Brazilian Journal of Medical and Biological Research (1997) 30: 41-50
ISSN 0100-879X

# A mutant cell line partially responsive to both IFN-α and IFN-γ

C.A. Bonjardim

Laboratório de Vírus, Departamento de Microbiologia,
Universidade Federal de Minas Gerais, 31270-901 Belo Horizonte, MG, Brasil

## Abstract

A recessive mutant cell line, B7, which is partially responsive to both interferon (IFN)-α and IFN-γ is described. B7 was FACS sorted from a cellular pool, which was obtained from the parental cell line 2C4, after several rounds of mutagenesis. The partial responsiveness to IFN was observed both in terms of expression of cell surface markers (CD2, class I and II HLAs) and mRNA expression of IFN-stimulated genes (9-27; 6-16; 2'-5' OAS; GBP and HLA-DRα). A genetic cross with the U4 mutant (JAK1$^-$, a member of the Janus family of nonreceptor tyrosine kinase) did not restore full IFN responsiveness to B7, and JAK1 cDNA transfection into B7 restored the wild phenotype of the cell line, defining B7 as a member of the U4 complementation group. Nevertheless, JAK1 mRNA was not detected in this mutant. Transcriptional regulator complexes such as IRF1/2 (IFN-regulatory factor) and ISGF3-γ (IFN-stimulated gene factor) were constitutively formed in the B7 mutant and co-migrated with the IFN-induced complexes expressed in the parental cell line 2C4. Thus, this cell line seems to be useful for understanding cis-acting elements governing JAK1 mRNA expression.

**Correspondence**
C.A. Bonjardim
Laboratório de Vírus
Departamento de Microbiologia
Instituto de Ciências Biológicas
Universidade Federal de
Minas Gerais
Av. Antonio Carlos, 6627
Bloco F4, 258
31270-901 Belo Horizonte, MG
Brasil
Fax: 55 (031) 498-1510
E-mail: claubonj@mono.icb.ufmg.br

The author was the recipient of a post-doctoral fellowship from CNPq.

Received December 28, 1995
Accepted October 16, 1996

**Key words**
- Interferons
- Transcriptional activation
- Molecular genetics
- JAK1
- FACS analysis

## Introduction

Interferons (IFNs) constitute a family of multifunctional cytokines first identified as antiviral agents. However, other activities have been associated with these molecules such as the control of cell growth and differentiation, besides their modulatory regulation of the immune system (1).

After binding to the specific cell surface receptor, type I IFN (mainly α and ß) and type II IFN (γ) activate a number of IFN-stimulated genes (ISGs), which are believed to be responsible for the different IFN actions (1,2). IFN-activated gene expression is mediated by latent cytoplasmic proteins which become phosphorylated at a tyrosine residue upon ligand-receptor binding, migrate to the nucleus, bind to DNA and initiate transcription (3-5).

It has been shown that the receptor to nucleus signalling triggered by IFN-α relies on the activation of the primary transcriptional activator IFN-stimulated gene factor 3 (ISGF3), which comprises the α and γ subunits (6,7). The ISGF3-α moiety is constituted of three polypeptides: 113 kDa (p113), 91 kDa (p91) and 84 kDa (p84). p91 and p84 are products of a differentially spliced gene (8-10) and the ISGF3-γ moiety is represented by p48, which is the DNA-binding protein (9). The ISGF3-α and -γ subunits associate after tyrosine phosphorylation of the α-subunit proteins, which in turn translocate to the nucleus, bind to cis-acting DNA elements termed interferon-stimulated re-

sponse element (ISRE) located at the promoter of ISGs and initiate transcription (11-13). Due to the dual role of p91, p84 and p113 as signal transducers and activators of transcription, these molecules were renamed STAT1α, STAT1ß and STAT2, respectively.

The nuclear signals resulting from the interaction of IFN-γ with its receptor occur in a fashion very similar to that described for IFN-α. After activation of STAT1α (the same component of ISGF3-α), and migration to the nucleus, STAT1α activates transcription by binding as a dimer (14) to a regulatory sequence termed gamma-activated sequence (GAS). GAS was initially identified at the promoter of the guanylate-binding protein (GBP) gene (15,16), but has now been found in a variety of other genes (17-19), and STAT1α turned out to be the previously described gamma-activated factor (GAF) (20).

It is known that the intracytoplasmic portions of both IFN-α/ß and IFN-γ receptors are devoid of an intrinsic tyrosine kinase activity (21,22), suggesting that a nonreceptor protein tyrosine kinase (NRPTK) is recruited to assemble the multisubunit receptor complexes in order to transduce the nuclear signals (23,24). By using a genetic approach, a number of IFN-unresponsive cell lines (U- and γ-mutants) have been generated (25-28) and genetic complementation of these mutants by cDNA transfection has proved valuable in dissecting both types of IFN signalling pathways (29-31).

The first evidence that a NRPTK is involved in these pathways came from the complementation of the U1 mutant which was transfected with the gene and cDNA encoding for TYK2 (29), a member of the Janus family of NRPTK (23,24). Complementation of the U3 mutant, a STAT1α$^-$/STAT1ß$^-$ cell line, established the fundamental role STAT1α plays in the IFN-α and IFN-γ transduction pathways (30), while complementation of the U4 mutant, a JAK1$^-$ (a member of the Janus family of NRPTK) cell line, clearly demonstrated the involvement of NRPTK-JAK1 in both signalling pathways (32). Another important finding was the restoration of the IFN-γ responsiveness to the JAK2$^-$ cell line (γ1A) upon JAK2 cDNA transfection (28). Thus, the converging biochemical and genetic approaches enable to decipher the emerging picture of both IFN-α and IFN-γ signalling cascades (5), which can be summarized as follows: IFN-α: two JAK family members are required: TYK2 and JAK1, plus the transcription factors p113 and/or STAT1α/STAT1ß, besides p48. IFN-γ: two JAK family members are required as well: JAK1 and JAK2 , plus the transcription activator STAT1α (dimer), probably associated with a DNA-binding protein.

In the present study, I describe the genetic characterization of a mutant cell line, B7, and its complementation by JAK1 cDNA. Although JAK1 mRNA expression was below detection levels in B7, a partial IFN-α and IFN-γ responsiveness was achieved. In addition, DNA-protein complexes are constitutively formed in this mutant. Thus, this cell line represents a useful model to analyze JAK1 structure-function relationships, concerning JAK1 expression.

## Material and Methods

### Cell culture, cell fusion and DNA transfection

Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal calf serum, 5 µM L-glutamine, and neomycin (G418, 700 µg/ml) and incubated at 37ºC, 5% $CO_2$. The parental cell line 2C4 was generated as described elsewhere (28). Briefly, human HT1080 cells were co-transfected with a selectable marker, pTKNeo, and pDW9-27CD2, a plasmid containing the promoter region of the IFN-stimulated gene 9-27 placed just upstream of a full-length CD2 cDNA. 2C4 cells were mutagenized

(five rounds) with the frameshift agent ICR191 (25) and the cell population was subdivided into different pools. B7 was FACS sorted from one of these pools (a gift from D. Watling, ICRF, London), cloned and characterized. U3 and U4 mutants were characterized as described elsewere (27), and both are unresponsive to type I and type II IFN and correspond to STAT1α⁻/1ß⁻ and JAK1⁻ cell lines, respectively. For cell fusion experiments a puromycin- or hygromycin-dominant selectable marker was transfected into the cells and the resistant hybrid cell population was maintained in the presence of the adequate drug selection. Cell fusion experiments employing PEG 4000 (Sigma) were performed as described elsewhere (27). DNA transfections using calcium phosphate were carried out essentially as described in Ref. 30.

**FACS analysis**

Fluorescence-activated cell sorter (FACSCAN-Becton Dickinson) analysis was performed as previously described (28). Briefly, 5 x $10^5$ cells were seeded in a 10-cm Petri dish and after an overnight incubation they were treated with $10^3$ IU/ml of a highly purified mixture of IFNs-α (Wellferon, 1.5 x $10^8$ IU per mg protein) from Wellcome Research Laboratories, Beckenham, Kent, UK, or recombinant human IFN-γ (2 x $10^7$ IU per mg protein), a generous gift from Dr. G. Adolf, Ernst Boehringer Institut für Arzneimittelforschung, Vienna, Austria. Cells were treated with IFN for 48-72 h and then incubated with antibodies as described in Ref. 28 for 1 h at 0ºC. Cells were pelleted, resuspended in PBS, fixed in 1% paraformaldehyde and analyzed in a FACS using a Consort 30 program (3000 data points).

**RNase protection assay**

Cytoplasmic RNA was obtained from monolayer cells by NP40 lysis and phenol/chloroform extraction. Probes were synthetized from SP6/T7 transcription vectors, pGEMs 3 and 4 (Promega), labelled with [$^{32}$P]αUTP (Amersham, UK) to a specific activity >$10^8$ cpm/µg DNA. Ten µg of RNA and 1-3 x $10^5$ cpm of each probe were employed in each assay. Single-stranded RNA was digested with ribonucleases and protected double-stranded RNA was resolved on a 6% polyacrylamide-urea gel as described elsewhere (30). Details on the construction and length of the protected fragments of the specific RNase protection probes (2'-5' OAS; 6-16; GBP; HLA class II DRα; 9-27 and γ-actin) used in this work have been described elsewhere (30). The 6-16 and GBP probes were gifts from S. Goodbourn and T. Decker, respectively.

**Electrophoretic mobility shift assay (EMSA)**

EMSA was carried out essentially as described in Ref. 30. Whole cell extracts were prepared by a modification of the method of Zimarino and Wu (33). Briefly, frozen-cell pellets were thawed on ice and lysed with an equal volume of extraction buffer (0.76 M NaCl, 1.5 mM $MgCl_2$, 10 mM HEPES, pH 7.9, 0.1 M EGTA, 5% glycerol, 0.5 mM dithiothreitol, and 0.2 mM phenylmethylsulfonylfluoride). Lysates were centrifuged at 100,000 *g* at 4ºC for 30 min. The supernatants were dialyzed against the same buffer as described above, except that 0.1 M NaCl was used, for approximately 4 h at 4ºC, and the concentration of protein was determined by the Bio-Rad assay. Ten µg of protein was pre-incubated with 1.2 µl poly dI-dC (Pharmacia) (5.4 mg/ml) at room temperature for 10 min, followed by the addition of 1.25 µg BSA, 0.125 µg *E. coli* DNA, 0.25 µg yeast tRNA, 2% Ficoll 400 and 0.32 ng labelled probe. The reactions were incubated at room temperature for 15 min and then analyzed by 6% PAGE. The 5' [$^{32}$P]-end-labelled probes 6-16, 9-27 and IRF-1 were used as described elsewhere (34,35).

**Northern blot analysis**

mRNA poly $A^+$ was obtained according to the manufacturer (Pharmacia). Five µg RNA per lane was electrophoresed on a denaturing 1.5% agarose-formaldehyde gel, transferred to Hybond $N^+$ membranes (Amersham, UK) as previously described (36) and hybridized with an αdCTP [$^{32}$P] human JAK1 cDNA probe, labelled to a specific activity >$10^8$ cpm/µg DNA, by using a multiprime DNA labelling system from Amersham, UK. Hybridization was performed at 65°C and subsequent washes were carried out according to Church and Gilbert (37). After exposure for 7 days, the membrane was stripped of the JAK1 probe and then reprobed with GAPDH, labelled at the same specific activity as described above, and exposed for 16 h.

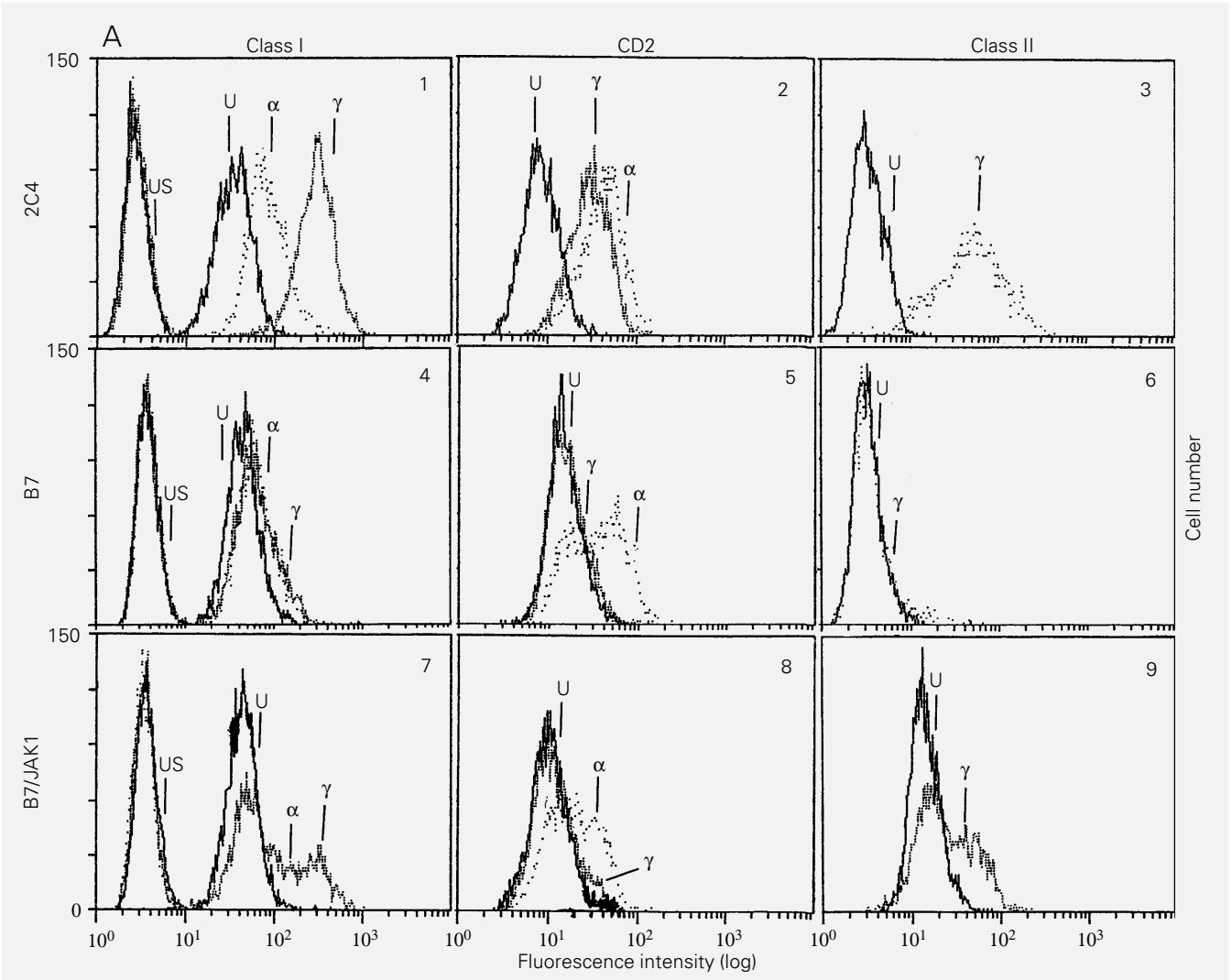


Figure 1 - FACS analysis of the parental and mutant cell lines. *A*, 2C4, B7 and B7/JAK1 transfectants and *B*, genetic crosses B7/U3 and B7/U4. The expression of the cell surface markers CD2 (panels 2,5,8,11,14) and endogenous class I (panels 1,4,7,10,13) and class II HLAs (panels 3,6,9,12,15) upon IFN-α or IFN-γ stimulation (500 IU/ml for 48 h) was monitored by FACS analysis as described in Material and Methods. Transfectants were analyzed as a population. *Panels 1-3*: Parental cell line 2C4; *panels 4-6*: B7 cell line; *panels 7-9*: B7/JAK1 transfectants; *panels 10-12*: genetic cross between B7 and the U3 mutant; *panels 13-15*: genetic cross between B7 and the U4 mutant. Untreated cells (U), IFN-α (α) or IFN-γ (γ) treated cells, and unstained cells (US) as indicated. US cells were left untreated or IFN-α treated, but were not incubated with any antibody.

## Results

### FACS analysis of the parental cell line 2C4 and the B7 mutant

FACS analysis of the B7 mutant indicated that this cell line responded partially to IFN-α and IFN-γ when the cell surface marker (CD2) and the endogenous class I HLA were investigated, whereas no response to IFN-γ was observed when the expression of endogenous class II HLA was analyzed (Figure 1A, panels 4-6). The expression of these genes in response to both IFN-α and IFN-γ in the parental cell line 2C4 is shown in Figure 1A (panels 1-3). B7 is a recessive mutant since crossing with 2C4 and with the wild type 2fTGH (25) restores the full responsiveness of the cells to both types of IFN (data not shown). The mutation(s) in B7 seems to be very stable since its phenotype remained the same after several passages in culture for more than three months, as confirmed by FACS analysis and RNase protection experiments.

### JAK1 complements B7 mutant

From the FACS analysis data (Figure 1A, panels 4-6), it was evident that the nature of the mutation(s) affecting both IFN-α and IFN-γ signalling pathways in this particular cell line may reside in a common polypeptide(s) which serves simultaneously in both the IFN-α and IFN-γ transduction cascades. To address this question, the mutant cell lines U3 and U4, which are known to be unresponsive to both types of IFN (27), were fused to the B7 mutant and cell surface expression of CD2, and class I and II HLAs were monitored by FACS analysis as described elsewhere (28). Full respon-

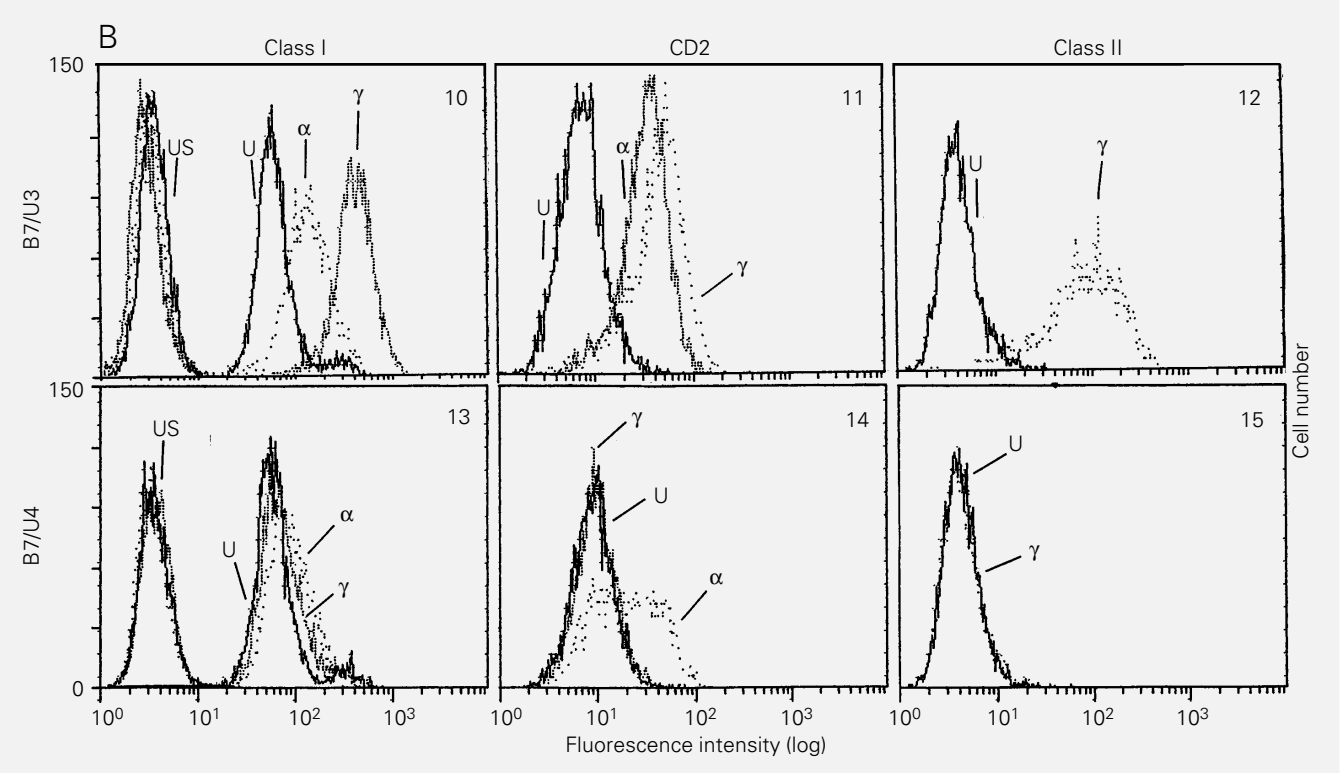


Figure 1 B.

siveness to both IFN-α and IFN-γ was obtained after fusing B7 with the U3 mutant (Figure 1B, panels 10-12). However, fusion with U4 just maintains the same original responsiveness of B7 to both types of interferon (Figure 1B, panels 13-15), indicating that B7 is a member of the U4 complementation group. Human JAK1 cDNA was then transfected into the B7 cell line, and cell surface expression (the same markers as described above) was monitored by FACS analysis (Figure 1A, panels 7-9). Although the experiment was done with a cell population, an enhancement of responsiveness to both IFN-α and IFN-γ was achieved, confirming B7 as a member of the U4 complementation group.

**IFN-inducible gene expression**

I next asked whether the IFN-α- and IFN-γ-partial responsiveness observed in this cell line by FACS analysis is correlated with the IFN-α- and IFN-γ-gene inducibility. Figure 2 shows mRNA expression of some IFN-stimulated genes (2'-5' OAS; 6-16; GBP; 9-27 and class II HLA-DRα) in response to either IFN-α or IFN-γ. Although the expression of these genes varied to some extent in response to the specific inducer, as observed in the parental cell line, the reduction in the mRNA expression levels of these genes in mutant B7 was remarkable (compare Figure 2, lanes 1-3 to 4-6 and 7-9 to 10-12), which agrees with the phenomenon of IFN-partial responsiveness observed by FACS analysis.

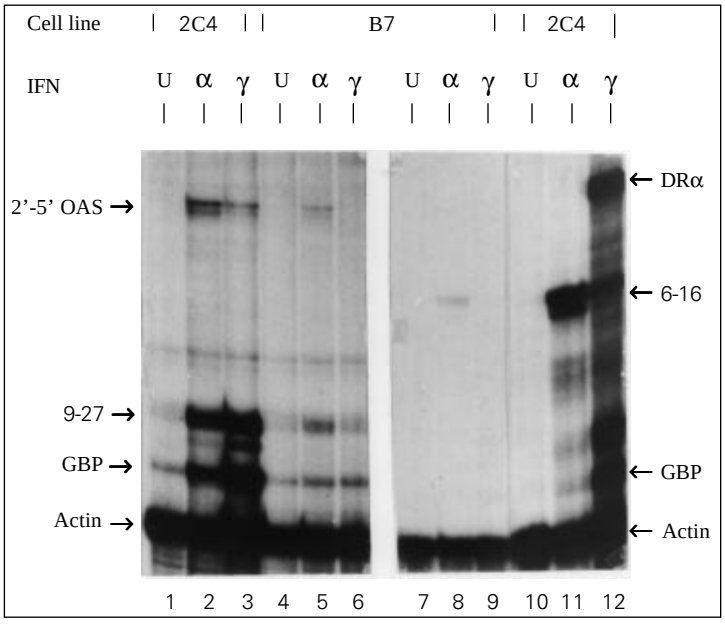


Figure 2 - IFN-stimulated gene expression in the parental cell line 2C4 and in the B7 mutant. The expression of 2'-5' oligo A synthetase (OAS), 9-27 and GBP (lanes 1-6), and class II HLA (DRα), 6-16 and GBP (lanes 7-12) mRNAs in the parental cell line 2C4 and in the B7 mutant was investigated upon IFN-α or IFN-γ treatment. Five hundred IU/ml of either IFN-α or IFN-γ was added to the cells for 6 h and 18 h, respectively, cytoplasmic RNA was isolated (10 µg per lane), hybridized with the specific probe and analyzed by the RNase protection assay as described in Material and Methods. γ-Actin was used as an internal standard for RNA loading.

**IFN-activated transcriptional complex formation**

Upon IFN treatment a number of transcriptional activator complexes are formed depending on the type of ISG promoter (34,38,39). Thus, when the promoter of an ISG possessing a classical ISRE, such as the 9-27 gene, is used as a probe (Figure 3A), two complexes are observed after IFN stimulation in cell extracts prepared from a variety of cell lines (34) including 2C4 cells (this work): i) ISGF3-α, which is activated/formed upon IFN-α treatment (Figure 3A, lane 2); ii) ISGF3-γ, represented by p48, which is expressed at a low constitutive level in untreated cells, but whose level increases significantly after either IFN-α or IFN-γ treatment (Figure 3A, lanes 1-3). In contrast, the B7 mutant was completely inefficient in terms of ISGF3-α formation upon IFN-α treatment (Figure 3A, lane 5), whereas the expression level of ISGF3-γ in untreated cells was similar to that observed in IFN-treated cells (Figure 3A, lanes 4-6).

By employing the promoter of the 6-16 gene as a probe, another transcriptional complex was found in cell extracts of untreated HT1080 cells, whose expression was increased upon IFN stimulation, mainly IFN-γ. Antibody studies identified this complex

which comprises IRF1/IRF2 (IFN-regulator factor) proteins (34), and this was also observed for 2C4 cells (HT1080-derived cell line) (Figure 3B, lanes 7-9). On the other hand, the B7 mutant showed a constitutive level of IRF1/IRF2 complex formation regardless of the IFN treatment (Figure 3B, lanes 10-12).

When the promoter of the IRF1 gene was employed as a probe (Figure 3C), a transcriptional complex was detected in cell extracts prepared from 2C4 cells after IFN-γ treatment (Figure 3C, lane 15), which was absent upon IFN-α treatment (Figure 3C, lane 14). Antibody characterization identified p91 and p48 (or related proteins) as components of this complex (data not shown), which may act through the GAS element (17,20) or a different motif other than an ISRE (30,40), since the IRF1 promoter is devoid of an ISRE. In contrast, this complex formation was not observed in the B7 mutant. On the other hand, a slightly faster migrating complex was observed in the mutant, which is expressed at a low constitutive level, regardless of the IFN treatment (Figure 3C, lanes 16-18). The significance of this observation remains to be investigated.

All the above DNA-protein complexes formed specifically competed for binding with the homologous oligonucleotide, at 50 molar excess, but did not compete with heterologous oligonucleotides (data not shown).

#### JAK1 mRNA expression in the B7 mutant

In order to investigate whether the partial responsiveness to both types of IFN in the B7 mutant (FACS analysis and ISGs mRNA expression) is correlated with the level of JAK1 mRNA expression in this cell line, poly $A^+$ mRNA was hybridized with the human JAK1 probe. As shown in Figure 4, no detectable JAK1 message was identified in the mutant cell line when compared to the parental cell line 2C4.

## Discussion

By combining genetic and biochemical approaches, impressive progress has been made towards understanding the nuclear signals triggered by IFN upon ligand-receptor binding (5). A converging approach employing target gene disruption, which affects specific genes such as IFN receptors (41), and regulatory proteins IRF1/IRF2 (42,43), has provided substantial evidence showing how the IFN system operates. On the other hand, structure-function analysis has also provided important information on this emerging picture, for example i) a single amino acid substitution changing tyrosine 701 at the phosphorylation site in the STAT1 to phenylalanine (44) abrogates IFN-α and -γ responsiveness, ii) the same substitution at position 690 in the STAT2 interferes with the phosphorylation of STAT1 (31,45), and iii) sequential deletions affecting different domains in the NRPTK-TYK2 restore the loss of kinase function (46).

In the present study I described a reces-

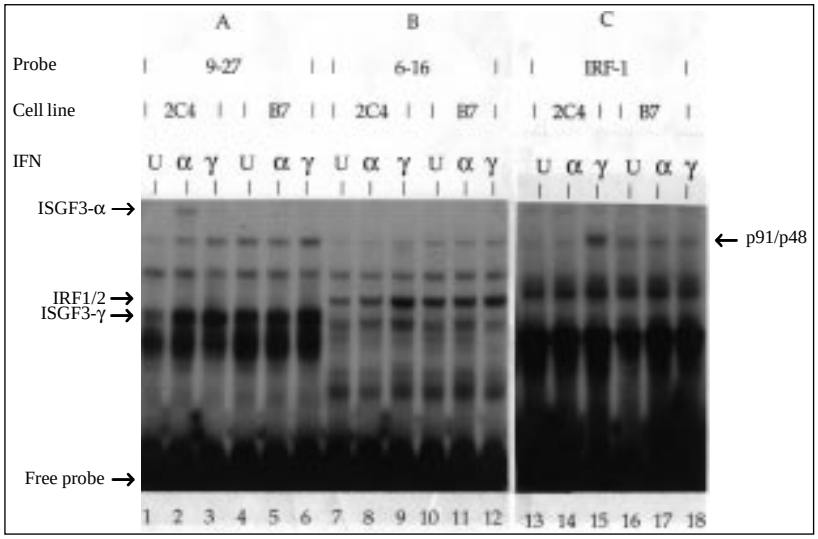


Figure 3 - IFN-activated transcriptional complex formation. EMSA of ISGF3-α, ISGF3-γ, IRF1/2 and p91/p48 in whole cell extracts from IFN-treated 2C4 and B7 cells. Cells were left untreated (U) or treated with 500 IU/ml of either IFN-α or IFN-γ for 6 h and 18 h, respectively. Cell extracts were incubated with an end-labelled oligodeoxynucleotide probe containing the promoter region of 9-27 (A, lanes 1-6); 6-16 (B, lanes 7-12) or IRF-1 (C, lanes 13-18) genes. DNA-protein complexes formed are indicated.

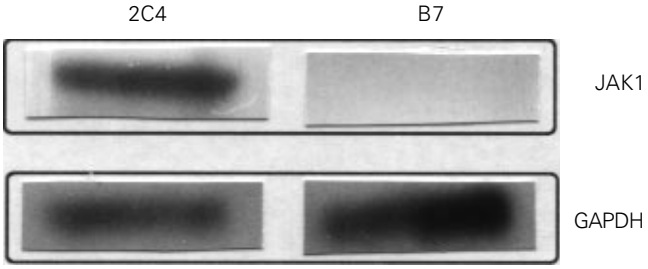


Figure 4 - Northern analysis of JAK1 mRNA. Five µg of poly A$^+$ RNA from the parental cell line 2C4 and the B7 mutant was hybridized with a human JAK1 cDNA probe as indicated. GAPDH was used as an internal standard for RNA loading.

sive mutant cell line, B7, which is partially responsive to both IFN-α and IFN-γ. The defect in this mutant was associated with the NRPTK-JAK1, because this cell line is not complemented by fusion with the U4 mutant (JAK1$^-$), and genetic transfer of human JAK1 cDNA into the cell line enhanced both IFN-α and IFN-γ responsiveness. Although the induction of ISGs, upon IFN-α and IFN-γ stimulation, was partially observed in this mutant, JAK1 mRNA expression was below detection levels. The nature of mutation(s) affecting JAK1 mRNA expression remains to be further investigated, but one may speculate that it may map either at the promoter region of the gene or at the 3' end, the latter contributing to the instability of the mRNA. However, the kinetics of ISG stimulation favors the former hypothesis. Whatever the mutation associated with the B7 phenotype, it seems that this mutant is particularly useful to understand cis-acting elements governing JAK1 expression. It is tempting to speculate that even under these circumstances partial ISG stimulation occurs through the JAK/STAT signalling pathway (5).

Transcriptional complex formation, involving IRF-family members (IRF1, IRF2 and p48), which are inducible in the parental cell line 2C4 upon IFN-α and IFN-γ treatment, becomes constitutively expressed in the B7 mutant. Whether this phenomenon is directly correlated with the nature of mutation(s) affecting JAK1 expression, or whether they are unrelated events remains to be determined. The possibility that additional mutation(s) contributes to this particular phenotype cannot be ruled out.

Thus, the mutant cell line B7 seems to be of particular importance for the understanding of cis-acting elements governing JAK1 mRNA expression and may represent regulatory mechanisms associated with JAK1 expression and transcriptional activation.

## Acknowledgments

I would like to thank Dr. I.M. Kerr, Imperial Cancer Research Fund, London, for the opportunity to carry out this work in his laboratory. I also thank A. Wilks for providing the human JAK1 cDNA, S. Goodbourn, T. Decker and M. Müller for kindly providing the protection probes, R. Pine, D. Levy and C. Schindler for the antibodies, and Dima Guschin and L.F.L. Reis for a critical reading of the manuscript.

## References


1. Pestka S, Langer JA, Zoon KC & Samuel C (1987). Interferons and their actions. *Annual Review of Biochemistry*, 56: 727-777.
2. Uzé G, Lutfalla G & Mogensen KE (1995). α and ß interferons and their receptor and their friends and relations. *Journal of Interferon and Cytokine Research*, 15: 3-26.
3. Ihle JN (1995). Cytokine receptor signalling. *Nature*, 377: 591-594.
4. Pellegrini S & Schindler C (1993). Early events in signalling by interferons. *Trends in Biochemical Sciences*, 18: 338-342.
5. Darnell Jr JE, Kerr IM & Stark GR (1994). JAK-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science*, 264: 1415-1421.
6. Levy DE, Kessler DS, Pine R & Darnell Jr JE (1989). Cytoplasmic activation of ISGF3, the positive regulator of interferon-α-stimulated transcription, reconstituted *in vitro*. *Genes and Development*, 3: 1362-1371.
7. Dale TC, Imam AMA, Kerr IM & Stark GR (1989). Rapid activation by interferon α of a latent DNA-binding protein present in the cytoplasm of untreated cells. *Proceedings of the National Academy of Sciences, USA*, 86: 1203-1207.
8. Fu XY, Kessler DS, Veals SA, Levy DE & Darnell Jr JE (1990). ISGF3, the transcriptional activator induced by interferon α, consists of multiple interacting polypeptide chains. *Proceedings of the National Academy of Sciences, USA*, 87: 8555-8559.
9. Kessler DS, Veals SA, Fu XY & Levy DE (1990). Interferon-alpha regulates nuclear translocation and DNA binding affinity of ISGF3, a multimeric transcriptional activator. *Genes and Development*, 4: 1753-1765.
10. Fu XY, Schindler C, Improta T, Aebersold R & Darnell Jr JE (1992). The proteins of ISGF-3, the interferon α-induced transcriptional activator, define a gene family involved in signal transduction. *Proceedings of the National Academy of Sciences, USA*, 89: 7840-7843.
11. Levy DE, Kessler DS, Pine R & Darnell Jr JE (1988). Interferon-induced nuclear factors that bind a shared promoter element correlate with positive and negative transcription control. *Genes and Development*, 2: 383-393.
12. Dale TC, Rosen JM, Guille MJ, Lewin AR, Porter AC, Kerr IM & Stark GR (1989). Overlapping sites for constitutive and induced DNA binding factors involved in interferon-stimulated transcription. *EMBO Journal*, 8: 831-839.
13. Schindler C, Shuai K, Prezioso V & Darnell Jr JE (1992). Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. *Science*, 257: 809-812.
14. Shuai K, Horvath CM, Tsai Huang LH, Qureshi SA, Cowburn D & Darnell Jr JE (1994). Interferon activation of the transcription factor STAT91 involves dimerization through SH2-phosphotyrosyl peptide interactions. *Cell,* 76: 821-828.
15. Decker T, Lew DJ & Darnell Jr JE (1991). Two distinct alpha-interferon-dependent signal transduction pathways may contribute to activation of transcription of the guanylate-binding protein gene. *Molecular and Cellular Biology*, 11: 5147-5153.
16. Lew DJ, Decker T, Strehlow I & Darnell Jr JE (1991). Overlapping elements in the guanylate-binding protein gene promoter mediate transcriptional induction by alpha and gamma interferons. *Molecular and Cellular Biology*, 11: 182-191.
17. Kanno Y, Kozak CA, Schindler C, Driggers PH, Ennist DL, Gleason SL, Darnell Jr JE & Ozato K (1993). The genomic structure of the murine ICSBP gene reveals the presence of the gamma interferon-responsive element, to which an ISGF3-α subunit (or similar) molecule binds. *Molecular and Cellular Biology*, 13: 3952-3963.
18. Khan KD, Shuai K, Lindwall G, Maher SE, Darnell Jr JE & Bothwell ALM (1993). Induction of the LY-6A/E gene by interferon α/ß and γ requires a DNA element to which a tyrosine-phosphorylated 91-kDa protein binds. *Proceedings of the National Academy of Sciences, USA*, 90: 6806-6809.
19. Pearse RN, Feinman RN, Shuai K, Darnell Jr JE & Ravetch JV (1993). Interferon gamma-induced transcription of the high affinity Fc receptor for IgG requires assembly of a complex that includes the 91-kDa subunit of the transcription factor ISGF3. *Proceedings of the National Academy of Sciences, USA*, 90: 4314-4318.
20. Shuai K, Schindler C, Prezioso V & Darnell Jr JE (1992). Activation of transcription by IFN-γ: tyrosine phosphorylation of a 91-kDa DNA binding protein. *Science*, 258: 1808-1812.
21. Aguet M, Dembic Z & Merli G (1988). Molecular cloning and expression of the human interferon-gamma receptor. *Cell*, 55: 273-280.
22. Uzé G, Lutfalla G & Gresser I (1990). Genetic transfer of a functional human interferon alpha receptor into mouse cells: cloning and expression of its cDNA. *Cell*, 60: 225-234.
23. Ziemiecki A, Harpur AG & Wilks AF (1994). JAK protein tyrosine kinases: their role in cytokine signalling. *Trends in Cell Biology*, 4: 207-212.
24. Ihle JN & Kerr IM (1995). JAKs and STATs in signaling by the cytokine receptor superfamily. *Trends in Genetics,* 11: 69-74.
25. Pellegrini S, John J, Shearer M, Kerr IM & Stark GR (1989). Use of a selectable marker regulated by alpha interferon to obtain mutations in the signaling pathway. *Molecular and Cellular Biology*, 9: 4605-4612.
26. John J, McKendry R, Pellegrini S, Flavell D, Kerr IM & Stark GR (1991). Isolation and characterization of a new mutant cell line unresponsive to alpha and beta interferons. *Molecular and Cellular Biology*, 11: 4189-4195.
27. McKendry R, John J, Flavell D, Müller M, Kerr IM & Stark GR (1991). High-frequency mutagenesis of human cells and characterization of a mutant unresponsive to both α and γ interferons. *Proceedings of the National Academy of Sciences, USA*, 88: 11455-11459.
28. Watling D, Guschin D, Müller M, Silvennoinen O, Witthuhn BA, Quelle FW, Rodgers NC, Schindler C, Stark GR, Ihle JN & Kerr IM (1993). Complementation by the protein tyrosine kinase JAK2 of a mutant cell line defective in the interferon gamma signal transduction pathway. *Nature*, 366: 166-170.
29. Velazquez L, Fellous M, Stark GR & Pellegrini S (1992). A protein tyrosine kinase in the interferon α/ß signaling pathway. *Cell*, 70: 313-322.
30. Müller M, Laxton C, Briscoe J, Schindler C, Improta T, Darnell Jr JE, Stark GR & Kerr IM (1993). Complementation of a mutant cell line: central role of the 91 kDa polypeptide of ISGF3 in the interferon-alpha and gamma signal transduction pathways. *EMBO Journal*, 12: 4221-4228.
31. Leung S, Qureshi SA, Kerr IM, Darnell Jr JE & Stark GR (1995). Role of STAT2 in the alpha interferon signaling pathway. *Molecular and Cellular Biology*, 15: 1312-1317.

32. Müller M, Briscoe J, Laxton C, Guschin D, Ziemiecki A, Silvennoinen O, Harpur AG, Barbieri G, Witthuhn BA, Schindler C, Pellegrini S, Wilks AF, Ihle JM, Stark GR & Kerr IM (1993). The protein tyrosine kinase JAK1 complements defects in interferon-α/ß and γ signal transduction. *Nature*, 366: 129-135.
33. Zimarino W & Wu C (1987). Induction of sequence-specific binding of *Drosophila* heat shock activator protein without protein synthesis. *Nature*, 327: 727-730.
34. Parrington J, Rodgers NC, Gewert D, Pine R, Veals SA, Levy DE, Stark GR & Kerr IM (1993). The interferon-stimulable response elements of two human genes detect overlapping sets of transcription factors. *European Journal of Biochemistry*, 214: 617-626.
35. Pine R, Canova A & Schindler C (1994). Tyrosine phosphorylated p91 binds to a single element in the ISGF2/IRF-1 promoter to mediate induction by IFNα and IFNγ, and is likely to autoregulate the p91 gene. *EMBO Journal*, 13: 158-167.
36. Sambrook J, Fritsch EF & Maniatis T (1987). *Molecular Cloning. A Laboratory Manual*. Vol. 1. 2nd edn. Cold Spring Harbor Laboratory Press, New York, 7.37-7.50.
37. Church GM & Gilbert W (1984). Genomic sequencing. *Proceedings of the National Academy of Sciences, USA*, 81: 1991-1995.
38. Stark GR & Kerr IM (1991). The control of interferon-inducible gene expression. *FEBS Letters*, 285: 194-198.
39. Williams BRG (1991). Transcriptional regulation of interferon-stimulated genes. *European Journal of Biochemistry*, 200: 1-11.
40. Haque SJ & Williams BRG (1994). Identification and characterization of an interferon (IFN)-stimulated response element-IFN-stimulated gene factor 3-independent signaling pathway for IFN-α. *Journal of Biological Chemistry*, 269: 19523-19529.
41. Müller U, Steiholff U, Reis LFL, Hemmi S, Pavlovic J, Zinkernagel RM & Aguet M (1994). Functional role of type I and type II interferons in antiviral defense. *Science*, 264: 1918-1924.
42. Matsuyama T, Kimura T, Kitagawa M, Pfeffer K, Kawakami T, Watanabe N, Kündig TM, Amakawa R, Kishihara K, Wakeham A, Potter J, Furlonger CL, Narendran A, Suzuki H, Ohashi PS, Palge C, Taniguchi T & Mak TW (1993). Targeted disruption of IRF-1 or IRF-2 results in abnormal type I IFN gene induction and aberrant lymphocyte development. *Cell*, 75: 83-97.
43. Reis LFL, Ruffner H, Stark G, Aguet M & Weissmann C (1994). Mice devoid of interferon regulatory factor 1 (IRF-1) show normal expression of type I interferon genes. *EMBO Journal*, 13: 4798-4806.
44. Shuai K, Stark GR, Kerr IM & Darnell Jr JE (1993). A single phosphotyrosine residue of Stat91 required for gene activation by interferon-γ. *Science*, 261: 1744-1746.
45. Improta T, Schindler C, Horvath CM, Kerr IM, Stark GR & Darnell Jr JE (1994). Transcription factor ISGF-3 formation requires phosphorylated STAT91 protein, but Stat113 protein is phosphorylated independently of STAT91 protein. *Proceedings of the National Academy of Sciences, USA*, 91: 4776-4780.
46. Velazquez L, Mogensen KE, Barbieri G, Fellous M, Uzé G & Pellegrini S (1995). Distinct domains of the protein tyrosine kinase tyk2 required for binding of interferon α/ß and for signal transduction. *Journal of Biological Chemistry*, 270: 3327-3334.