MOLECULAR AND CELLULAR BIOLOGY, Mar. 1995, p. 1312–1317 Vol. 15, No. 3
0270-7306/95/$04.00+0

Role of STAT2 in the Alpha Interferon Signaling Pathway

STEWART LEUNG,[1] SAJJAD A. QURESHI,[2] IAN M. KERR,[3] JAMES E. DARNELL, JR.,[2] AND GEORGE R. STARK[1]*

Department of Molecular Biology, Research Institute, The Cleveland Clinic Foundation, Cleveland, Ohio 44195[1]; Laboratory of Molecular Cell Biology, Rockefeller University, New York, New York 10021[2]; and Imperial Cancer Research Fund, London WC2A 3PX, United Kingdom[3]

Received 29 August 1994/Returned for modification 27 September 1994/Accepted 18 November 1994

We have isolated U6A, a mutant cell line which lacks the STAT2 subunit of the transcription factor interferon (IFN)-stimulated gene factor 3 (ISGF3). The response of U6A cells to IFN-α is almost completely defective, but the response to IFN-γ is normal. Complementation of U6A cells with a cDNA encoding STAT2 restores the IFN-α response, proving that STAT2 is required in this pathway. Binding of IFNs to their receptors triggers tyrosine phosphorylation and activation of the receptors, JAK family kinases, STAT1, and STAT2. In IFN-α-treated U6A cells, phosphorylation of the essential tyrosine kinases TYK2 and JAK1 is normal, but the phosphorylation of STAT1 is weak. A mutant STAT2 protein in which the phosphorylated tyrosine at position 690 is changed to phenylalanine does not restore normal phosphorylation of STAT1 in response to IFN-α. The dependence of STAT1 phosphorylation on the presence of STAT2 but not vice versa (T. Improta, C. Schindler, C. M. Horvath, I. M. Kerr, G. R. Stark, and J. E. Darnell, Jr., Proc. Natl. Acad. Sci. USA 91:4776–4780, 1994) indicates that in the formation of ISGF3, these two proteins may be phosphorylated sequentially in response to IFN-α and that phosphorylated STAT2 may be required to allow unphosphorylated STAT1 to bind to the activated IFN-α receptor.

Binding of the interferons (IFNs) to their cognate receptors stimulates phosphorylation on tyrosine of several proteins (reviewed by Darnell et al. [5]), including the receptors (12, 30), members of the JAK family of tyrosine kinases (22, 26, 43, 44), and transcription factor subunits called STATs (signal transducers and activators of transcription) (8, 39). Phosphorylated STATs, which also contain Src homology 2 (SH2) domains, associate to form homo- and heterodimers (40, 51). These dimers, with or without additional cofactors, migrate to the nucleus, where they activate the transcription of IFN-responsive genes (5).

IFN-α and -β induce formation of the transcription factor IFN-stimulated gene factor 3 (ISGF3) (4, 20). The binding sites on DNA for ISGF3, called IFN-stimulated response elements (ISREs), are found near promoters of most IFN-α/β-responsive genes (7, 19, 31, 33, 34, 48). The ISGF3 transcription factor is an oligomeric protein with three subunits: STAT1, STAT2, and a 48-kDa DNA-binding protein (9, 10, 38, 46). STAT1 exists in two alternatively spliced forms of 91 kDa (STAT1α) and 84 kDa (STAT1β); either is capable of participating in ISGF3 formation (27). STAT2 is a 113-kDa protein having approximately 40% homology with STAT1α (10). Unlike IFN-α/β, IFN-γ triggers the phosphorylation on tyrosine of STAT1 but not STAT2 (41), leading to formation of the gamma-activated transcription factor GAF, which binds to the gamma-activated sequences (GAS) (6, 21, 42). GAF is formed when STAT1α subunits dimerize through reciprocal SH2 domain-phosphotyrosine interactions (40). Phosphorylation of STAT1 and formation of STAT1 homodimers can also be activated by IFN-α. Before the STAT proteins were recognized, this IFN-α-activated factor was called AAF (6).

* Corresponding author. Mailing address: Research Institute—NC11, The Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195. Phone: (216) 444-3900. Fax: (216) 444-3279. Electronic mail address: starkg@ccsmtp.ccf.org.

Unphosphorylated STAT1α binds to a specific phosphotyrosine near the C terminus of the IFN-γ receptor α chain (12). Phosphorylation of this tyrosine upon binding of IFN-γ to the receptor is an early step in the IFN-γ signaling pathway, creating a binding site for the unphosphorylated transcription factor subunit in proximity to the receptor-bound tyrosine kinases JAK1 and JAK2 (26, 49). The IFN-α receptor may employ a similar mechanism. However, although phosphorylation of the IFN-α receptor has been observed (30), the site is not yet known.

Genetic studies using mutant cell lines unresponsive to the IFNs (17, 24, 29, 49) have established the functional importance of JAKs and STATs in the pathways. Two JAK family kinases, TYK2 (47) and JAK1 (26), as well as STAT1 (27) and the 48-kDa DNA-binding protein (17) are required for the response of most genes to IFN-α. However, the situation is somewhat complex in that mutants lacking TYK2 retain a weak response to IFN-β (29), probably mediated by formation of ISGF3 (15). Furthermore, some genes (IRF-1, for example) respond to IFN-α/β independently of ISGF3, utilizing AAF to activate a GAS-like element (6, 13). The availability of mutant cell lines lacking the individual proteins of ISGF3 allows analysis of the function of each protein separately. Study of U3A cells, which lack STAT1α and -β, has shown that STAT1α (but not STAT1β) is required in the IFN-γ pathway, that either STAT1α or STAT1β can function in the IFN-α pathway, and that Y-701 (the phosphorylation site in STAT1) and R-602 (a conserved residue in the SH2 domain of STAT1) are required in both pathways (27, 42). The STAT1-independent phosphorylation of STAT2 on Y-690 in U3A cells in response to IFN-α has also been shown (16).

We have now obtained and begun to characterize the mutant cell line U6A, which lacks STAT2. The properties of these cells prove that STAT2 is needed to form ISGF3 and show a required and ordered interaction between STAT2 and STAT1 for efficient activation of STAT1 in response to IFN-α.

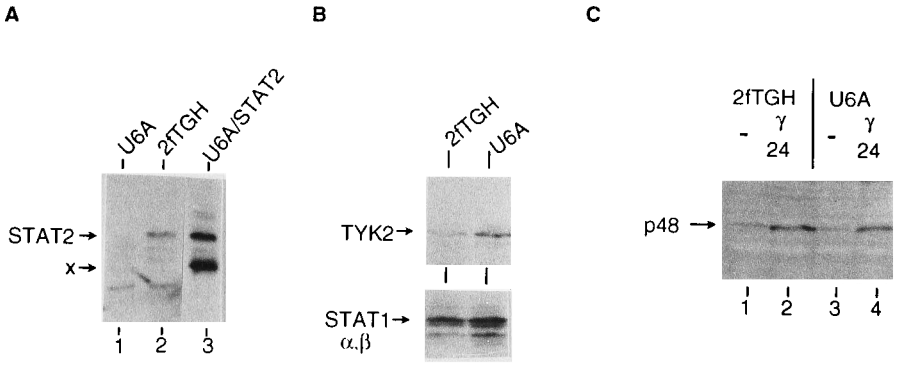

FIG. 1. Western blot analysis of proteins present in cell line U6A. (A) U6A cells lack STAT2. Extracts of cell lines U6A (lane 1), 2fTGH (lane 2), and U6A transfected with STAT2 cDNA (lane 3) were analyzed with an anti-STAT2 antibody. The band with higher mobility seen in lane 3 (X) is not consistently observed and is likely to be a degradation product of STAT2. (B) U6A cells contain TYK2 and STAT1α/β. Extracts of cell lines 2fTGH and U6A were analyzed with antibodies to TYK2 and STAT1α/β. (C) U6A cells have IFN-γ-inducible p48. Equal amounts of extracts from the cell lines 2fTGH (lane 1), 2fTGH treated for 24 h with IFN-γ (lane 2), U6A (lane 3), and U6A treated for 24 h with IFN-γ (lane 4) were analyzed with antibodies to p48.

MATERIALS AND METHODS

Cells and IFNs. The 2fTGH cells used for mutagenesis have been described elsewhere (29). IFN-α (10^8 IU/mg) was obtained from Wellcome Research Laboratories. IFN-γ (8.3×10^6 IU/mg) was from Genentech. IFN-β (10^8 IU/mg) was from Biogen. The IFNs were used at a final concentration of 500 IU/ml unless stated otherwise.

Mutagenesis and selection. Mutagenesis with ICR 191 (Polysciences Inc.) was performed as described by Pellegrini et al. (29) and McKendry et al. (24). Seventeen independent pools of 2fTGH cells were subjected to four rounds of mutagenesis, and a total of 1.6×10^7 cells were selected in 500 IU of IFN-α per ml and 30 μM 6-thioguanine. Twelve days later, the colonies in each pool were combined separately. About 500 cells of the combined populations from nine pools were selected again in 2,500 IU of IFN-α per ml and 6-thioguanine. Individual colonies were analyzed by Western blotting (immunoblotting). Mutants lacking TYK2 were isolated from seven of the pools, one of which also yielded a mutant cell line lacking STAT1. One pool did not give any mutants. The mutant cell line U6A was isolated from the ninth pool.

Analysis and complementation. Extracts prepared from individual mutant clones were analyzed by Western blotting (14). The antisera used were directed against TYK2 (26), STAT1α and -β (38), STAT2 (10), and p48 (46). Bands were detected by chemiluminescence using the Renaissance reagents (DuPont NEN) according to the company's protocol.

U6A cells were stably transfected by the calcium phosphate method (3), using a construct based on the eukaryotic expression plasmid pMNC113 in which the open reading frame of STAT2 is expressed under control of the cytomegalovirus promoter (32). To distinguish between *cis* and *trans* mutants (29), individual clones were monitored for the induction by IFN-α of class I major histocompatibility complex (MHC) on the cell surface by using a fluorescence-activated cell scanner (FACScan). Cells were stained with a class I MHC ABC monoclonal antibody and a fluorescein-conjugated secondary antibody (Dako) after 48 h of IFN-α or -β treatment.

RNase protection assay. Total RNA was prepared from IFN-α- or IFN-γ-treated cells, and protection experiments were performed as described by Sambrook et al. (37). The probes used were 6-16 (protects 190 bases) and 9-27 (160 bases), both described by Ackrill et al. (1); ISG54 (250 bases, derived from exon 2) and ISG15 (150 bases, derived from the 3′ untranslated region), both gifts from David Levy; and IRF-1 (175 bases), guanylate-binding protein (GBP; 138 bases), and γ-actin (130 bases), described by Müller et al. (27).

Immunoprecipitation. Tyrosine phosphorylation of TYK2, JAK1 (50), and STAT1 was monitored as described by Schindler et al. (39). The antiphosphotyrosine monoclonal antibodies used were PY20 (Signal Transduction Laboratories) and 4G10 (Upstate Biotechnology Incorporated).

Band shift assays. Cells were lysed in whole-cell extraction buffer (16) and assayed with a ^{32}P-labeled oligonucleotide corresponding to the GAS element of the LyE/6 gene (41).

RESULTS

STAT2 is missing in U6A cells and is required for IFN-α/β signaling. The fibrosarcoma cell line 2fTGH contains an *Escherichia coli* guanine phosphoribosyltransferase gene whose expression is regulated by the upstream region of the IFN-inducible human gene 6-16. This cell line has been used to isolate several complementation groups of mutants with defective responses to IFN-α/β (5, 17, 24, 29). To identify new complementation groups rapidly, individual clones selected with IFN-α and 6-thioguanine were screened with antisera directed against TYK2, STAT1, STAT2, and p48. Mutant U6A, which lacks STAT2 protein (Fig. 1A), was isolated from one of nine mutagenized pools. The TYK2, STAT1, and p48 proteins were present in U6A cells (Fig. 1B and C). Analysis of total RNA by Northern (RNA) blotting indicated that the 4.8-kb STAT2 mRNA, detected in 2fTGH cells, was missing in U6A (data not shown).

U6A cells are complemented by STAT2. We analyzed the expression in U6A cells of several IFN-α-inducible genes which are regulated by ISREs: 6-16, 9-27, ISG54, ISG15, and class I MHC (Fig. 2 and 3A and B). U6A cells did not respond to IFN-α in any of these assays, confirming that STAT2 is absolutely required for the IFN-α response of the genes tested. FACScan analysis also revealed that expression of class I MHC genes was not induced by IFN-β in U6A cells (data not shown). To verify that the defect in U6A is due solely to the lack of STAT2, we transfected a construct expressing STAT2 cDNA into these cells and isolated several clones. The expression level of STAT2 was high in clones that survived selection in IFN-α plus hypoxanthine-aminopterin-thymidine medium (see example in Fig. 1A), which selects for restoration of the response (29). As expected, the IFN-α/β response was restored fully in these clones (Fig. 2B and C, Fig. 3C, and data not shown). Thus, we have proved that STAT2 is required for expression of ISRE-containing genes in response to IFN-α/β, as shown previously for STAT1, another component of ISGF3 (27).

STAT2 is not required for the IFN-γ response. STAT1, but not STAT2, is phosphorylated in response to IFN-γ. Thus, we expected that the IFN-γ response would not be affected in U6A cells. IRF-1, GBP, and class I MHC genes all have binding sites for STAT1 that are required for IFN-γ-induced expression (6, 13, 21, 28), and these genes are not induced by IFN-γ in U3A cells, which lack STAT1 (27). Induction of class I MHC, IRF-1, and GBP by IFN-γ was not defective in U6A cells (Fig. 3E and data not shown), confirming that STAT2 is not required for the IFN-γ response. Furthermore, the 9-27 mRNA (Fig. 2A) and the p48 protein (Fig. 1C) were also induced by IFN-γ in U6A cells, although the response was weaker than in 2fTGH cells. The induction of class II MHC by IFN-γ was also reduced in U6A cells by more than twofold (data not shown). This property is not related to the lack of

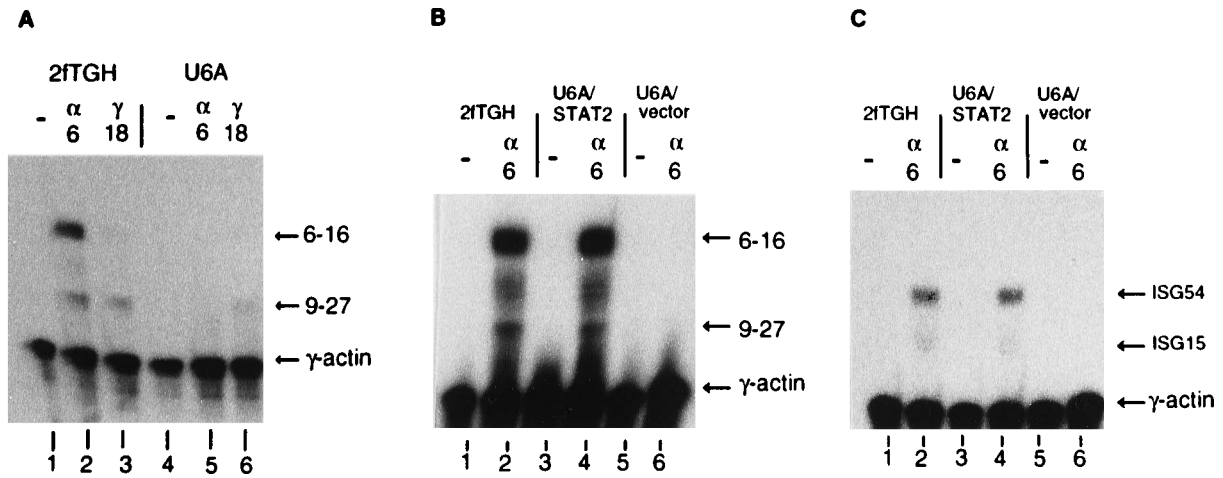

FIG. 2. STAT2 is essential for IFN-α/β signaling. (A) Total RNA samples from cell lines 2fTGH (lanes 1 to 3) and U6A (lanes 4 to 6) were analyzed by RNase protection using probes for 6-16 and 9-27 mRNAs. The cells analyzed in lanes 2 and 5 were treated with IFN-α for 6 h, and those analyzed in lanes 3 and 6 were treated with IFN-γ for 18 h. (B and C) Total RNAs prepared from cell lines 2fTGH (lanes 1 and 2), U6A transfected with STAT2 cDNA (lanes 3 and 4), and U6A transfected with the LNCX vector (lanes 5 and 6) were analyzed with 6-16 and 9-27 probes (B) or with ISG54 and ISG15 probes (C). The cells analyzed in lanes 2, 4, and 6 were treated with IFN-α for 6 h. A γ-actin probe was used for normalization. U6A cells transfected with the LNCX vector (25) are essentially the same as untransfected U6A cells in their response to interferon (data not shown).

STAT2, because full class II MHC induction was not restored in U6A clones complemented by STAT2 cDNA (data not shown). The U6A cells may contain a second mutation that affects expression of class II genes.

STAT1 is phosphorylated on tyrosine very weakly in response to IFN-α in U6A cells. In U3A cells, which lack STAT1, treatment with IFN-α still allows a normal level of phosphorylation of STAT2 on the correct tyrosine (16). To test whether the reciprocal phosphorylation occurs in U6A cells treated with IFN-α, cell extracts were immunoprecipitated with anti-STAT1 and the precipitates were tested for tyrosine phosphorylation. STAT1 was phosphorylated only very weakly on tyrosine in U6A cells (Fig. 4A and B). The level of phosphorylation was at most 5% of that seen in response to IFN-γ in the same cells or in U6A cells complemented with STAT2 cDNA, in which IFN-α and IFN-γ induced similar levels of STAT1 phosphorylation. Several lines of evidence show that the STAT1 in U6A cells is fully functional: it was phosphorylated on tyrosine normally in response to IFN-γ (Fig. 4A and B), GAF was formed normally (Fig. 4C), the IRF-1 and GBP genes were induced normally by IFN-γ (data not shown), and both the phosphorylation-activation of STAT1 and the IFN-α response were restored fully in U6A cells transfected with STAT2 cDNA. The transcription factor complexes ISGF3 and AAF (the IFN-α-induced complex containing STAT1) were not detected in IFN-α-treated U6A cells (Fig. 4C and data not shown). Transfection of STAT2 cDNA into U6A cells restored both the phosphorylation of STAT1 (Fig. 4A and B) and the formation of AAF (Fig. 4C) and ISGF3 (data not shown). Thus, STAT2 is required for efficient phosphorylation of

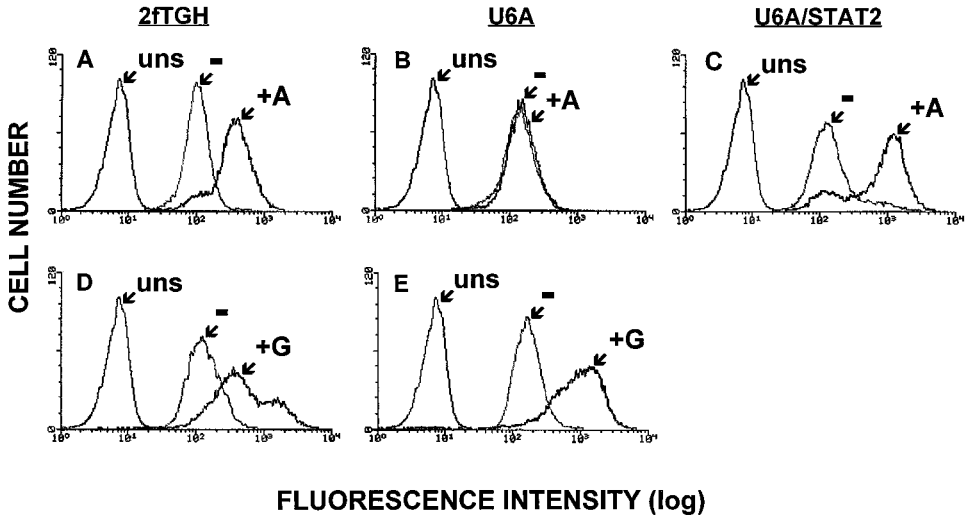

FIG. 3. Analysis of class I MHC induction in response to IFN-α or IFN-γ. Cells were treated with IFN-α or IFN-γ for 48 h, stained with anti-class I MHC monoclonal and fluorescein isothiocyanate-conjugated antibodies, and analyzed by flow cytometry. uns, unstained cells; −, no IFN treatment; +A, IFN-α treatment; +G, IFN-γ treatment. (A) 2fTGH cells respond to IFN-α. (B) U6A cells do not respond to IFN-α. (C) The IFN-α response is restored in U6A cells transfected with STAT2 cDNA. (D) 2fTGH cells respond to IFN-γ. (E) U6A cells respond to IFN-γ.

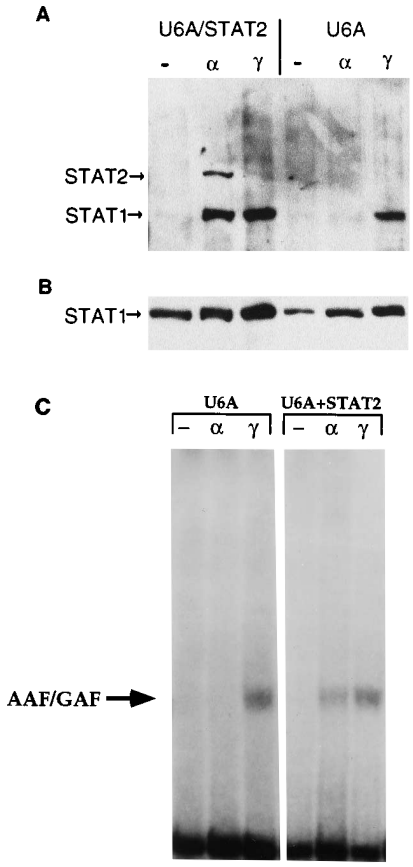

FIG. 4. STAT1 is not phosphorylated in IFN-α-treated U6A cells. (A) Cell lines U6A and U6A transfected with STAT2 cDNA were treated with IFN-α (α) or IFN-γ (γ) for 45 min or left untreated (−). Cell lysates were treated with an anti-STAT1 antibody. After fractionation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the immunoprecipitated proteins were blotted onto nitrocellulose. The filter was probed with antiphosphotyrosine antibody 4G10. (B) The filter was subsequently probed with an anti-STAT1 antibody. (C) AAF/GAF activity is not detected in IFN-α-treated U6A cells and is restored by STAT2. The band shift assay was performed with a ^{32}P-labeled GAS probe from the LyE/6 gene (41).

STAT1 in IFN-α-treated cells. The induction of the IRF-1 and GBP genes (two genes regulated by AAF through GAS-like elements) was greatly reduced in IFN-α-treated U6A cells and was restored to normal levels by complementation with STAT2

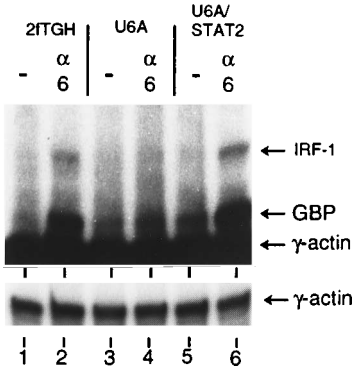

FIG. 5. The induction of IRF-1 and GBP by IFN-α is impaired in U6A cells and is restored by STAT2. Total RNA samples from cell lines 2fTGH (lanes 1 and 2), U6A (lanes 3 and 4), and U6A transfected with STAT2 cDNA were analyzed with IRF-1 and GBP protection probes. The cells in lanes 2, 4, and 6 were treated with IFN-α for 6 h. A lighter exposure of the γ-actin band is also shown.

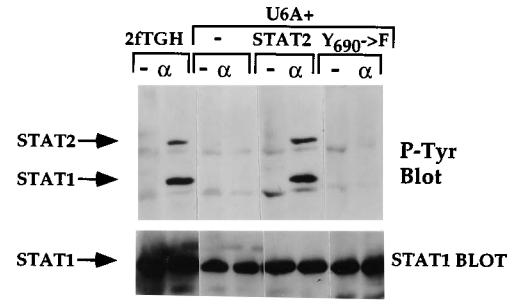

FIG. 6. The Y-690→F mutant of STAT2 does not support tyrosine phosphorylation of STAT1 in U6A cells. STAT proteins were immunoprecipitated from lysates of cells. −, untreated cells; α, cells treated with IFN-α for 45 min. After transfer, the proteins were first probed with antiphosphotyrosine (P-Tyr) antibody 4G10 and then with anti-STAT1. The cell lines tested were 2fTGH, U6A, U6A expressing a full-length STAT2 cDNA (STAT2), and U6A expressing a STAT2 cDNA in which tyrosine 690 has been mutated to phenylalanine by site-directed mutagenesis (Y-690→F).

(Fig. 5). The Y-690→F mutant of STAT2, which is not phosphorylated in response to IFN-α, failed to support phosphorylation of STAT1 in U6A cells (Fig. 6), showing that STAT2 function, rather than simply the presence of the STAT2 protein, is required.

Phosphorylation of TYK2 and JAK1 in IFN-α-treated U6A cells. When HeLa, 2fTGH, 3T3, or lymphoid cells are treated with IFN-α, both TYK2 and JAK1 are phosphorylated on tyrosine and thus activated (22, 26, 43, 44). We determined whether the absence of STAT2 affects this step in the IFN-α pathway. Extracts of IFN-α-treated cells were precipitated separately with an antiserum against JAK1 or TYK2, and the precipitates were tested for phosphorylation of tyrosine in the appropriate ~120-kDa bands. The lack of STAT2 in U6A cells had no effect on the phosphorylation of JAK1 or TYK2 (Fig. 7) in response to IFN-α. The lack of STAT2 in U6A cells apparently does not affect the early steps of IFN-α receptor activation, including activation of the receptor-associated tyrosine kinases. Thus, the deficit in STAT1 phosphorylation in U6A cells treated with IFN-α cannot be ascribed to failure of kinase activation.

DISCUSSION

The mutant cell line U6A, lacking STAT2, is the first member of the new complementation group U6. Analysis of U6A

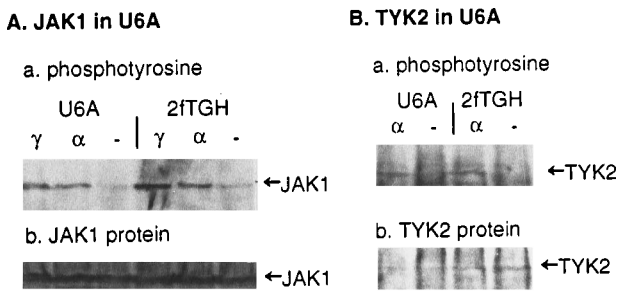

FIG. 7. The IFN-α-induced phosphorylation of JAK1 and TYK2 is normal in U6A cells. (a) Extracts were prepared from cell lines 2fTGH and U6A, and immunoprecipitation was performed with anti-JAK1 (A) or anti-TYK2 (B); the precipitates were analyzed by Western blotting using the antiphosphotyrosine antibody PY20 or 4G10. The cells were treated with either IFN-α or IFN-γ for 15 min. (b) The antiphosphotyrosine antibody was removed, and the Western blot was reprobed with anti-JAK1 (A) or anti-TYK2 (B).

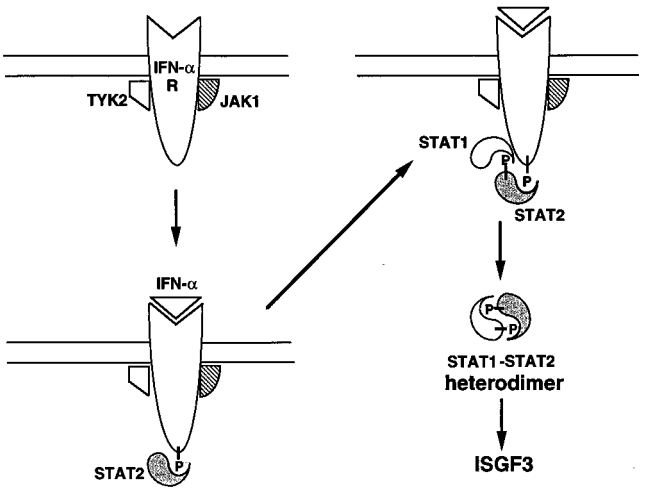

FIG. 8. A model for ISGF3 formation in response to IFN-α. R, receptor.

cells proves the necessity for STAT2 in all tested transcriptional responses for IFN-α, both for genes that possess an ISRE and are activated by ISGF3 and for genes such as IRF-1, which respond to IFN-α through STAT1 homodimers (AAF) and an inverted repeat (GAS) element (6, 13, 41). The formation of AAF is not observed in IFN-α-treated U6A cells (Fig. 4C), but a small amount may be present, accounting for the slight activation of the IRF-1 and GBP genes observed (Fig. 5).

The experiments have revealed a vital role for STAT2 in the activation of STAT1. The pathway through which ligands trigger the activation of STATs begins by inducing dimerization (or oligomerization) of receptor chains, leading to JAK activation (reviewed by Darnell et al. [5] and Kishimoto et al. [18]). These early steps in the IFN-α-induced pathway are intact in U6A cells. The binding site for STATs on the IFN-α receptor–kinase complex is unknown but is likely to be a tyrosine phosphate since the SH2 domain is conserved in all STATs and has been proven to be required for STAT1. In the case of the IFN-γ receptor, the required tyrosine has been identified on the α chain, and a phosphopeptide representing this residue and flanking sequences binds specifically to STAT1 (12). An apparently homologous tyrosine is present on an IFN-α receptor chain but has not yet been proven to bind a STAT protein (28). However, the IFN-α receptor is known to be phosphorylated in response to IFN-α (30). If activation of STAT2 does indeed occur through the binding of its SH2 domain to a phosphotyrosine of the IFN-α receptor, we can understand the requirement for STAT2 for efficient phosphorylation of STAT1. In U6A cells, the IFN-α receptor–kinase complex is active, but since STAT2 is absent, the phosphotyrosine docking site for STAT1 is not available. Since phosphorylated STAT1 and STAT2 form a heterodimer (40, 51) and coprecipitate from extracts made from IFN-α-treated cells (39), this site might be the phosphotyrosine of STAT2 itself, as suggested by results showing that the phosphorylated tyrosine of STAT2 is required for STAT1 phosphorylation (Fig. 6). Additional unpublished experiments with U6A cells and altered STAT2 proteins reveal that the SH2 domain of STAT2 is also required to support STAT1 phosphorylation (14a) and that the N-terminal region of STAT2 is important as well (21a).

The proposed mechanism of ISGF3 formation is shown in Fig. 8. First, STAT2 binds to the phosphorylated receptor and is itself phosphorylated. STAT1 then binds to phosphorylated STAT2; this step is followed by STAT1 phosphorylation and STAT1-STAT2 heterodimer formation, perhaps coincident with release from the receptor. Binding of the heterodimer to p48 completes the formation of ISGF3. Such a sequential, nonequilibrium mechanism of heterodimer formation could direct a specific subset of interactions between STAT family members, whereas the alternative random association of activated STATs would allow all possible interactions to occur. It is not at all clear from the mechanism now proposed how the formation of STAT1 homodimers would be facilitated by STAT2 in wild-type cells.

Single STAT proteins may suffice for some signalling pathways, but it seems very likely that combinations of STATs will be required in others, as for ISGF3 in the IFN-α pathway. For example, STAT1 (11, 35, 36, 45) and STAT3 (2, 51) are both activated in response to epidermal growth factor and participate in the formation of complexes with the serum-inducible element of the c-*fos* gene. The same two factors are also activated in response to IL-6 (23). Sequential activation may be necessary to favor specific heterodimer formation in order to generate specific transcription factors in at least some cases.

ACKNOWLEDGMENTS

We thank David Levy for providing RNase protection probes, Rick Peppler for assistance in FACScan analysis, Sandra Pellegrini for the anti-TYK2 antibody, and Andrew Ziemiecki for the anti-JAK1 antibody.

S.A.Q. was supported by the Cancer Research Institute.

REFERENCES

1. **Ackrill, A. M., L. E. Reid, C. S. Gilbert, D. R. Gewert, A. C. G. Porter, A. R. Lewin, G. R. Stark, and I. M. Kerr.** 1991. Differential response of the human 6-16 and 9-27 genes to α and γ interferons. Nucleic Acids Res. **19:**591–598.
2. **Akira, S., Y. Nishio, M. Inoue, X.-J. Wang, S. Wei, T. Matsusaka, K. Yoshida, T. Sudo, M. Naruto, and T. Kishimoto.** 1994. Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. Cell **77:**63–71.
3. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. A. Smith, J. G. Seidman, and K. Struhl (ed.).** 1987. Current protocols in molecular biology. John Wiley & Sons, New York.
4. **Dale, T. C., A. M. A. Imam, I. M. Kerr, and G. R. Stark.** 1989. Rapid activation by interferon α of a latent DNA-binding protein present in the cytoplasm of untreated cells. Proc. Natl. Acad. Sci. USA **86:**1203–1207.
5. **Darnell, J. E., Jr., I. M. Kerr, and G. R. Stark.** 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science **264:**1415–1421.
6. **Decker, T., D. J. Lew, and J. E. Darnell, Jr.** 1991. Two distinct alpha-interferon-dependent signal transduction pathways may contribute to activation of transcription of the guanylate-binding protein gene. Mol. Cell. Biol. **11:**5147–5153.
7. **Friedman, R. L., and G. R. Stark.** 1985. α-Interferon-induced transcription of HLA and metallothionein genes containing homologous upstream sequences. Nature (London) **314:**637–639.
8. **Fu, X.-Y.** 1992. A transcription factor with SH2 and SH3 domains is directly activated by an interferon α-induced cytoplasmic protein tyrosine kinase(s). Cell **70:**323–335.
9. **Fu, X.-Y., D. S. Kessler, S. A. Veals, D. E. Levy, and J. E. Darnell, Jr.** 1990. ISGF3, the transcriptional activator induced by interferon α, consists of multiple interacting polypeptide chains. Proc. Natl. Acad. Sci. USA **87:**8555–8559.
10. **Fu, X.-Y., C. Schindler, T. Improta, R. Aebersold, and J. E. Darnell, Jr.** 1992. The proteins of ISGF-3, the interferon α-induced transcriptional activator, define a gene family involved in signal transduction. Proc. Natl. Acad. Sci. USA **89:**7840–7843.
11. **Fu, X.-Y., and J.-J. Zhang.** 1993. Transcription factor p91 interacts with the epidermal growth factor receptor and mediates activation of the c-*fos* gene promoter. Cell **74:**1135–1145.
12. **Greenlund, A. C., M. A. Farrar, B. L. Viviano, and R. D. Schreiber.** 1994. Ligand-induced IFNγ receptor tyrosine phosphorylation couples the receptor to its signal transduction system (p91). EMBO J. **13:**1591–1660.
13. **Haque, S. J., and B. R. G. Williams.** 1994. Identification and characterization of an interferon (IFN)-stimulated response element-IFN-stimulated gene factor 3-independent signaling pathway for IFN-α. J. Biol. Chem. **269:**19523–19529.
14. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

14a. **Heim, M., I. M. Kerr, G. R. Stark, and J. E. Darnell, Jr.** Contributions of STAT SH2 groups to specific interferon signal transduction in the Jak-STAT pathway. Science, in press.

15. **Holland, S., S. Pellegrini, G. R. Stark, and I. M. Kerr.** Unpublished data.

16. **Improta, T., C. Schindler, C. M. Horvath, I. M. Kerr, G. R. Stark, and J. E. Darnell, Jr.** 1994. Transcription factor ISGF-3 formation requires phosphorylated Stat91 protein, but Stat113 protein is phosphorylated independently of Stat91 protein. Proc. Natl. Acad. Sci. USA **91:**4776–4780.

17. **John, J., R. McKendry, S. Pellegrini, D. Flavell, I. M. Kerr, and G. R. Stark.** 1991. Isolation and characterization of a new mutant human cell line unresponsive to alpha and beta interferons. Mol. Cell. Biol. **11:**4189–4195.

18. **Kishimoto, T., T. Taga, and S. Akira.** 1994. Cytokine signal transduction. Cell **76:**253–262.

19. **Levy, D. E., D. S. Kessler, R. Pine, and J. E. Darnell, Jr.** 1988. Interferon-induced nuclear factors that bind a shared promoter element correlate with positive and negative transcription control. Genes Dev. **2:**383–393.

20. **Levy, D. E., D. S. Kessler, R. Pine, and J. E. Darnell, Jr.** 1989. Cytoplasmic activation of ISGF3, the positive regulator of interferon-α-stimulated transcription, reconstituted in vitro. Genes Dev. **3:**1362–1371.

21. **Lew, D. J., T. Decker, I. Strehlow, and J. E. Darnell.** 1991. Overlapping elements in the guanylate-binding protein gene promoter mediate transcriptional induction by alpha and gamma interferons. Mol. Cell. Biol. **11:**182–191.

21a. **Li, X., S. Leung, S. A. Qureshi, I. M. Kerr, J. E. Darnell, Jr., and G. R. Stark.** Unpublished data.

22. **Loh, J. E., C. Schindler, A. Ziemiecki, A. G. Harpur, A. F. Wilks, and R. A. Flavell.** 1994. Mutant cell lines unresponsive to alpha/beta and gamma interferon are defective in tyrosine phosphorylation of ISGF-3α components. Mol. Cell. Biol. **14:**2170–2179.

23. **Lütticken, C., U. M. Wegenka, J. Yuan, J. Buschmann, C. Schindler, A. Ziemiecki, A. G. Harpur, A. F. Wilks, K. Yasukawa, T. Taga, T. Kishimoto, G. Barbieri, S. Pellegrini, M. Sendtner, P. C. Heinrich, and F. Horn.** 1994. Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. Science **263:**89–92.

24. **McKendry, R., J. John, D. Flavell, M. Müller, I. M. Kerr, and G. R. Stark.** 1991. High-frequency mutagenesis of human cells and characterization of a mutant unresponsive to both α and γ interferons. Proc. Natl. Acad. Sci. USA **88:**11455–11459.

25. **Miller, A. D., and G. J. Rosman.** 1989. Improved retroviral vectors for gene transfer and expression. BioTechniques **7:**980–990.

26. **Müller, M., J. Briscoe, C. Laxton, D. Guschin, A. Ziemiecki, O. Silvennoinen, A. G. Harpur, G. Barbieri, B. A. Witthuhn, C. Schindler, S. Pellegrini, A. F. Wilks, J. N. Ihle, G. R. Stark, and I. M. Kerr.** 1993. The protein tyrosine kinase JAK1 complements defects in interferon-α/β and -γ signal transduction. Nature (London) **366:**129–135.

27. **Müller, M., C. Laxton, J. Briscoe, C. Schindler, T. Improta, J. E. Darnell, Jr., G. R. Stark, and I. M. Kerr.** 1993. Complementation of a mutant cell line: central role of the 91 kDa polypeptide of ISGF3 in the interferon-α and -γ signal transduction pathways. EMBO J. **12:**4221–4228.

28. **Novick, D., B. Cohen, and M. Rubinstein.** 1994. The human interferon α/β receptor: characterization and molecular cloning. Cell **77:**1–20.

29. **Pellegrini, S., J. John, M. Shearer, I. M. Kerr, and G. R. Stark.** 1989. Use of a selectable marker regulated by alpha interferon to obtain mutations in the signaling pathway. Mol. Cell. Biol. **9:**4605–4612.

30. **Platanias, L. C., S. Uddin, and O. R. Colamonici.** 1994. Tyrosine phosphorylation of the α and β subunits of the type I interferon receptor. J. Biol. Chem. **269:**17761–17764.

31. **Porter, A. C. G., Y. Chernajovsky, T. C. Dale, C. S. Gilbert, G. R. Stark, and I. M. Kerr.** 1988. Interferon response element of the human gene 6-16. EMBO J. **7:**85–92.

32. **Qureshi, S. A., S. Leung, G. R. Stark, and J. E. Darnell, Jr.** Unpublished data.

33. **Reich, N., B. Evans, D. Levy, D. Fahey, E. Knight, Jr., and J. E. Darnell, Jr.** 1987. Interferon-induced transcription of a gene encoding a 15-kDa protein depends on an upstream enhancer element. Proc. Natl. Acad. Sci. USA **84:**6394–6398.

34. **Reid, L. E., A. H. Brasnett, C. S. Gilbert, A. C. G. Porter, D. R. Gewert, G. R. Stark, and I. M. Kerr.** 1989. A single DNA response element can confer inducibility by both α- and γ-interferons. Proc. Natl. Acad. Sci. USA **86:**840–844.

35. **Ruff-Jamison, S., K. Chen, and S. Cohen.** 1993. Induction by EGF and interferon-γ of tyrosine phosphorylated DNA binding proteins in mouse liver nuclei. Science **261:**1733–1736.

36. **Sadowski, H. B., K. Shuai, J. E. Darnell, Jr., and M. Z. Gilman.** 1993. A common nuclear signal transduction pathway activated by growth factor and cytokine receptors. Science **261:**1739–1744.

37. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

38. **Schindler, C., X.-Y. Fu, T. Improta, R. Aebersold, and J. E. Darnell, Jr.** 1992. Proteins of transcription factor ISGF-3: one gene encodes the 91- and 84-kDa ISGF-3 proteins that are activated by interferon α. Proc. Natl. Acad. Sci. USA **89:**7836–7839.

39. **Schindler, C., K. Shuai, V. R. Prezioso, and J. E. Darnell, Jr.** 1992. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. Science **257:**809–813.

40. **Shuai, K., C. M. Horvath, L. H. Tsai Huang, S. A. Qureshi, D. Cowburn, and J. E. Darnell, Jr.** 1994. Interferon activation of the transcription factor Stat91 involves dimerization through SH2-phosphotyrosyl peptide interactions. Cell **76:**821–828.

41. **Shuai, K., C. Schindler, V. R. Prezioso, and J. E. Darnell, Jr.** 1992. Activation of transcription by IFN-γ: tyrosine phosphorylation of a 91-kD DNA binding protein. Science **258:**1808–1812.

42. **Shuai, K., G. R. Stark, I. M. Kerr, and J. E. Darnell, Jr.** 1993. A single phosphotyrosine residue of Stat91 required for gene activation by interferon-γ. Science **261:**1744–1746.

43. **Shuai, K., A. Ziemiecki, A. F. Wilks, A. G. Harpur, H. B. Sadowski, M. Z. Gilman, and J. E. Darnell.** 1993. Polypeptide signalling to the nucleus through tyrosine phosphorylation of Jak and Stat proteins. Nature (London) **366:**580–583.

44. **Silvennoinen, O., J. N. Ihle, J. Schlessinger, and D. E. Levy.** 1993. Interferon-induced nuclear signalling by Jak protein tyrosine kinases. Nature (London) **366:**583–585.

45. **Silvennoinen, O., C. Schindler, J. Schlessinger, and D. E. Levy.** 1993. Ras-independent growth factor signaling by transcription factor tyrosine phosphorylation. Science **261:**1736–1739.

46. **Veals, S. A., C. Schindler, D. Leonard, X.-Y. Fu, R. Aebersold, J. E. Darnell, Jr., and D. E. Levy.** 1992. Subunit of an alpha-interferon-responsive transcription factor is related to interferon regulatory factor and Myb families of DNA-binding proteins. Mol. Cell. Biol. **12:**3315–3324.

47. **Velazquez, L., M. Fellous, G. R. Stark, and S. Pellegrini.** 1992. A protein tyrosine kinase in the interferon α/β signaling pathway. Cell **70:**313–322.

48. **Vogel, J., M. Kress, G. Khoury, and G. Jay.** 1986. A transcriptional enhancer and an interferon-responsive sequence in major histocompatibility complex class I genes. Mol. Cell. Biol. **6:**3550–3554.

49. **Watling, D., D. Guschin, M. Müller, O. Silvennoinen, B. A. Witthuhn, F. W. Quelle, N. C. Rogers, C. Schindler, G. R. Stark, J. N. Ihle, and I. M. Kerr.** 1993. Complementation by the protein tyrosine kinase JAK2 of a mutant cell line defective in the interferon-γ signal transduction pathway. Nature (London) **366:**166–170.

50. **Wilks, A. F., A. G. Harpur, R. R. Kurban, S. J. Ralph, G. Zürcher, and A. Ziemiecki.** 1991. Two novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase. Mol. Cell. Biol. **11:**2057–2065.

51. **Zhong, Z., Z. Wen, and J. E. Darnell, Jr.** 1994. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science **264:**95–98.