Stimulated Gene Factor 3γ (ISGF3γ/p48) cDNA and Chromosomal Localization of the Gene[1]

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Interferon stimulated gene factor 3 (ISGF3) is a trimeric transcription factor activated on treatment of cells with interferon-α and β (type I IFNs). Upon stimulation, the regulatory subunits, p84/91 and p113, present in the cytoplasm are phosphorylated at specific tyrosine residues and assemble with the DNA binding subunit, ISGF3γ, into the active ISGF3 in the nucleus. Thus, ISGF3 plays a primary role in the transmission of a signal from the cell surface to the nucleus. In this report, we describe the cloning of a mouse cDNA encoding a polypeptide homologous to human ISGF3γ. Comparison of the deduced amino acid sequences revealed the middle region was significantly different between mouse and man. The mouse cDNA was shown to encode a functional ISGF3 subunit by means of an *in vitro* reconstitution assay. Furthermore, the locus of the ISGF3γ gene, designated as *Isgf3g*, was mapped to distal mouse chromosome 14 by linkage analysis using an intersubspecific backcross typing panel.

Key words: amino acid sequence, chromosomal localization, interferon, interferon stimulated gene factor 3, reconstitution.

Interferon-α and β (type I IFNs) are cytokines with biological functions including the induction of an antiviral state and growth inhibition (*1*). Type I IFNs exert their effects through the induction of a set of cellular genes. Upon interaction of the cell surface type I IFN receptor with a ligand, two JAK family tyrosine kinases (JAK1 and TYK2) (*2, 3*) are activated and phosphorylate the regulatory subunits of ISGF3 (ISGF3α: p84/91 and p113, also designated as Stat1α/β and Stat2, respectively) in the cytoplasm (*4, 5*). The activated ISGF3α subunits then assemble with another subunit, ISGF3γ (p48), to form the active transcription factor, ISGF3 (*6*) and which is translocated into the nucleus (*7-9*). The ISGF3γ subunit within the complex is responsible for the specific interaction with the promoter element, interferon stimulation response element (ISRE) or interferon consensus sequence (ICS) (*10-12*). A series of human cell mutants defective in the type I IFN-stimulated signalling pathway has been studied, and it has been demonstrated that the above mentioned signalling cascade is critical for expression of the biological functions of type I IFNs.

Human ISGF3γ cDNA has been isolated and revealed to belong to the interferon regulatory factor (IRF) family of transcription factors (*7*). The DNA binding domain of ISGF3γ is located in its N-terminal region, and is homologous to those of IRF-1, IRF-2, ICSBP, and Pip (*13-17*). Limited homology is also observed with ICSBP and Pip in the C-terminal region of ISGF3γ, the domain that interacts with ISGF3α subunits.

To elucidate the function of ISGF3γ in a mouse system, we attempted to isolate mouse ISGF3γ cDNA and to analyze its structure. A cDNA library was prepared from poly A$^+$ RNA extracted from mouse L929 cells infected with Newcastle Disease Virus (NDV) for 9 h (λgt22A, Super Script Lambda System, GIBCO BRL). The cDNA library was screened with human ISGF3γ cDNA as a probe, and two positive clones containing cDNA inserts of 1.95 and 1.4 kbp were isolated from 3.2×10^5 recombinants. The longer cDNA was subcloned into pBluescript SK(−) for sequence analysis. To determine the primary structure unambigously, both strands were sequenced and the additional two cDNA clones obtained by RT-PCR were also sequenced. The cDNA consisted of a single large open reading frame encoding a polypeptide of 398 amino acids (a.a.), with a calculated molecular weight of 44.5 kDa. Comparison with the human ISGF3γ amino acid sequence showed highly conserved regions in their amino and carboxyl termini (Fig. 1, a and b) (*7*). The DNA binding domain of human ISGF3γ (a.a. 10 to 117) (*18*) is highly homologous (86% identity) to the corresponding region (a.a. 10 to 117) of the mouse polypeptide. Similarly, the domain that interacts with ISGF3α of human ISGF3γ (a.a. 217 to 377) (*18*) is highly conserved (83% identity) in mouse (a.a. 211 to 371). In contrast, the intermediate region of about 100 a.a. (a.a. 118 to 211) is significantly different between man and mouse (34% identity). This divergence is not due to an artifact or an error in sequencing because the sequences of independent clones were identical, and the nucleotide sequences in the two species also exhibited low sequence conservation.

To determine whether the cloned mouse cDNA encodes a

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mouse homolog of ISGF3γ or another IRF family member, we examined its function using an *in vitro* reconstitution system (*18*). For the assay, a HeLa cell extract which contained the activated ISGF3α proteins but no detectable mature ISGF3 complex was used. When the ISGF3α fraction was incubated with human ISGF3γ produced in 293T cells on transient transfection, a complex corresponding to human ISGF3 was detected on electrophoresis mobility shift assay (EMSA) (Fig. 2, lane 5). Similarly, when the fraction containing the polypeptide encoded by the mouse cDNA was incubated with the ISGF3α fraction, a new complex exhibiting slightly slow mobility was observed (Fig. 2, lane 6). The mobility of the complex was very similar to that of the mouse ISGF3 induced in L929 cells on IFN-β treatment (Fig. 2, lane 8). The slower mobility of mouse ISGF3 is likely due to the larger molecular mass of mouse ISGF3γ. With the control fractions from 293T cells transfected with the vector alone (Fig. 2, lane 4) or from mock-treated HeLa cells (Fig. 2, lanes 1 to 3), we failed to reconstitute the ISGF3 complex. In the same assay, with ICSBP, we failed to reconstitute ISGF3 (unpublished observation). The above results indicate that the isolated mouse cDNA encodes the functional ISGF3γ. The less conserved intermediate region may function as a spacer maintaining the distance between the two functionally distinct domains, the DNA binding and ISGF3α interaction domains (*18*).

Next, we investigated the chromosomal localization of the mouse ISGF3γ gene. For this purpose, genomic DNA of an intersubspecific backcross typing panel was used (see

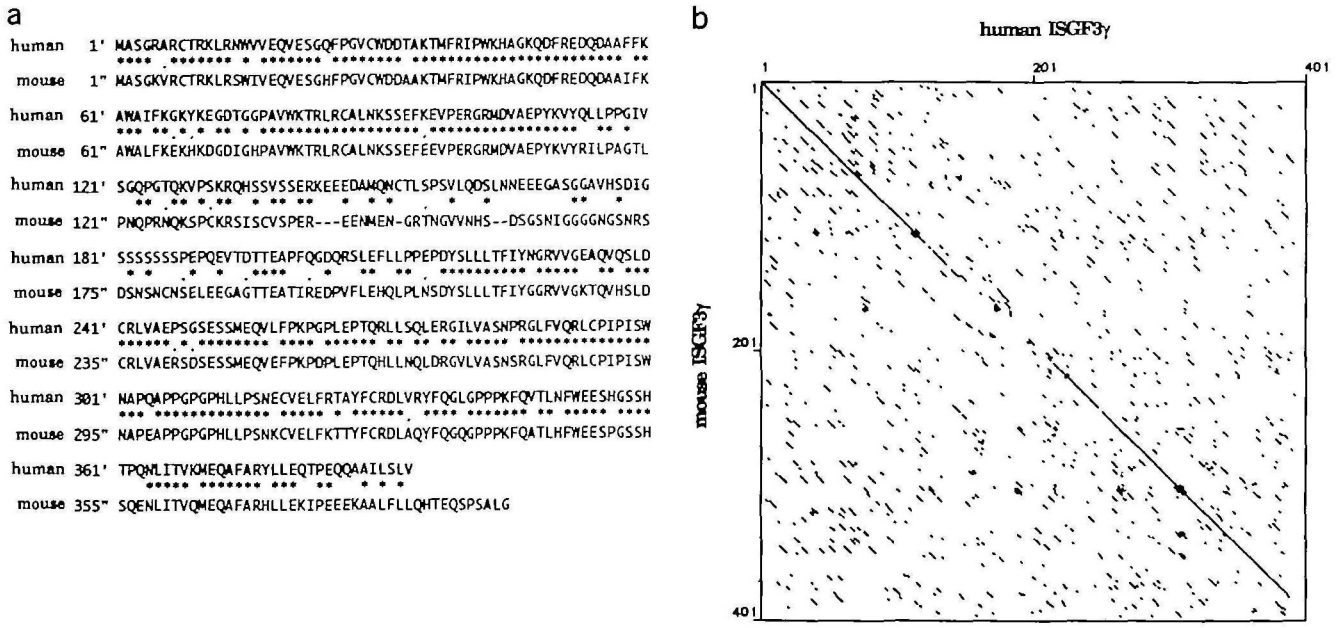

Fig. 1 **Primary structure of mouse ISGF3γ deduced from the structure of the isolated mouse cDNA.** (a) Sequence comparison of human ISGF3γ with the polypeptide encoded by the isolated mouse cDNA. The amino acid sequence of human ISGF3γ (top) (*7*) is compared with the deduced amino acid sequence of the mouse polypeptide. Asterisks and dots indicate identical and related amino acids, respectively (b) Harrplot analysis of the homology between the mouse and human ISGF3γ proteins The horizontal and vertical axes indicate the amino acids of the human (a.a. 1-393) and mouse ISGF3γ (a a 1-398) proteins, respectively

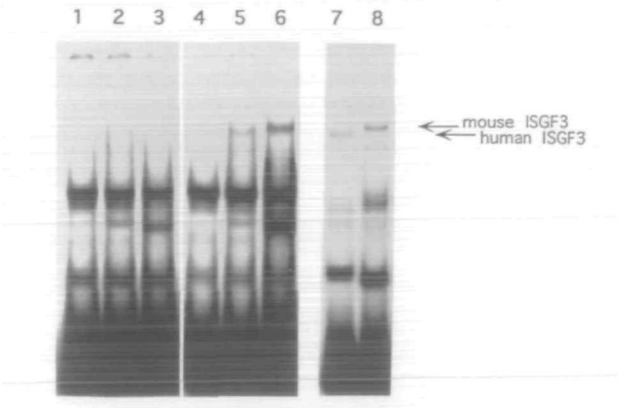

Fig 2. **Reconstitution of ISGF3 from ISGF3α and the product encoded by the mouse cDNA.** HeLa cells were mock-treated or treated with human IFN-α (1,000 U/ml) for 15 min, and then a whole cell extract was prepared with lysis buffer (20 mM Hepes, pH 7.9, 50 mM NaCl, 10 mM EDTA, 2 mM EGTA, 1 mM DTT, 0.1% NP40, 10% glycerol, 100 mM sodium orthovanadate, 1 mM PMSF, and 100 μg/ml leupeptin). The lysates were centrifuged to remove insoluble materials (100,000 rpm, 15 min). The lysate prepared from IFN-treated HeLa cells contained no significant ISGF3 activity, as seen on EMSA (lane 4), and the lysate was used as the ISGF3α fraction A vector, pEF-BOS, containing the strong promoter of elongation factor (*27*) was used to construct an expression vector for human ISGF3γ (pEF-hp48) and mouse ISGF3γ (pEF-mp48) Human 293T cells were transfected with these vectors by the calcium phosphate method (*28*). The recombinant proteins were extracted with the lysis buffer at 72 h after transfection The control (lanes 1 to 3) or ISGF3α fraction (lanes 4 to 6) obtained from HeLa cells was mixed with 293T lysates of cells either transfected with the empty vector (lanes 1 and 4), human ISGF3γ expression vector (lanes 2 and 5), or mouse ISGF3γ expression vector (lanes 3 and 6), and then incubated at 25°C for 20 min subjected to EMSA (*29*). A ^{32}P-labeled double-stranded oligonucleotide probe containing ISRE of ISG15 (5′-GGGAAACCGAAACTG-3′) was used as the probe (*30*). For comparison, extracts from HeLa cells treated with IFN-γ and IFN-α (lane 7), and L929 cells treated with IFN-β (lane 8) were subjected to EMSA. The positions of human and mouse ISGF3 are indicated at the right

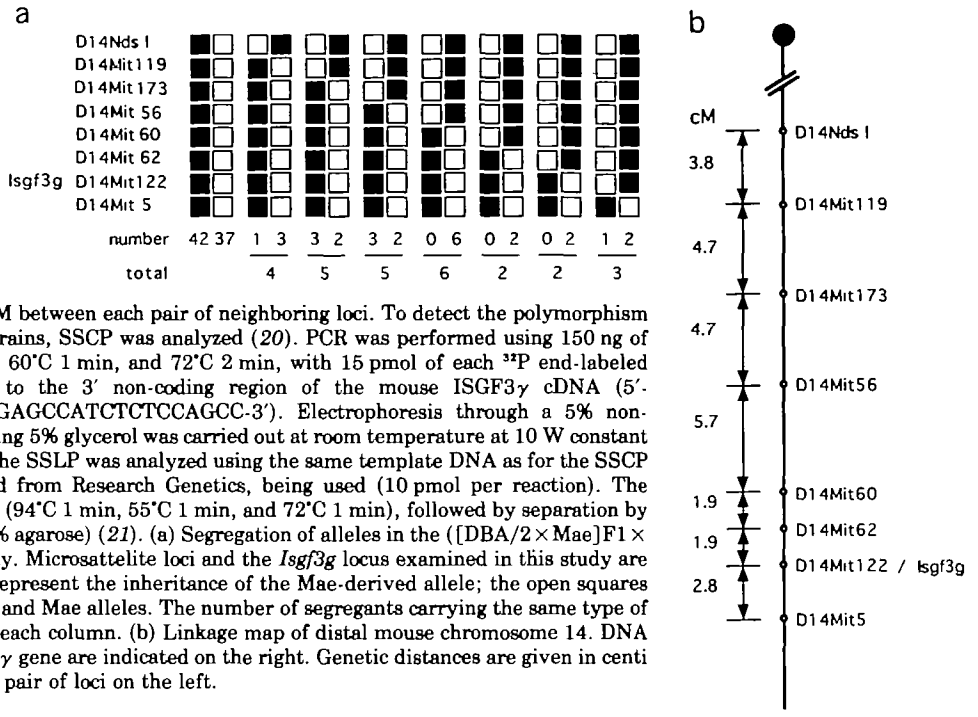

Fig. 3. **Chromosomal localization of the mouse ISGF3γ gene.** To construct an intersubspecific backcross typing panel, two mouse strains, DBA/2J and Mae, descended from a pair of Japanese wild mice, *Mus musculus molossinus*, were used as parents, and the F1 females were backcrossed to Mae males. The resultant backcross segregants were typed for 3 or more microsatellite markers per chromosome with an average distance of 20 cM between each pair of neighboring loci. To detect the polymorphism in the *Isgf3g* locus between the two strains, SSCP was analyzed (*20*). PCR was performed using 150 ng of genomic DNA (30 cycles: 94°C 1 min, 60°C 1 min, and 72°C 2 min, with 15 pmol of each ^{32}P end-labeled primer). The primers corresponded to the 3′ non-coding region of the mouse ISGF3γ cDNA (5′-CCCAGTCCTCACTAGAC-3′ and 5′-GAGCCATCTCTCCAGCC-3′). Electrophoresis through a 5% non-denaturing polyacrylamide gel containing 5% glycerol was carried out at room temperature at 10 W constant power to separate the PCR products. The SSLP was analyzed using the same template DNA as for the SSCP analysis, a pair of primers, purchased from Research Genetics, being used (10 pmol per reaction). The templates were amplified for 35 cycles (94°C 1 min, 55°C 1 min, and 72°C 1 min), followed by separation by gel electrophoresis (3% Nusieve and 1% agarose) (*21*). (a) Segregation of alleles in the ([DBA/2×Mae]F1×Mae) intersubspecific backcross progeny. Microsattelite loci and the *Isgf3g* locus examined in this study are shown on the left. The black squares represent the inheritance of the Mae-derived allele; the open squares represent the presence of both DBA/2 and Mae alleles. The number of segregants carrying the same type of chromosome is given at the bottom of each column. (b) Linkage map of distal mouse chromosome 14. DNA markers and the position of the ISGF3γ gene are indicated on the right. Genetic distances are given in centi Morgans (cM), and are shown for each pair of loci on the left.

Fig. 3 legend for details). The alleles at the ISGF3γ gene locus, hereafter designated as *Isgf3g*, were typed by means of radioactive PCR with a primer pair for the mouse ISGF3γ cDNA sequence, followed by analysis of single-stranded conformation polymorphism (SSCP) (*19, 20*). The analysis revealed the close linkage between *Isgf3g* and the microsatellite locus, *D14Mit5*, in mouse chromosome 14 [1Q95 release of the Whitehead Institute/MIT Center of Genome Research mouse genetic map (MIT mouse genetic map) obtained from URL: "http://www-genome.wi.mit.edu/genome data/mouse/mouse index.html"]. To confirm this, detailed linkage analysis was carried out using several additional microsatellite markers, such as *D14Nds1, D14Mit119, D14Mit173, D14Mit56, D14Mit60, D14Mit62*, and *D14Mit122* (MIT mouse genetic map), by the simple sequence length polymorphism (SSLP) method (*21-23*). No recombinant was obtained between *Isgf3g* and the *D14Mit122* locus, showing that the two loci are tightly linked to each other (Fig. 3a). Based on this linkage analysis, a linkage map was constructed for the distal part of mouse chromosome 14 (Fig. 3b). There was no discordance in the order of the loci examined between this map and the chromosome 14 map obtained with PCR-SSLP markers (MIT mouse genetic map).

Comparison of this map with the other map compiled for mouse chromosome 14 (*24*) shows that the waved coat (*Wc*) locus (*25*) is also closely linked to the *Isgf3g* locus, suggesting that the *Isgf3g* locus is a strong positional candidate responsible for the *Wc* locus. To examine this, we compared the cDNA structure between normal mice (+/+) and mice carrying the *Wc* locus (*Wc*/+) by PCR-SSCP. However, no difference was observed in the PCR-SSCP patterns (data not shown). Furthermore, primary kidney cells derived from either *Wc*/+ or +/+ mice indistinguishably induced ISGF3 on treatment with type I IFN (unpublished observations). These results strongly suggest that the *Isgf3g* gene is not responsible for the Wc mutation.

Since ISGF3γ is essential for the formation of ISGF3, the primary regulator of type I IFN responses, and ISGF3γ may also be involved in type II IFN-induced signal transduction (*26*), an aberration of the ISGF3γ gene may result in an abnormal response to infections by viruses or other agents. So far there has been no report of a naturally occurring mouse mutant exhibiting the above phenotype. It is interesting to relate the susceptibility to viral infections and the polymorphism of the identified ISGF3γ locus.

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