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Isolation and Characterization of a New Mutant Human Cell Line Unresponsive to Alpha and Beta Interferons

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Previously we described human cell line 2fTGH, in which expression of guanine phosphoribosyltransferase is tightly controlled by the upstream region of interferon (IFN)-stimulated human gene 6-16. After mutagenesis of 2fTGH and selection with 6-thioguanine and IFN-α, we isolated 11,1, a recessive mutant that does not respond to IFN-α. We now describe U2, a second recessive mutant, selected similarly, that complements 11,1. U2 had no response to IFN-α or IFN-β, and its response to IFN-γ was partially defective. Although many genes did respond to IFN-γ in U2, the 9-27 gene did not and the antiviral response of U2 cells to IFN-γ was greatly reduced. Band shift assays showed that none of the transcription factors normally induced in 2fTGH cells by IFN-α (E and M) or IFN-γ (G) were induced in U2. However, extracts of untreated U2 cells gave rise to a novel band that was increased by treatment with IFN-γ but not IFN-α. Band shift complementation assays revealed that untreated and IFN-γ-treated U2 cells lack the functional Eγ subunit of transcription factor E and that IFN-α-treated U2 cells do contain the functional Eα subunit.

Pathways through which extracellular proteins such as cytokines and growth factors induce expression of mammalian genes are under intensive study in many laboratories, but none has been defined fully. In the case of alpha interferon (IFN-α), a cDNA that encodes an IFN-binding protein likely to be part of a complex cell surface receptor has recently been cloned (24). Latent transcription factor E, present in the cytoplasm, is activated rapidly in response to IFN-α or IFN-β by an unknown mechanism. Active E translocates to the nucleus, where it binds to the IFN-stimulated regulatory elements of inducible genes (5) and almost certainly initiates their transcription (5, 6, 13). Levy et al. (14) have described very similar properties for the factor they call ISGF3. It is likely that E and ISGF3 are the same. Levy et al. (15) have shown that ISGF3 is composed of subunits ISGF3α (Eα) and ISGF3γ (Eγ) and can be reconstituted from the subunits in vitro. In the case of IFN-γ, a cloned cDNA encodes a binding protein that is part of a complex receptor (1), and some work has been done to identify transcription factors that act at later stages of the IFN-γ signalling pathways (see reference 9 for a recent discussion and references).

Cell lines carrying mutations in signalling pathways can be expected to yield information that complements the results of biochemical studies. For example, such mutations can define steps carried out by proteins (e.g., kinases or phosphatases) present at very low concentrations that act on substrates which have not been isolated. If the mutated gene can be cloned by complementation, its function in the pathway can be established clearly. The combination of mutant cells plus a gene that can be manipulated should be very powerful in elucidating details of the mechanism of signal transduction.

To obtain mutants in IFN signalling pathways that can be characterized and complemented relatively easily, we constructed 2fTGH, a human cell line in which the *Escherichia coli* guanine phosphoribosyltransferase (GPT) gene is controlled by the upstream region of the human 6-16 gene (19). GPT was chosen because established protocols can be used to select either for or against its expression in mammalian cells. 2fTGH, a clone in which the transfected GPT genes are regulated tightly by IFN-α, was treated with frameshift mutagen ICR 191 and selected in 6-thioguanine and IFN-α for failure to induce expression of GPT, yielding mutant 11,1 (19). We now describe the isolation and properties of U2, a second unresponsive mutant derived from 2fTGH cells.

MATERIALS AND METHODS

Cells and IFNs. 2fTGH cells (19) and mutants 11,1 and U2 were grown in Dulbecco's modification of Eagle medium with 10% heat-inactivated fetal calf serum (Bockneck), which gave a plating efficiency at low cell density of 80%. Cells were treated with 500 IU of a highly purified mixture of human IFN-αs per ml (specific activity, 10^8 IU/mg; Wellcome Research Laboratories [2]) or with the same amount of recombinant IFN-β (a gift of Triton Biosciences, Inc.). Human recombinant IFN-γ was used at 500 IU/ml and was a gift of G. R. Adolph, Ernst-Boehringer-Institut für Arzneimittelforschung, Vienna, Austria.

Mutagenesis and selection. Mutagenesis with ICR 191 (Polysciences Inc.) was performed (19) on a total of 4×10^7 cells in eight independent pools. After 15 days of recovery, a total of 2×10^7 cells were mutagenized again and allowed to recover for 2 days before a third round of treatment. Four days after the third treatment, the cells were placed in selective medium containing 6-thioguanine (30 μM) and IFN-α. After 12 days, all wild-type cells had died and several resistant colonies were evident, one of which was U2.

Cell fusions. 11,1 and U2 cells (1.5×10^6 of each) were fused in suspension with polyethylene glycol (molecular weight, 1,000) and seeded at a density of 3×10^5/10-cm-diameter dish (19). Selection was applied 2 days later by adding hypoxanthine-aminopterin-thymidine (HAT) medium and IFN-α. In a separate experiment, U2 cells were fused

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TABLE 1. Induction of mRNAs in U2 cells[a]

mRNA	Induction			
	2fTGH cells plus:		U2 cells plus:	
	IFN-α	IFN-γ	IFN-α	IFN-γ
9-27	+	+	−	−
6-16	+	Very low	−	−
2-5AS	+	N	−	N
ISG54	+	N	−	N
IFI-56K	+	N	−	N
HLA 8ABC5 (class I)	+	+	−	+
GBP	+	+	−	+
β_2-M	+	+	−	+
RING4	+	+	−	+
HLA DRα (class II)	N	+	N	+
INV	N	+	N	+
ICAM-1	N	+	N	+

[a] Northern analyses were performed by using 10 μg of total RNA from 2fTGH or U2 cells treated with IFN-α for 6 h or IFN-γ for 8 or 24 h. Symbols: +, induced; −, not induced; N, not induced in 2fTGH or U2. The very low induction of 6-16 by IFN-γ was seen only for the 24-h time point. The 9-27, 6-16, 2',5'-oligoadenylate synthetase (2-5AS), and human actin cDNA probes used were described earlier (10). The following additional probes were kindly provided by our colleagues, whom we thank: IDO, Sohan Gupta (4); ICSBP, Keiko Ozato (8); IP-10 (16) and IP-30 (17), Jeffrey Ravetch; guanylate-binding protein (GBP), Thomas Decker (7); IFI-56K, Marc Wathelet (25); ISG54, David Levy (12); HLA DRα (class II; reference 11), β_2-microglobulin (β_2-M; reference 20), invariant chain (INV; reference 3), ICAM-1 (21), HLA 8ABC5 (class I; reference 23) and RING4, a new probe from the class II region (22), John Trowsdale and Adrian Kelly.

with HPRT$^-$ HT1080 cells resistant to G418 (Neo^r). Selection was carried out either with hygromycin and G418 or with HAT and IFN-α.

RNA analyses. For quick analysis of cytoplasmic RNAs, the method of White and Bancroft (27) was used. Cells, 5×10^4 per well in 24-well plates, were treated with IFN-α for 6 h or left untreated. After incubation in phosphate-buffered saline without calcium for 15 min, they were detached with a squirt of buffer, pelleted, washed with buffer twice, and lysed with 60 μl of buffer containing 0.5% Nonidet P-40. The nuclei were separated by centrifugation, and the supernatant solution, after treatment with formaldehyde, was transferred to a GeneScreen nylon membrane (New England Nuclear) which was washed with phosphate buffer, air dried, and baked at 80°C for 2 h. Hybridization was carried out as described before (19).

For Northern (RNA) transfers, total RNA was analyzed by using 10 μg per gel track, as described before (19). Cells were treated with IFN-α or IFN-β for 6 h, unless noted otherwise, or with IFN-γ for 24 h, unless noted otherwise. The probes used are described in Table 1.

Band shift assays. Cells collected by centrifugation were frozen immediately in dry ice. Whole-cell extracts were prepared as described by Zimarino and Wu (29), and 5-μl portions were diluted with 5 μl of extraction buffer with no added NaCl {10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.9], 1.5 mM $MgCl_2$, 0.1 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid], 0.5 mM dithiothreitol, 5% glycerol, 0.5 mM phenylmethylsulfonyl fluoride}. The final concentration of NaCl was adjusted to 100 mM. Binding reactions were carried out as described by Dale et al. (6). Competition assays were performed by adding 1.5 μl of unlabelled DNA in 75 mM NaCl to a mixture containing 1 ng of end-labelled probe, 30,000 cpm, before adding the extract. The complexes were separated by electrophoresis in a 6% acrylamide gel in 45 mM Tris-borate buffer (pH 8.0)–0.1 mM EDTA.

RESULTS

Isolation and complementation analysis of mutant U2. Recessive mutations are rare in mammalian cells, probably requiring inactivation of both alleles of the target gene. Therefore, it was not surprising that many more than 10^8 mutagenized 2fTGH cells had to be screened, in 10 different experiments, to isolate our first mutant, 11,1 (19). To increase the frequency of unresponsive mutants, we performed three successive rounds of mutagenesis with ICR 191 before selection in 6-thioguanine and IFN-α. Mutant U2 was isolated from about 2×10^7 cells in a single experiment, an apparent improvement in the frequency. Recently we have found that five rounds of mutagenesis improves the frequency further, to about 10^{-6} (unpublished data). To eliminate *cis* mutants directly affected in the transfected GPT genes, we analyzed clones that grew in 6-thioguanine plus IFN-α for expression of the endogenous 6-16 gene. Mutant U2 was the only clone found (of about 25 analyzed) in which expression of 6-16 and other genes regulated by IFN-α was defective.

The spontaneous reversion frequency of mutant U2, measured as the frequency of resistance in HAT plus IFN-α, was $<10^{-7}$ (data not shown), making these cells suitable for complementation analyses. To test for dominance, U2 cells (resistant to hygromycin B) were fused with G418-resistant, HPRT$^-$ HT1080 cells and selected either with G418 plus hygromycin or with HAT plus IFN-α, in which neither cell line can grow. About 20 colonies per plate were observed in both selections (frequency, about 10^{-4}). Self-fusion controls yielded no colonies. We conclude that U2 is a recessive mutant, complemented by fusion with wild-type cells with a frequency about equal to that obtained for coincidence of the two drug resistance markers in stable hybrid cells.

U2 and the previously described mutant 11,1 (19) are in different complementation groups. Equal numbers of cells of each were fused and selected in HAT plus IFN-α. Resistant hybrid colonies were found at a frequency of about 10^{-4}. No resistant hybrid cells were obtained from the two self-fusion controls (1.5×10^6 cells each). The response of the endogenous 6-16 gene to IFN-α and IFN-β was restored both in pools of 11,1 × U2 hybrids and in individual hybrid clones (Fig. 1).

Induction of gene expression by IFN-α, IFN-β, and IFN-γ in mutant U2. Many cDNAs corresponding to IFN-responsive genes were used as probes on Northern transfers of RNAs from mutant and wild-type cells. The results are summarized in Table 1, and examples of the Northern analyses are shown in Fig. 2. All of the genes tested that responded well to IFN-α in parental 2fTGH cells and one of those that responded to IFN-γ (9-27) failed to respond in mutant U2. A time course study with IFN-α from 6 to 24 h revealed no induction of 6-16 in mutant U2 (data not shown). The 6-16 gene completely failed to respond to IFN-β in mutant U2 (Fig. 2A), in contrast to mutant 11,1 in which a partial response to IFN-β was observed (see Fig. 5B of Pellegrini et al. [19]). Nor was there any response of 6-16 to combinations of IFN-α, IFN-β, and IFN-γ (Fig. 2A). The response of 9-27 to IFN-γ was defective in mutant U2, whereas the gene for human leukocyte antigen (HLA) DRα, a class II gene, responded well (Fig. 2B). The relatively high induction of DRα in U2 compared with 2fTGH was not seen consistently. Invariant chain, induced by IFN-γ in 2fTGH

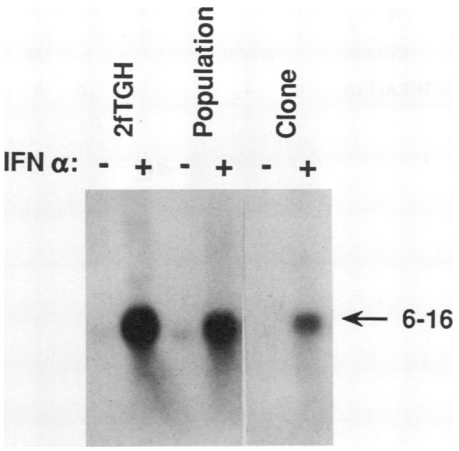

FIG. 1. Complementation of mutants 11,1 and U2. Equal numbers of cells were fused and selected in HAT plus IFN. Analyses are shown for induction of 6-16 mRNA by IFN-α in a pool of about 20 colonies and in one representative clone. Self-fusions of U2 or 11,1, like unfused mutant cells, showed no induction of 6-16 mRNA in response to IFN-α (data not shown).

cells, responded normally in mutant U2 (Fig. 2C), as did all of the other genes tested that respond to IFN-γ in 2fTGH cells (Table 1). Like class II genes, the invariant-chain gene did not respond to IFN-α in any cell type. Several genes induced by IFNs in other cell types (IP-10, IP-30, ICSBP, and IDO—see the footnote to Table 1 for references) were not induced in 2fTGH cells or in mutant U2. Most of the experiments summarized for U2 in Table 1 were carried out in parallel with mutant 11,1. As expected (19), no gene tested responded to IFN-α in mutant 11,1 and all responded to IFN-γ, as well as in parental 2fTGH cells (data not shown). The results shown in Table 1 for the HLA class I genes for 8ABC5 and β_2-microglobulin were confirmed for other class I genes by using pan-class I antibody W632 (18) and a second antibody labelled with fluorescein. The level of expression was measured with a fluorescence-activated cell scanner. There was a substantial increase for 2fTGH cells treated with IFN-α for 24 h but no detectable change with mutant U2. Both cell lines had similar and substantial responses to IFN-γ at 24, 48, and 60 h (data not shown).

U2 cells are also partially defective in their antiviral response to IFN-γ. In an assay with Semliki Forest virus used at 10 PFU per cell essentially as described by Pellegrini et al. (19), control 2fTGH cells were half-protected at 10 IU of IFN-γ per ml, while U2 cells were half-protected at 2,000 IU/ml. As expected, U2 cells were not protected by 10,000 IU of IFN-α per ml whereas 2fTGH cells were half-protected at 10 IU/ml.

Several IFN-inducible genes respond directly to double-stranded RNA (dsRNA), as well as indirectly through the action of induced IFNs (26). The 6-16 gene was induced rapidly by dsRNA in 2fTGH cells and in both of the unresponsive mutants (Fig. 3). However, the response in 2fTGH cells was greater, probably reflecting a combination of direct and indirect effects. The small response to dsRNA in the two mutants must be due to direct stimulation of the 6-16 gene by dsRNA rather than indirect stimulation by induced IFNs, since neither mutant responded to IFN-α, U2 did not respond to IFN-β, and the response of 11,1 to IFN-β was weak. Induction of IFN-β mRNA by dsRNA, assayed by RNase protection, was equivalent in 2fTGH and U2 cells

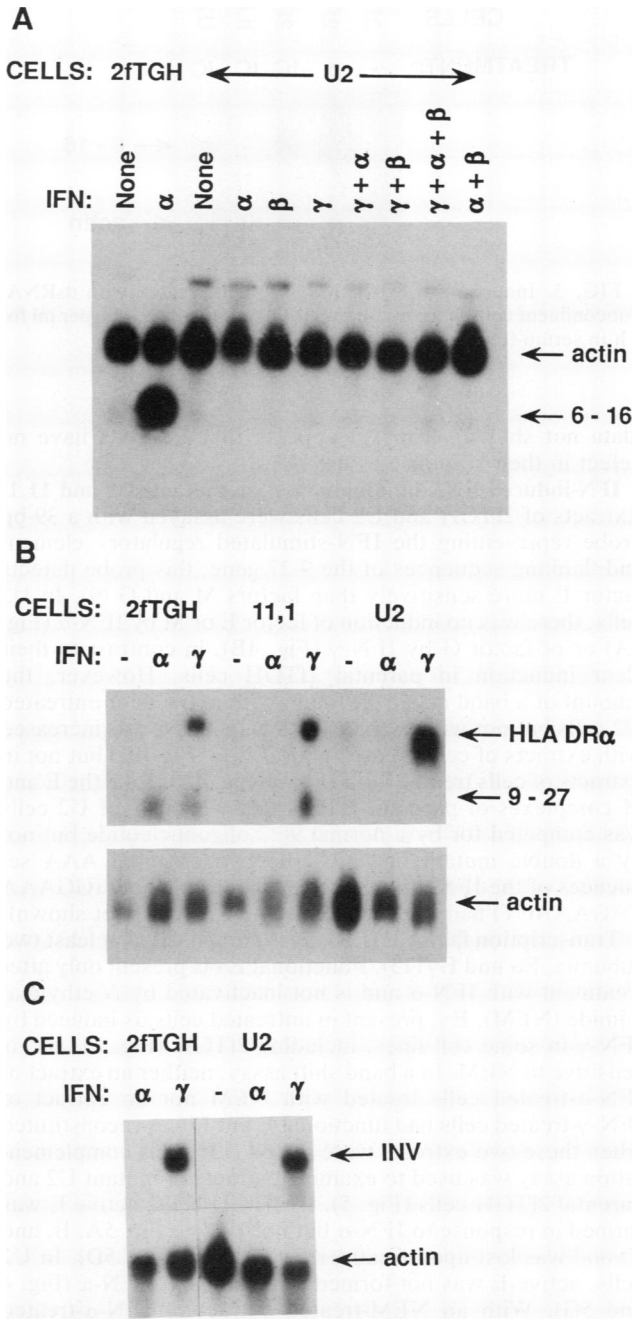

FIG. 2. Accumulation of mRNAs in mutant U2 in response to IFN-α, IFN-β, or IFN-γ. Northern transfers were probed sequentially in the order given; probes used earlier were not removed before later ones were applied. (A) 6-16, actin. Cells were treated with IFN-α or IFN-β for 6 h or IFN-γ for 8 h. In combined treatments, IFN-γ was added alone for 2 h before IFN-α, IFN-β, or both, were added for 6 h more. In parallel experiments, the IFN-β and IFN-γ preparations used were shown to be effective on 2fTGH cells (data not shown). (B) 9-27, HLA DRα, actin. Cells were treated with IFN-α or IFN-γ for 24 h. (C) Invariant chain (INV), actin. Treatments with IFN-α and IFN-γ were for 6 and 24 h, respectively. Invariant-chain mRNA was not detected in untreated 2fTGH cells (data not shown).

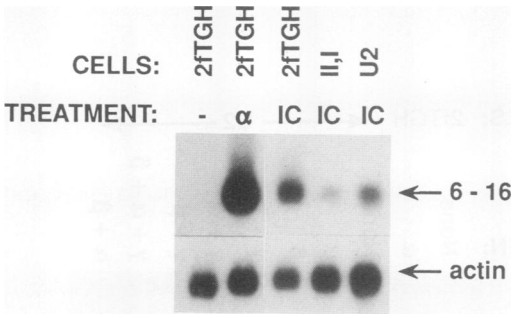

FIG. 3. Induction of 6-16 mRNA in cells treated with dsRNA. Subconfluent cells were treated with 100 μg of poly(rI-rC) per ml for 4 h in serum-free medium before Northern analysis.

(data not shown). Thus, it appears that U2 cells have no defect in their response to dsRNA.

IFN-induced DNA-binding factors in mutants U2 and 11,1. Extracts of 2fTGH and U2 cells were assayed with a 39-bp probe representing the IFN-stimulated regulatory element and flanking sequences of the 9-27 gene; this probe detects factor E more sensitively than factors M and G (9). In U2 cells, there was no induction of factor E or M by IFN-α (Fig. 4A) or of factor G by IFN-γ (Fig. 4B), in contrast to their clear induction in parental 2fTGH cells. However, the amount of a band called X, found with extracts of untreated U2 cells but not with extracts of 2fTGH cells, was increased with extracts of cells treated with IFN-γ (Fig. 4B) but not in extracts of cells treated with IFN-α (Fig. 4A). Like the E and M complexes of parental 2fTGH cells, band X of U2 cells was competed for by a normal 9-27 oligonucleotide but not by a double mutant in which the two essential AAA sequences of the IFN-stimulated regulatory element (GGAAA TAGAAACT) had been changed to ACA (data not shown).

Transcription factor E (ISGF3) is composed of at least two subunits, Eα and Eγ (13). Functional Eα is present only after treatment with IFN-α and is not inactivated by *N*-ethylmaleimide (NEM). Eγ, present in untreated cells, is induced by IFN-γ in some cell lines, including 2fTGH (Fig. 5), and is sensitive to NEM. In a band shift assay, neither an extract of IFN-α-treated cells treated with NEM nor an extract of IFN-γ-treated cells had functional E but E was reconstituted when these two extracts were mixed (13). This complementation assay was used to examine extracts of mutant U2 and parental 2fTGH cells (Fig. 5). In 2fTGH cells, active E was formed in response to IFN-α but not IFN-γ (Fig. 5A, B, and C) and was lost upon treatment with NEM (Fig. 5D). In U2 cells, active E was not formed in response to IFN-α (Fig. 4 and 5G). With an NEM-treated extract of IFN-α-treated wild-type cells as a source of Eα (D), a small amount of E was generated in a control complementation assay in which untreated 2fTGH cells supplied Eγ (Fig. 5A and D). Much more E was formed with extracts of 2fTGH cells in which the level of Eγ had been increased by treatment with IFN-γ (Fig. 5B and D). Therefore, extracts of 2fTGH cells contain active Eγ and the amount is increased upon treatment with IFN-γ. However, no complementation was seen when extracts of untreated (E) or IFN-γ-treated (F) U2 cells were combined with NEM-treated extracts of IFN-α-treated 2fTGH cells (Fig. 5D and E and D and F). Therefore, extracts of untreated or IFN-γ-treated U2 cells do not contain active Eγ. Finally, extracts of U2 cells treated with IFN-α (G) were able to complement extracts of untreated

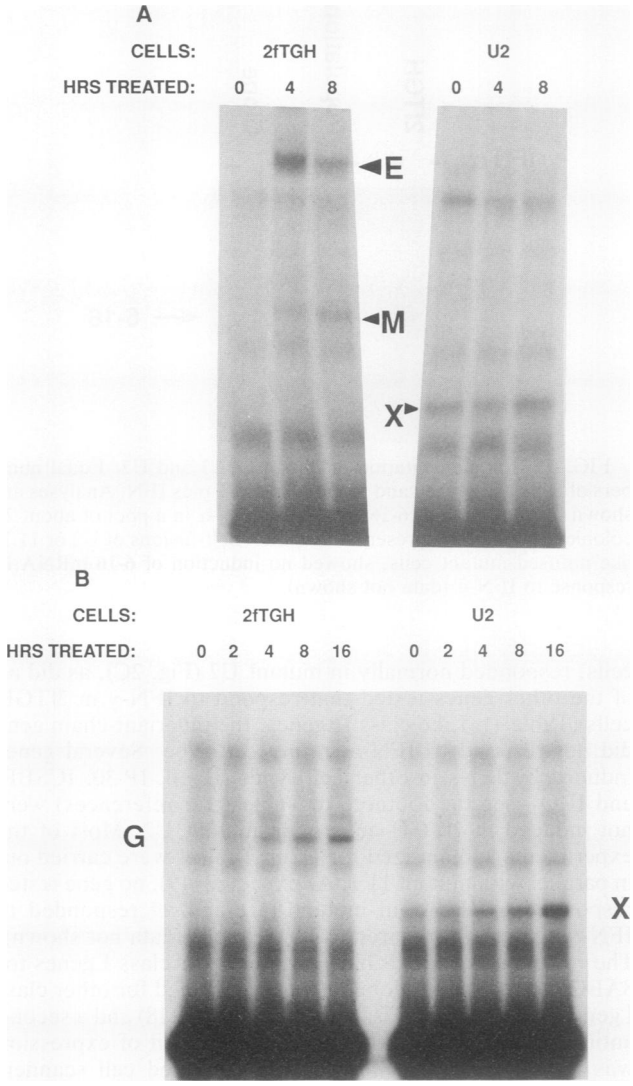

FIG. 4. Band shift assays with extracts of mutant U2 cells after treatment with IFN-α or IFN-γ. A 39-bp probe (TTTACAAACAG CAGGAAATAGAAACTTAAGAGAAATACA), representing the IFN-stimulated regulatory element and flanking sequences of the 9-27 gene, was used (9). (A) Complexes E and M, formed with extracts of IFN-α-treated control cells, were not detected with extracts of U2 cells. Complex X, a novel band formed with untreated extracts of U2 cells, was not induced by IFN-α. (B) Complex G, formed with extracts of IFN-γ-treated control cells, was not detected with extracts of U2 cells. Complex X was induced by IFN-γ.

(A) or IFN-γ-treated (B) 2fTGH cells (Fig. 5G and A and G and B) equivalently to extracts of IFN-α-treated 2fTGH cells after reaction of the latter with NEM (Fig. 5D and A and D and B). Therefore, IFN-α-treated U2 cells and IFN-α-treated 2fTGH cells contain similar amounts of active Eα. Since U2 cells accumulated active Eα in response to IFN-α so well, it is likely that they have an approximately normal complement of functional IFN-α receptors.

Band shift assays were also performed with mutant 11,1. Consistent with the partial induction of 6-16 transcription in this mutant in response to IFN-β (19), there was significant induction of E and M in response to IFN-β (Fig. 6). There

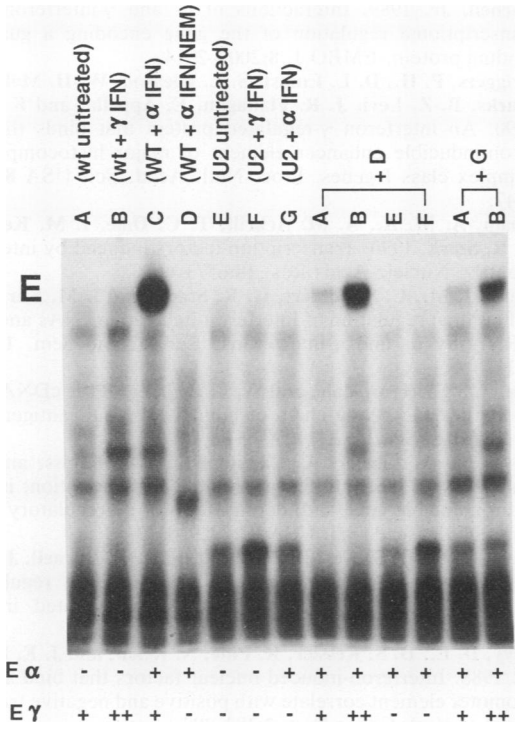

FIG. 5. Band shift complementation assays with extracts of 2fTGH and U2 cells. The cells were treated with IFN-α for 4 h or with a small amount (20 IU/ml) of IFN-γ for 20 h. In lane D, the extract was treated with NEM as described by Levy et al. (13). Complementation assays were performed by mixing half of the usual amount of each extract before adding the 9-27 probe. wt or WT, wild type. ++, induced level; +, normal level; −, not present.

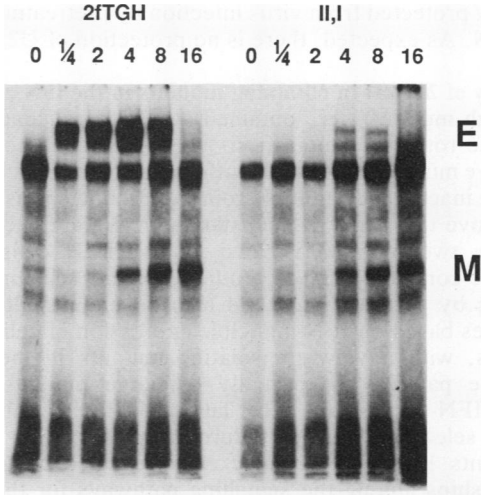

FIG. 6. Band shift assay with extracts of mutant 11,1 cells. The 9-27 probe was used. Cells were treated with 400 IU of IFN-β per ml for the number of hours shown above each lane.

was no detectable induction of E or M by IFN-α (data not shown), as expected for a mutant defective in the binding of IFN-α.

DISCUSSION

Nature of the mutation in U2 cells. As shown in the band shift complementation assays of Fig. 5, U2 cells lack the functional Eγ subunit of oligomeric transcription factor E. The mutation may be in the gene that encodes Eγ or in a gene that encodes a factor required for expression of Eγ. Another possibility is that the Eγ protein is present in an inactive form and the defect is in an enzyme required for its activation. These and other possibilities can be distinguished when antibodies to Eγ become available or, better, when the gene mutated in U2 has been cloned and identified. Since mutant U2, unlike 11,1, is fully defective in its response to IFN-β, the Eγ subunit is probably essential for the response to all type I IFNs. It is highly unlikely that either the IFN-α or IFN-γ receptor is affected in mutant U2, since Eα is induced normally in response to IFN-α and since most genes respond well to IFN-γ.

It seemed possible that the mutation in U2 might be in the IRF-1 gene, which encodes a protein that seems to be involved in both induction of IFN genes and responses to IFNs (for a recent summary, see reference 28). To test this, we transfected U2 cells with a mixture of a mammalian expression plasmid containing an IRF-1 cDNA, kindly provided by Simon Whiteside (Imperial Cancer Research Fund, London, England), and pSV2neo. Approximately 50 G418-resistant colonies were tested, and they gave no colonies that grew in HAT plus IFN-α, indicating that IRF-1 does not complement the mutation in U2. However, since normal levels of IRF-1-encoded protein and mRNA were present in untransfected U2 cells, we could not prove that the IRF-1-containing plasmid was expressed after transfection. By using an antibody kindly provided by Richard Pine (The Rockefeller University, New York, N.Y.) to probe Western blots (immunoblots) of proteins separated in sodium dodecyl sulfate-polyacrylamide gels, we found that the IRF-1-encoded protein was the same size in 2fTGH and U2 cells and was present at the same level (17a). IRF-1 mRNA was also present at similar levels in 2fTGH and U2 cells (17a). In summary, these results strongly suggest (but do not prove) that the mutation in U2 lies outside the IRF-1 gene. The issue will be resolved when the affected gene has been cloned.

It is interesting that the mutation in U2 affects transcription factors M and G, as well as E. Our recent work indicates strongly that M is a positively acting factor, prolonging the response to type I IFNs but incapable of initiating transcription in the absence of E (9). This conclusion contrasts with the previous idea that ISGF2, a factor likely to be similar to M, acts negatively (14). Factor G, induced in response to IFN-γ, is also likely to act positively (reference 9 and references therein). Two of several possible explanations for the coordinate effect of the U2 mutation on all three factors are (i) that a modifying enzyme essential for the proper function of all three has been mutated and (ii) that a factor required for the transcription of all three is absent. It may be that X, induced by IFN-γ in U2 cells similarly to the induction of G in normal cells, is derived from G, missing either all or part of a subunit or lacking a covalent modification.

The primary responses to IFN-γ are likely to be normal in mutant U2 and may be sufficient to activate most IFN-γ-regulated genes. In contrast, the failure of the 9-27 gene to respond to IFN-γ in mutant U2 is presumably related to the defect in G, although the reason why this defect should affect 9-27 preferentially remains to be established. U2 cells show another defect in their response to IFN-γ: they are only

partially protected from virus infection by pretreatment with this IFN. As expected, there is no protection of U2 cells by IFN-α.

Utility of 2fTGH in obtaining mutants in the IFN response. Although mutants were obtained at a low frequency when only one round of mutagenesis was used, as expected for recessive mutations in which both copies of the affected gene must be inactivated, multiple rounds of mutagenesis seemed to improve the frequency substantially. We are encouraged that the two mutants isolated from 2fTGH cells are in different complementation groups. We are selecting more mutants by this approach and also developing alternative strategies based on IFN-inducible expression of cell surface markers, with a view to isolating mutants in the IFN-γ response pathway or partially defective mutants in the IFN-α–IFN-β pathway. The latter would be unlikely to survive selection based on failure to express GPT.

Mutants 11,1 and U2 have each revealed unsuspected relationships among the signalling pathways for the IFNs. Mutant 11,1, completely defective in its response to IFN-α but still partially responsive to IFN-β, shows that these two type I IFNs probably do not work through entirely identical receptors or pathways (19). Mutant U2, selected with IFN-α, reveals an unexpected connection among the α, β, and γ signalling pathways. However, the greatest utility of these mutants will come when the wild-type versions of the mutated genes have been cloned, making it possible to manipulate these genes in vitro, introduce them into mutant cells that lack them, and study the phenotypes of such transfectants. Experiments of this type can reveal the detailed functions of the individual proteins and the molecular bases of the subtle interactions between the pathways that have been uncovered in 11,1 and U2. Since both of these mutants are stable, transfection with genomic DNA or cDNA, followed by selection for restoration of the response with HAT plus IFN-α, provides a straightforward way to clone the genes.

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REFERENCES

1. **Aguet, M., Z. Dembic, and G. Merlin.** 1988. Molecular cloning and expression of the human interferon-γ receptor. Cell **55**:273–280.
2. **Allen, G., K. H. Fantes, D. C. Burke, and J. Morser.** 1982. Analysis and purification of human lymphoblastoid (Namalwa) interferon using a monoclonal antibody. J. Gen. Virol. **63**:207–212.
3. **Claesson, L., D. Larhammar, L. Rask, and P. A. Peterson.** 1983. cDNA clone for the human invariant γ chain of class II histocompatibility antigens and its implications for the protein structure. Proc. Natl. Acad. Sci. USA **80**:7395–7399.
4. **Dai, W., and S. L. Gupta.** 1990. Molecular cloning, sequencing and expression of human interferon-γ-inducible indoleamine 2,3-dioxygenase cDNA. Biochem. Biophys. Res. Commun. **168**:1–8.
5. **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.
6. **Dale, T. C., J. M. Rosen, M. J. Guille, A. R. Lewin, A. C. G. Porter, I. M. Kerr, and G. R. Stark.** 1989. Overlapping sites for constitutive and induced DNA binding factors involved in interferon-stimulated transcription. EMBO J. **8**:831–839.
7. **Decker, T., D. J. Lew, Y.-S. E. Cheng, D. E. Levy, and J. E. Darnell, Jr.** 1989. Interactions of α- and γ-interferon in the transcriptional regulation of the gene encoding a guanylate-binding protein. EMBO J. **8**:2009–2014.
8. **Driggers, P. H., D. L. Ennist, S. L. Gleason, W.-H. Mak, M. S. Marks, B.-Z. Levi, J. R. Flanagan, E. Appella, and K. Ozato.** 1990. An interferon γ-regulated protein that binds the interferon-inducible enhancer element of major histocompatibility complex class I genes. Proc. Natl. Acad. Sci. USA **87**:3743–3747.
9. **Imam, A. M. A., A. M. Ackrill, T. C. Dale, I. M. Kerr, and G. R. Stark.** 1990. Transcription factors induced by interferons α and γ. Nucleic Acids Res. **18**:6573–6580.
10. **Kelly, J. M., C. S. Gilbert, G. R. Stark, and I. M. Kerr.** 1985. Differential regulation of interferon-induced mRNAs and c-myc mRNA by α- and γ-interferons. Eur. J. Biochem. **153**:367–371.
11. **Lee, J. S., J. Trowsdale, and W. F. Bodmer.** 1982. cDNA clones coding for the heavy chain of human HLA-DR antigen. Proc. Natl. Acad. Sci. USA **79**:545–549.
12. **Levy, D., A. Larner, A. Chaudhuri, L. E. Babiss, and J. E. Darnell, Jr.** 1986. Interferon-stimulated transcription: isolation of an inducible gene and identification of its regulatory region. Proc. Natl. Acad. Sci. USA **83**:8929–8933.
13. **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.
14. **Levy, D. E., D. S. Kessler, R. Pine, N. Reich, and J. E. Darnell, Jr.** 1988. Interferon-induced nuclear factors that bind a shared promoter element correlate with positive and negative transcriptional control. Genes Dev. **2**:383–393.
15. **Levy, D. E., D. J. Lew, T. Decker, D. S. Kessler, and J. E. Darnell, Jr.** 1990. Synergistic interaction between interferon-α and interferon-γ through induced synthesis of one subunit of the transcription factor ISGF3. EMBO J. **9**:1105–1111.
16. **Luster, A. D., and J. V. Ravetch.** 1987. Genomic characterization of a gamma-interferon-inducible gene (IP-10) and identification of an interferon-inducible hypersensitive site. Mol. Cell. Biol. **7**:3723–3731.
17. **Luster, A. D., R. L. Weinshank, R. Feinman, and J. V. Ravetch.** 1988. Molecular and biochemical characterization of a novel γ-interferon-inducible protein. J. Biol. Chem. **263**:12036–12043.
17a. **McKendry, R., and M. Guille.** Unpublished data.
18. **Parham, P., C. J. Barnstable, and W. F. Bodmer.** 1979. Use of a monoclonal antibody (W6/32) in structural studies of HLA-A,B,C antigens. J. Immunol. **123**:342–349.
19. **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.
20. **Rosa, F., H. Berissi, J. Weissenbach, L. Marolteaux, M. Fellous, and M. Revel.** 1983. The β_2-microglobulin mRNA in human Daudi cells has a mutated initiation codon but is still inducible by interferon. EMBO J. **2**:239–243.
21. **Simmons, D., M. W. Makgoba, and B. Seed.** 1988. ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. Nature (London) **331**:624–627.
22. **Trowsdale, J., I. Hanson, I. Mockridge, S. Beck, A. Townsend, and A. Kelly.** 1990. Sequences encoded in the class II region of the MHC related to the 'ABC' superfamily of transporters. Nature (London) **348**:741–744.
23. **Trowsdale, J., J. Lee, A. Kelly, J. Carey, J. Jenkins, P. Travers, and W. F. Bodmer.** 1984. Isolation and sequencing of a cDNA clone for a human HLA-A,B,C antigen. Mol. Biol. Med. **2**:53–61.
24. **Uzé, G., G. Lutfalla, and I. Gresser.** 1990. Genetic transfer of a functional human interferon α receptor into mouse cells: cloning and expression of its cDNA. Cell **60**:225–234.
25. **Wathelet, M., S. Moutschen, P. Defilipi, A. Cravador, M. Collet, G. Huez, and J. Content.** 1986. Molecular cloning, full-length sequence and preliminary characterization of a 56 kDa protein induced by human interferons. Eur. J. Biochem. **155**:11–17.
26. **Wathelet, M. C., I. M. Clauss, F. C. Paillard, and G. A. Huez.**

1989. 2-Aminopurine selectively blocks the transcriptional activation of cellular genes by virus, double-stranded RNA and interferons in human cells. Eur. J. Biochem. **184**:503–509.

27. **White, B. A., and F. C. Bancroft.** 1982. Cytoplasmic dot hybridization. J. Biol. Chem. **257**:8569–8572.
28. **Yamada, G., M. Ogawa, K. Akagi, H. Miyamoto, N. Nakano, S. Itoh, J.-I. Miyazaki, S.-I. Nishikawa, K.-I. Yamamura, and T. Taniguchi.** 1991. Specific depletion of the B-cell population induced by aberrant expression of human interferon regulatory factor 1 gene in transgenic mice. Proc. Natl. Acad. Sci. USA **88**:532–536.
29. **Zimarino, V., and C. Wu.** 1987. Induction of sequence-specific binding of Drosophila heat shock activator protein without protein synthesis. Nature (London) **327**:727–730.