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Presence of Human Chromosome 21 Alone Is Sufficient for Hybrid Cell Sensitivity to Human Interferon

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Human/mouse somatic cell hybrids with chromosome 21 as the only detectable human genetic material were sensitive to both human leukocyte and fibroblast interferons. The presence of additional human chromosomes decreased the amount of interferon needed to attain a given level of virus resistance. Decreased cytopathic effects, decreased virus yields, and the appearance of a specific phosphorylated protein associated with interferon treatment were all observed in hybrids maintaining only human chromosome 21. The phosphorylated protein found in extracts of these human interferon-treated hybrid cells was of mouse origin.

Somatic cell genetic techniques have allowed the identification of distinct genetic elements involved in the interferon systems of several species (7). The genes for the biosynthesis of interferon and for sensitivity to its action have been shown to be asyntenic in a number of studies (3, 23). Chromosome assignments for these functions have been made in the human system through the use of human/mouse hybrid cells (20, 23).

A number of experiments have implicated human chromosome 21 as bearing a locus governing sensitivity to human interferon. The original assignment of a gene for the antiviral response to interferon in the human system was made by Tan et al., using hybrid cells selectively losing human chromosomes (23). Sensitivity to human interferon was found to segregate concordantly with the expression of the dimeric form of indophenol oxidase (superoxide dismutase, cytoplasmic form; EC 1.15.1.1), and both traits were assigned to chromosome 21.

Further support for this assignment came from studies with cells having different numbers of copies of chromosome 21. Monosomy 21 cells were less sensitive to human interferon than were normal diploid cells, whereas trisomy 21 cells were markedly more sensitive (6, 19, 22). Recent work with cell lines carrying translocations for part of chromosome 21 has permitted subregional localization of the gene for interferon sensitivity to the distal portion of the long arm of this chromosome (10, 21).

Revel et al. have proposed a model with chromosome 21 coding for a cell surface component specifically required for response to human interferon (17). Antibodies made in mice against hybrid cells containing chromosome 21 could block interferon action in sensitive human and hybrid cells; this antibody activity could be removed by preadsorption on 21^+ but not 21^- cells.

Chany and co-workers have also done experiments with hybrid cells and have suggested that interferon species specificity is due to receptor interactions, whereas the actual antiviral machinery in a primate/mouse hybrid is probably of mouse origin (4, 6). They found, however, that if only chromosome 21 is present in a hybrid, it does not respond to human interferon. The presence of any other human chromosomes in addition to chromosome 21 rendered a hybrid susceptible; this need for nonspecific "helper chromosomes" has been explained as providing human material necessary for receptor accessibility.

We have studied a series of human/mouse somatic cell hybrids with only chromosome 21 present or with chromosome 21 and one or two additional human chromosomes in order to assess the differential susceptibility of such cells to human leukocyte and fibroblast interferon. Our experiments indicate that chromosome 21 alone is sufficient for expression of human interferon-mediated virus growth inhibition and the changes in protein phosphorylation observed in interferon-treated cell extracts. A mouse-specific protein is phosphorylated in extracts from hybrid cells containing only chromosome 21 after treatment with human interferon.

MATERIALS AND METHODS

Cell lines. Normal human diploid foreskin cells, FS4 and FS7, were kindly provided by J. Vilcek and maintained in Eagle minimum essential medium

(Grand Island Biological Co. [GIBCO]) with 5% fetal bovine serum (International Biological Laboratories). Mouse L cells were maintained in Dulbecco's modified Eagle medium (GIBCO) with 10% fetal bovine serum. WAV hybrids were derived from a fusion of the human diploid fibroblast WI38 with mouse A9 cells and were maintained in Dulbecco's modified Eagle medium with 10% fetal bovine serum.

Isozyme analysis. Assays for enzymes mapped to 17 different human chromosomes were done essentially as described by Nichols and Ruddle (16).

Chromosome analysis. Metaphase spreads of hybrid cells were analyzed for the presence of human chromosomes by the alkaline Giemsa method of Friend et al. (11) and by Giemsa banding followed by Hoechst 33258 centromeric staining (14).

Viruses. Vesicular stomatitis virus (VSV), grown in chicken embryo fibroblasts and titered on mouse L cells, was provided by C. A. Kozak.

Interferon. Human leukocyte interferon, induced in human buffy coats by Newcastle disease virus, was provided by S. Shaila and P. Lengyel. Fibroblast interferon was prepared in FS4 cells by superinduction with polyriboinosinic acid:polyribocytidylic acid [poly(rI:rC)] essentially by the method of Havell and Vilcek (12). These preparations were titered against the National Institutes of Health (NIH) reference standard of human interferon (National Institute of Allergy and Infectious Diseases catalog no. G023-901-527). Mouse interferon was produced by Newcastle disease virus induction of L cells.

Interferon assays. Interferon assays were done in 96-well microtiter trays (Linbro) by a modification of the microassay protocol of Armstrong (1). A dilution series of interferon samples in 0.05 ml of Dulbecco's modified Eagle medium with 10% fetal bovine serum was placed in duplicate wells, and then ~2.5×10^4 cells (enough to constitute a confluent monolayer in each well) were added in another 0.05 ml. After incubation at 37°C for 20 h, the medium was removed, and 0.1 PFU of VSV per cell was added in 0.1 ml of Dulbecco's modified Eagle medium for 20 to 24 h at which time virus-induced cytopathic effect was scored. The NIH human reference standard (20,000 U/ml) gave a titer of 25,000 U/ml in this assay on FS7 cells. Visual appraisal of 50% reduction in cytopathic effect corresponded to at least a 99% reduction in VSV yield when determined by quantitation of virus production.

Protein phosphorylation assays. Analysis of protein phosphorylation in crude cell extracts (S10) was carried out as described by Zilberstein et al. (24). Buffer B is 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.4)–5 mM $MgCl_2$–120 mM KCl–7 mM 2-mercaptoethanol–10% glycerol.

RESULTS

Extensive karyotypic and isozyme analysis of the three subclones of WAV used in this study revealed the presence of the following human chromosomes: WAVR4d, chromosomes 4, 21, and 22; WAVR4d-A19, chromosomes 21 and 22; WAVR4d-F9-4a, chromosome 21. It was especially important to determine the frequency of chromosome 21 and its stability in the WAVR4d-F9-4a hybrids. In 35 cells observed after alkaline Giemsa staining, a G-group chromosome was the only visible human material. Analysis of 70 cells with Giemsa banding and Hoechst centromeric staining (Fig. 1) revealed that chromosome 21 was the only human chromosome present, with 80% of the cells examined bearing at least one copy. In monolayer culture, this high frequency of chromosome 21 could be maintained in subcultures for several months. The other two hybrid lines retained chromosome 21 at a lower frequency (70% for WAVR4d-A19 and 40% for WAVR4d). Isozyme analysis for enzymes mapped to 17 different human chromosomes revealed only the dimeric form of indophenol oxidase.

The availability of human/mouse hybrid cell lines with only human chromosome 21 or with a small number of additional human chromosomes made possible a study of the interferon response as a function of the amount of human genetic material present. The amount of human interferon needed to reduce virus-induced cytopathic effects by at least 50% as compared with control cultures is shown in Table 1. Titers of leukocyte and fibroblast interferon are expressed in NIH units, normalized for assays on FS7 cells. The relatively high doses of human interferon did not seem to affect the viability of the stationary-phase cells used in the assay. The WAV hybrids were sensitive to mouse interferon at levels similar to those effective in the parental A9 cells (data not shown).

WAVR4d-F9-4a cells were treated with different concentrations of human fibroblast interferon, and the yields of virions were quantitated after one cycle of VSV growth (Fig. 2). A characteristic dose-response curve was observed for this hybrid line, whereas mouse L cells did not respond significantly to human interferon.

Figure 3 shows autoradiographs of ^{32}P-labeled proteins from crude extracts of control (human, mouse, and hybrid) cells and cells pretreated with human or mouse interferon. The concentration of human interferon used in these assays is lower than that determined to give a 50% reduction in VSV-induced cytopathic effect. Different interferon preparations were used during

FIG. 1. *(A) Hoechst centromeric staining of a chromosome spread from WAVR4d-F9-4a. Human chromosome 21 (arrow) lacks the brightly fluorescent centromere characteristic of mouse chromosomes. (B) Giemsa banding of the same spread. Chromosome 21 is again indicated by an arrow.*

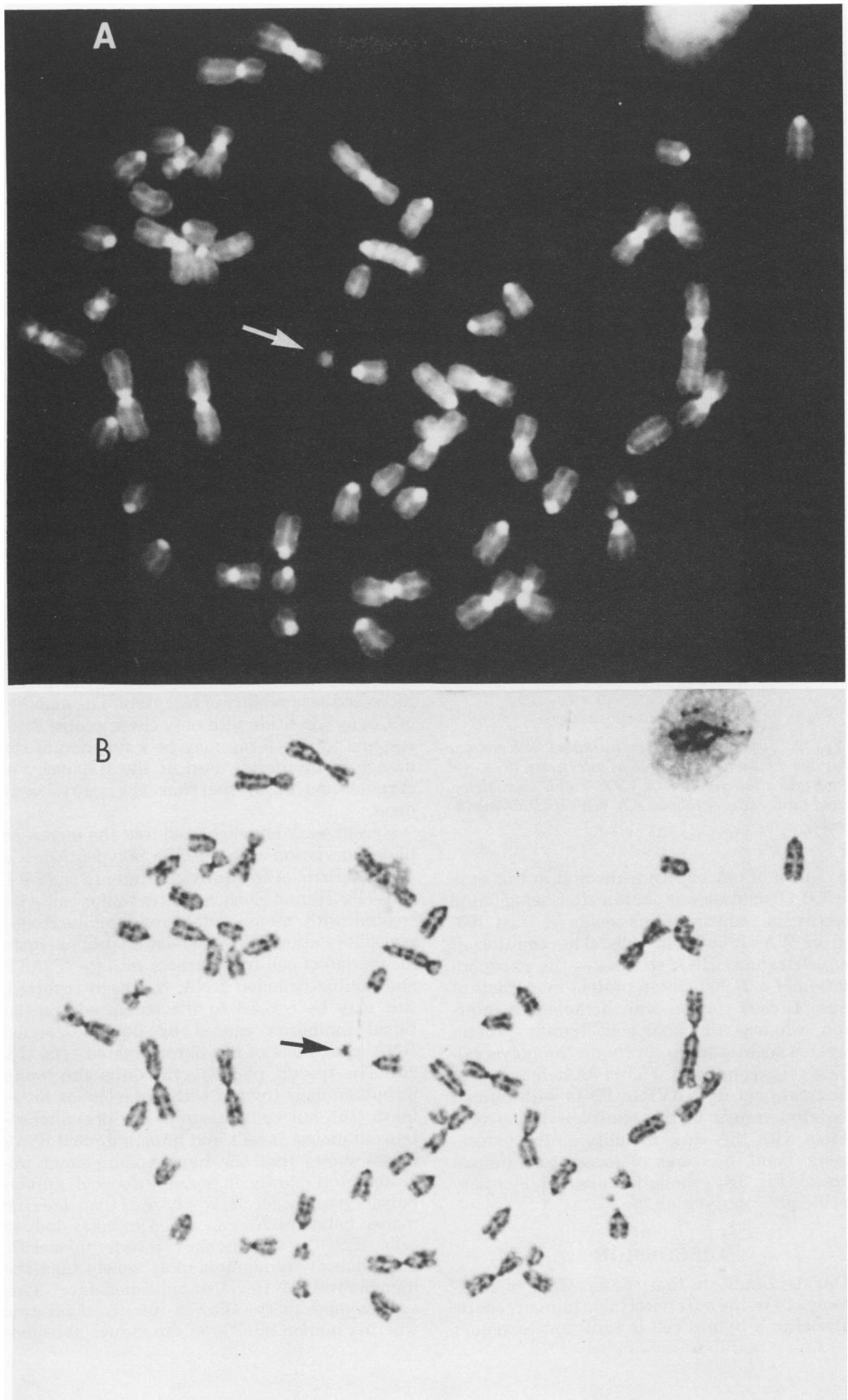
A
B

TABLE 1. *Amount of human interferon needed to reduce virus-induced cytopathic effects by at least 50%*[a]

Cell line	Human chromosomes	Leukocyte (U/well)	Fibroblast (U/well)
FS7		1	1
WAVR4d (passage 10)	4, 21, 22	250	350
WAVR4d-A19 (passage 9)	21, 22	275	600
WAVR4d-F9-4a (passage 13)	21	550	800

[a] These values represent the average of four experiments, each using internal reference standards. VSV (0.1 PFU/cell) was used as the challenge virus.

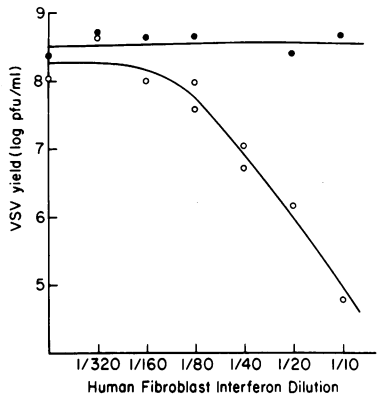

FIG. 2. *Virus yields from cells treated with various dilutions of human fibroblast interferon (2 × 10⁴ U/ml) and challenged with VSV. Yields were determined on L cells. Symbols:* (○) *WAVR4d-F9-4a;* (●) *L cells.*

the course of our experiments, and in this case the 500-U/ml dose was chosen after being found effective in reducing virus yields at least 100-fold in WAVR4d-F9-4a cells. The addition of double-stranded RNA stimulates the phosphorylation of a 67,000-dalton protein in extracts of mouse L cells treated with homologous interferon, whereas the analogous human protein migrates slightly more slowly on the polyacrylamide gel system used. Figure 3A demonstrates that treatment of WAVR4d-F9-4a with human interferon results in the phosphorylation of a protein with the same mobility as the mouse-specific band. Mixtures of mouse and human extracts (Fig. 3B) give both mouse- and human-specific phosphorylation.

DISCUSSION

Our data indicate that the presence of chromosome 21 as the only detectable human genetic material in a hybrid cell is sufficient to render the hybrid sensitive to human interferon. Both leukocyte and fibroblast interferons are able to elicit the antiviral state in these hybrids, but the amount of each type of interferon needed to give an equivalent level of protection in a given cell line is different when titer is expressed in terms of the NIH reference standard. This may be due to differences in the dose-response curves of the two types of interferon, as described by Edy et al. (9).

The amount of human interferon needed to elicit an antiviral response in a hybrid is about two orders of magnitude higher than that required in the human foreskin line FS7. This is consistent with the findings of Tan et al., who used at least 200 U of interferon per well in screening hybrid cells for human interferon sensitivity (23). If chromosome 21 codes for a cell surface receptor for interferon, as proposed by several investigators (2, 6, 17), this high interferon requirement is not unexpected. The number of human-specific receptors in the predominantly mouse-coded membrane is probably quite small. The presence of additional human material in a hybrid increases interferon sensitivity, as can be seen by comparing the three WAV subclones. Chany and co-workers have speculated that this type of result was due to a nonspecific "helper" effect (4, 6), probably involving increased accessibility of receptors. The inability of Chany's hybrids with only chromosome 21 to respond to interferon may be a function of the dosage of interferon used or the frequency of chromosome 21 at the time the assays were done.

Recent work has suggested that the increased phosphorylation of a specific polypeptide is a characteristic of the antiviral state. In mouse L cells (18, 24) and Ehrlich ascites tumor cells (15) treated with mouse interferon, the increased phosphorylation of a 67,000-dalton protein, upon incubation of cell-free extracts with [γ-^{32}P]ATP and double-stranded RNA, has been reported; this may be related to the enhanced translational inhibition caused by double-stranded RNA in extracts of interferon-treated cells (18, 24). The specific phosphorylation is also found in human cells treated with homologous interferon (15), but comparison of the phosphoproteins in mouse L cells and human diploid fibroblasts shows that the human component migrates more slowly in sodium dodecyl sulfate-polyacrylamide gel electrophoresis than does the mouse band. In African green monkey kidney cells (BSC-1) the monkey interferon-specific phosphoprotein migrates more slowly than the human form (M. Revel, unpublished data). This species specificity allowed us to determine whether human interferon can induce phospho-

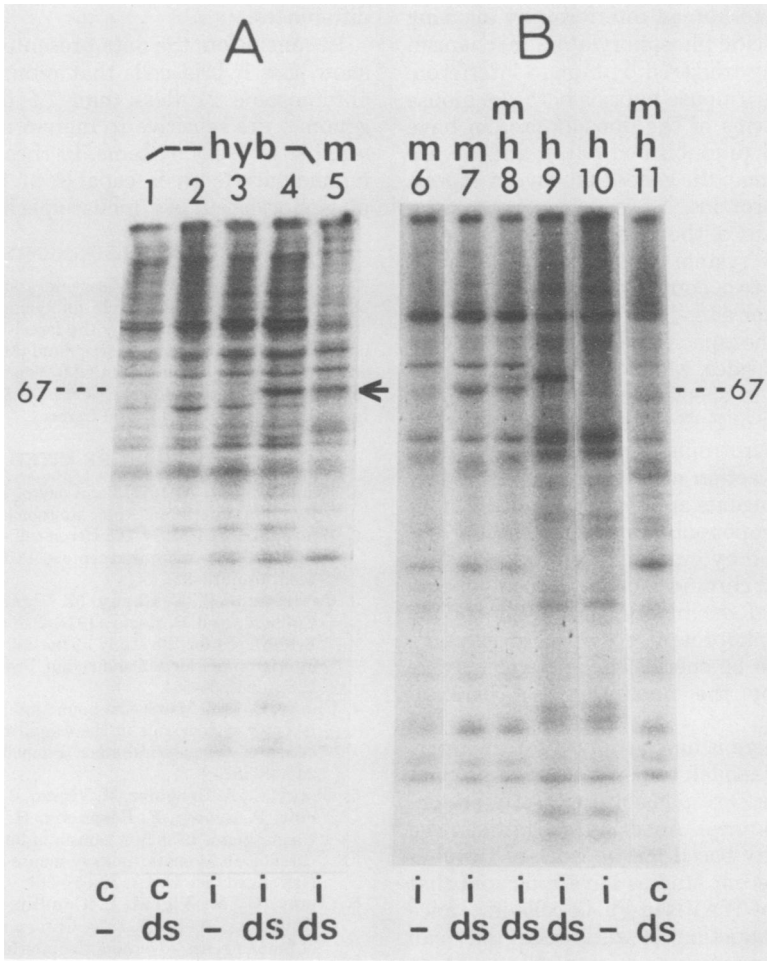

FIG. 3. *Sodium dodecyl sulfate(SDS)-polyacrylamide gels of ^{32}P-labeled proteins from cell extracts. (A) Cultures of WAVR4d-F9-4a cells (2×10^7 cells) were treated with 500 U of human fibroblast interferon per ml for 20 h or were left untreated. After trypsinization and washing with phosphate-buffered saline (PBS), the cells were lysed by hypotonic shock and homogenization. After centrifugation at 10,000 × g for 10 min, the extracts (S10) were incubated with [γ-^{32}P]ATP and analyzed by SDS-polyacrylamide slab gel electrophoresis. Total incubation volume was 0.025 ml. Slots 1 and 2 each contained 0.4 A_{260} (absorbance at 260 nm) unit of S10 from control hybrid cells. Slots 3 and 4: 0.13 A_{260} unit of S10 from hybrid cells treated with human interferon. Slot 5: 0.13 A_{260} unit of S10 from a suspension culture of mouse L cells (CCL 1) treated with 200 U of mouse interferon per ml. Slots 2, 4, and 5 had doublestranded (ds) RNA [poly(rI:rC), 0.4 μg/ml] included in the reaction. (B) Monolayer cultures of L cells (5×10^6 cells) were treated for 24 h with 200 U of mouse interferon per ml or left untreated. After washing with PBS and lysis in 0.5% Nonidet P-40 in buffer B, S10 fractions were prepared by centrifugation and passed through a 10-ml syringe of Sephadex G-25 in buffer B. Confluent monolayers of FS7 cells (8×10^6 cells) were treated with 50 U of human fibroblast interferon per ml for 20 h or left untreated. Extracts were made as above. Slots 6 and 7: 0.3 A_{260} unit of S10 from interferon-treated L cells. Slot 8: Mixture of 0.15 A_{260} unit of S10 from interferon-treated L cells and 0.11 A_{260} unit of S10 from interferon-treated FS7 cells. Slots 9 and 10: 0.21 A_{260} unit of S10 from interferon-treated FS7 cells. Slot 11: Mixture of 0.13 A_{260} unit of control mouse S10 and 0.10 A_{260} unit of control FS7 S10. Slots 7, 8, 9, and 11 had ds RNA (poly(rI:rC), 0.4 μg/ml) added to the incubation mix. Position of the mouse 67,000-dalton phosphoprotein is indicated.*

rylation changes in a hybrid retaining only human chromosome 21 and, if so, whether the human- or mouse-specific phosphoprotein appears. Human interferon treatment of WAVR4d-F9-4a cells resulted in the phosphorylation of the 67,000-dalton protein typical of mouse extracts, the same one phosphorylated when mouse interferon is used on these cells. We con-

clude that chromosome 21 makes these hybrid cells sensitive to human interferon by allowing the mouse-specific phosphorylation mechanism to be efficiently triggered by human interferon. In other human/mouse hybrids both the mouse and human forms of the phosphoprotein have been observed (unpublished data), and we are attempting to map the genes that govern expression of these proteins.

Chany's model of the membrane-bound interferon receptor system (4, 5) proposes the involvement of two components, a binding site and an activator site. The former is responsible for relatively nonspecific interactions and may involve gangliosides, whereas the latter is probably involved in species specificity determination. The work of Kohn et al. (13) has indicated that adding thyrotropin or cholera toxin to cells can block the action of interferon, presumably because these agents are restricting accessibility of receptor components. Once the receptor system is activated by human interferon in hybrid cells containing chromosome 21, the components of the antiviral machinery, perhaps involving the new phosphoprotein observed in extracts, do not have to be species specific and may be contributed by the nonsegregating parental mouse genome.

Although there is much support for the interferon receptor model, the specific gene product of chromosome 21 responsible for interferon sensitivity is not known. A cell surface antigen is a likely possibility based on the work of Revel et al. (17), and recent studies have indicated that the injection of WAVR4d-F9-4a cells into mice elicits the production of antibodies that can block interferon action on human cells, and that antiserum against WAVR4d loses its blocking activity when preadsorbed onto WAVR4d-F9-4a (unpublished data).

DeClercq et al. have recently concluded that chromosome 21 does not code for an interferon receptor (8). Using brief interferon exposures and interferon binding assays on human cell lines with different numbers of copies of chromosome 21, they found that the responses of these cells did not fit those expected from a model of gene dosage of interferon receptors. There are several possible explanations for their data: the initial interactions of interferon with cells probably involve non-species-specific interactions with gangliosides (4, 13), and this binding step may therefore differ from interaction with the chromosome 21-coded receptor, which requires much longer exposure time (17). Quantitation of interferon binding is very difficult with impure, unlabeled interferon preparations, and the nature of the assays used to measure interferon activity makes it difficult to find small differences.

In conclusion, the data presented here clearly show that hybrid cells that retain only human chromosome 21 (less than 1% of the human genome) are sensitive to human interferon in a number of assay systems. In these hybrid cells human interferon is capable of triggering the phosphorylation of a mouse-specific protein.

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