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Activation of the Human β-Interferon Gene Requires an Interferon-Inducible Factor

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β-Interferon (β-IFN) gene expression can be induced by poly(I)-poly(C) or virus, but there is considerable variation in the extent of induction between different cell lines. We characterized two poorly inducible human cell lines, HeLa and 143 thymidine kinase negative (143 tk⁻), to define cellular factors involved in the activation of the β-IFN gene. We show that the deficiency in β-IFN induction in these cells can be complemented by fusion to highly inducible mouse cells. We conclude that the human cells are deficient in a *trans*-acting factor required for β-IFN gene activation. The level of induction of the β-IFN gene in HeLa and 143 tk⁻ cells can also be increased by priming with IFN before induction. If IFN priming is carried out in the presence of cycloheximide, a ~200-fold increase in induction is observed. We conclude that activation of the β-IFN gene requires an IFN-inducible factor that is only expressed at low levels in unprimed HeLa and 143 tk⁻ cells.

Interferons (IFNs) are secreted polypeptides defined by their ability to protect cells of the same species from infection by viruses. There are three types of IFN: α (leukocyte), β (fibroblast), and γ (immune) (reviewed in references 32 and 42). α- and β-IFN expression is inducible in many cultured cell lines by infection with virus and, in the case of β-IFN, by treatment with poly(I)-poly(C). Most fibroblast and epithelial cell lines express β-IFN, and in some cases α-IFN is also produced (27, 29, 52; reviewed in reference 42). In the absence of induction, β-IFN mRNA is usually undetectable; however, upon induction as many as 5,000 β-IFN mRNA transcripts can accumulate per cell. The induction is due at least in part to an increase in the rate of transcription of the β-IFN gene (35, 37; K. Zinn, Ph.D. thesis, Harvard University, Cambridge, Mass. 1983). Induction of β-IFN mRNA can occur within 90 min after treatment with inducer, and this induction does not require protein synthesis (10, 39). These observations suggest that the β-IFN gene is activated by a preexisting factor that is altered in response to the induction signal.

The amount of IFN produced upon induction can be increased up to 50-fold by treating cells with protein synthesis inhibitors at the time of induction, a phenomenon known as superinduction (25; reviewed in reference 42). This increase may be due to stabilization of the β-IFN mRNA (10, 39). Many other inducible genes can be superinduced (12, 17, 28, 46), and in some cases this increase has also been shown to be due to posttranscriptional stabilization of the inducible transcript (reviewed in reference 46). In some cell lines β-IFN induction by virus or poly(I)-poly(C) can also be increased by treating cells with IFN before induction, a phenomenon known as priming (43). Priming can increase IFN production by up to 20-fold in cell lines in which it is observed (19, 25, 43, 44), and in some cases it can also accelerate the kinetics of IFN induction (1, 15, 18). The stimulation of IFN production observed upon priming results from an increase in the rate of β-IFN gene transcription (35).

There is a lag period between the time cells are exposed to inducer and the time when IFN mRNA is produced. The exact length of this lag period depends on the inducer-cell combination, but there are generally 1 to 4 h between the time inducer is added and the time when IFN mRNA can be detected. During this time, a sequence of events takes place which begins with uptake of the inducer and culminates in the transcriptional activation of the IFN gene. The early events in this pathway are likely to be different for different classes of inducers, since a wide range of viruses and many synthetic compounds can act as inducers, and these inducers must enter the cell by different mechanisms (reviewed in reference 42). However, it seems likely that the final steps in the β-IFN induction pathway are common to many inducers.

The presumptive cellular factors involved in β-IFN gene induction have not been identified. Factors involved in the regulation of other genes have been identified by analyzing mutant or variant cell lines that fail to express the gene (4, 30, 48). For the β-IFN gene many cell lines are inducible, but the extent of induction can vary widely (2, 8, 44). Cell lines that are poorly inducible might provide the opportunity to characterize and isolate the cellular factors involved in β-IFN gene regulation.

In this paper we describe the characterization of two poorly inducible human cell lines, HeLa and human 143 thymidine kinase negative (143 tk⁻). We find that the deficiency in β-IFN gene activation in these cells can be complemented in *trans* by fusion to highly inducible cells. In addition, we show that the extent of β-IFN mRNA induction can be increased 200-fold by treating HeLa and 143 tk⁻ cells with a combination of IFN and cycloheximide (CHX) before induction. We conclude that efficient expression of the β-IFN gene in HeLa and 143 tk⁻ cells requires an activating factor which is itself IFN inducible.

MATERIALS AND METHODS

Cell culture and induction procedure. Poly(I)-poly(C) inductions of HeLa, 143 tk⁻, and MG-63 cells were done as described previously (25). Cells were incubated in serum-free medium with 100 μg of poly(I)-poly(C) (P-L Biochemicals, Inc., Milwaukee, Wis.) per ml and 50 μg of CHX (Sigma

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Chemical Co., St. Louis, Mo.) per ml for 90 min, and then the medium was replaced with medium containing only CHX for an additional 2.5 h. The cells were then washed with phosphate-buffered saline (PBS) and incubated in medium containing 2% serum until the RNA was harvested. C-127 cells were induced with poly(I)-poly(C) as described previously (33). Virus inductions were carried out with Sendai virus (Hazleton Research Products, Denver, Pa.) as described previously (20). Cells were incubated in serum-free medium containing ~300 hemagglutinating units of virus per ml for 1.5 h and then washed with PBS and transferred to medium containing 2% serum. CHX inductions were done by incubating the cells in the continuous presence of 50 μg of CHX per ml in medium containing 10% serum. β-IFN mRNA levels were examined at various times (3 to 24 h) after addition of each of the inducers, and an optimal time point was chosen for each inducer. All the human cell lines responded to inducers with similiar kinetics; 3 to 6 h was found to be optimal for poly(I)-poly(C) and virus inductions; 12 h was optimal for CHX. Priming experiments were carried out with the α- and β-IFN obtained from Lee Biomolecular (San Diego, Calif.). Cells were preincubated in Dulbecco modified Eagle medium plus 10% serum containing the indicated amounts of IFN and, where indicated, 50 μg of CHX per ml. After 6 to 24 h the cells were washed three times with PBS and exposed to inducing solutions as described above.

Transfection of cloned β-IFN genes. HeLa, 143 tk^- and C-127 cells were each transfected with the plasmid pβ-IFN*-NEO. This plasmid was constructed by inserting an 8-base-pair (bp) *Cla* linker into the *Pvu*II site of pIFR (51) to generate pIFR*. A restriction fragment containing the bacterial TN5-neomycin^r resistance coding sequence linked to the simian virus 40 early promoter and polyadenylation sequences was isolated from pKO-NEO (D. Hanahan, unpublished data) and inserted between the *Bam*HI and *Hin*dIII sites of pIFR* to form pβ-IFN*-NEO. This plasmid was introduced into each cell line by calcium phosphate coprecipitation (47), and G418-resistant colonies were selected (40). Approximately 100 to 300 colonies of each cell type were pooled, expanded, and used for RNA analysis.

Preparation and analysis of cellular RNA. Cellular RNA was isolated 4 to 6 h after poly(I)-poly(C) or virus induction and 12 h after CHX induction. RNA was prepared by a modification of the procedure of Chirgwin et al. (11). Human β-IFN and actin transcripts were analyzed by quantitative RNase mapping as described previously (50) with single-stranded, uniformly labeled RNA probe synthesized in vitro with SP6 RNA polymerase (34). Human β-IFN mRNA from the endogenous gene was measured with a probe prepared from pSP65'IF cleaved with *Eco*RI (see Fig. 6 in reference 50). Correctly initiated transcripts protect a 277-bp fragment of this probe. RNA transcribed from the marked transfected human β-IFN* genes was detected with a probe transcribed from pSP6IFR* linearized with *Eco*RI. This plasmid was constructed by cloning the *Bgl*II-*Eco*RI fragment from pIFR* (see above) into the polylinker of pSP6-PL2 (34). Transcripts from the marked β-IFN gene protect 644 bp of this probe. The human β-IFN probes do not hybridize with mouse β-IFN mRNA under these conditions (50). Actin message was detected with a probe transcribed from pSP6 γ-actin linearized with *Hin*fI. This template was made by subcloning a *Bam*HI-*Hin*dIII fragment from the 3' noncoding region of the γ-actin cDNA clone pHF1 (23) into pSP64 (34). Human actin mRNA protects the entire probe, generating a 145-bp fragment. Mouse actin RNA, which is only homologous to part of the probe, protects a smaller fragment about 65 bp long. The sizes of the protected fragments were verified by running the samples on gels with end-labeled DNA markers (data not shown). The relative amounts of β-IFN mRNA in each sample were determined by densitometry of the autoradiogram.

Cell fusion. A total of 8×10^6 cells were trypsinized and plated on 100-mm tissue culture plates which had been pretreated for 30 to 60 min with a 10-μg/ml solution of poly-L-lysine (molecular weight, > 70,000; Sigma) in PBS. When two cell types were used, five times more mouse than human cells were mixed together before plating. At 3 to 6 h after plating, cells were fused by a modification of the Ca^{2+}-free technique described in reference 38. A preweighed sample of Koch-Light polyethylene glycol (PEG) 1000 (RPI, Mt. Prospect, Ill.) was melted in a microwave oven. An equal volume of PBS was added to the melted PEG, and the solution was mixed thoroughly. After the solution had cooled to room temperature, cells were washed twice with PBS, and after the second wash the last drops of PBS were removed by aspiration. About 1 to 1.5 ml of the 50% PEG-PBS solution was added, and the plate was tilted to spread the PEG evenly. After a carefully timed interval (45 to 60 s depending on the batch of PEG), the PEG was removed by aspiration, and the cells were gently washed five times with PBS. After washing, the cells were incubated for 30 to 60 min at 37°C in PBS, and then the PBS was replaced with fresh Dulbecco modified Eagle medium plus 10% serum. Inductions were done 0 to 48 h after fusion as described above.

In situ hybridization. In situ hybridizations were carried out with uniformly labeled ^{32}P-labeled single-stranded RNA probes synthesized with SP6 polymerase (34). Human β-IFN mRNA was detected with a probe made from pSP6-IF* truncated at an *Nco*I site at −12. The template for the mouse β-IFN probe was the plasmid pSP6mifβ which contains a 480-bp *Bam*HI-*Bgl*II fragment from the cDNA clone described in reference 26. These probes were hydrolyzed in bicarbonate buffer as described previously (16) to generate probe pieces an average of 50 bp in length. Actin RNA was detected with the same probe used for RNA mapping experiments (see above) and was not hydrolyzed.

Cells for in situ hybridization were cultured on 12-mm diameter circular cover slips and then fused and induced as described above. If the cells were to be fused, the cover slips were pretreated with poly-L-lysine as described above. At 3 to 5 h after induction, the cover slips were transferred to 24-microwell tissue culture trays (Costar, Cambridge, Mass.) and fixed and prepared for hybridization by a modification of the procedure described by Brigati et al. (5). The cover slips were handled with dissecting forceps (VWR). The various solutions were added directly to the cover slips in the microwells and removed by aspiration.

Hybridizations were carried out by a modification of the procedure described by Cox et al. (16). Hybridization solution was prepared as described previously (16) except that dextran sulfate was omitted. Hybridizations were carried out in an inverted lid of a 24-well tissue culture tray. Drops (3 μl) of hybridization solution containing 2 to 3 μg of probe per ml were placed in the centers of the 24 circular rims found on the inner side of the lid of a 24-well tissue culture tray. Each cover slip to be analyzed was placed cell-side down on a droplet of probe. The cover slip was covered with mineral oil to prevent evaporation and hybridized for 12 to 18 h at 51°C in a dry oven. At the end of the hybridization, the lid of the 24-well tray was carefully filled with 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and the cover slips

were pried off with dissecting forceps. The cover slips were then placed cell-side up in microwells in a fresh 24-well tissue culture tray. Posthybridization washes and RNase treatment were done by adding the solutions described by Cox et al. (16) to the individual microwells. When the washes were complete, the cover slips were mounted cell-side up on standard microscope slides with a drop of Permount (Fisher Scientific Co., Pittsburgh, Pa.). After the Permount had set (3 to 10 h), the slides were dipped in Kodak NTB-2 nuclear emulsion diluted 1:1 with 0.6 M ammonium acetate and exposed and developed by standard procedures (41). The developed slides were stained with Hoechst 33258 was described by Blau et al. (3). The cover slips were removed from the slide on which they were mounted by soaking the slides in xylene until the cover slips could be pried off with dissecting forceps. They were then rinsed successively in 95% ethanol, 60% ethanol, and distilled water and mounted cell-side down in a drop of water on a fresh slide. The samples were examined by using a combination of fluorescence and dark-field microscopy with a Zeiss fluorescence microscope equipped with a G 365 filter set.

RESULTS

Analysis of β-IFN gene regulation in HeLa and 143 tk⁻ cells. Previous studies demonstrated that HeLa cells do not produce β-IFN when treated with virus of poly(I)-poly(C) (8, 22, 44). We found that another human cell line, human 143 tk⁻ (21), is also not inducible. A comparison of β-IFN gene transcription in these two cell lines and MG-63 cells, a highly inducible human osteosarcoma cell line (2, 14), is shown in Fig. 1. The levels of β-IFN and actin mRNA were measured by quantitative RNase mapping (50). The β-IFN RNA hybridization probe detected accurately initiated RNA, while the actin probe was homologous to an internal region of the mRNA. In the absence of inducer, β-IFN mRNA was not detected in HeLa, 143 tk⁻, or MG-63 cells (data not shown). When MG-63 cells were treated with poly(I)-poly(C) (Fig. 1, lane C) or Sendai virus (Fig. 1, lane F), high levels of β-IFN mRNA were observed. In contrast, when HeLa (Fig. 1, lanes A and D) or 143 tk⁻ (Fig. 1, lanes B and E) cells were treated with the same inducers, only low levels of β-IFN were produced. Similar levels of actin mRNA were observed in each case, indicating that roughly equal amounts of cellular RNA were present in all of the samples. Quantitation of the levels of β-IFN mRNA produced by these cell lines in a number of experiments indicates that MG-63 cells are 300 to 500 times more inducible than HeLa or 143 tk⁻ cells, depending on the inducer-cell combination. The uninducibility of HeLa cells that we observed may depend on the subline, since there is a recent report that β-IFN mRNA can be induced in HeLa cells by virus (27).

Since HeLa and 143 tk⁻ cells are poorly inducible by both virus and poly(I)-poly(C), it seems unlikely that the poor induction is due to a failure of the inducer to enter the cell, since these inducers presumably enter cells by different mechanisms. Further evidence that the deficiency in β-IFN induction is not at the level of inducer uptake was provided by an analysis of CHX induction. β-IFN mRNA can be induced in a number of cell lines by prolonged exposure to high levels of CHX, an inhibitor of protein synthesis (45); K. Zinn and S. Goodbourn, unpublished observations). β-IFN gene transcription in MG-63 cells was inducible with CHX (Fig. 1, lane I). The level of induction was about 1/10th of that observed when the cells were treated with virus or poly(I)-poly(C). In contrast, when HeLa and 143 tk⁻ cells were treated with CHX, β-IFN gene transcription was not induced (Fig. 1, lanes G and H, respectively). In summary, HeLa and 143 tk⁻ cells respond poorly to three different inducers that efficiently activate β-IFN gene transcription in MG-63 cells. It therefore seems likely that these cells have a general rather than an inducer-specific deficiency in β-IFN gene induction. This deficiency must affect a step in the induction pathway which is common to all three inducers.

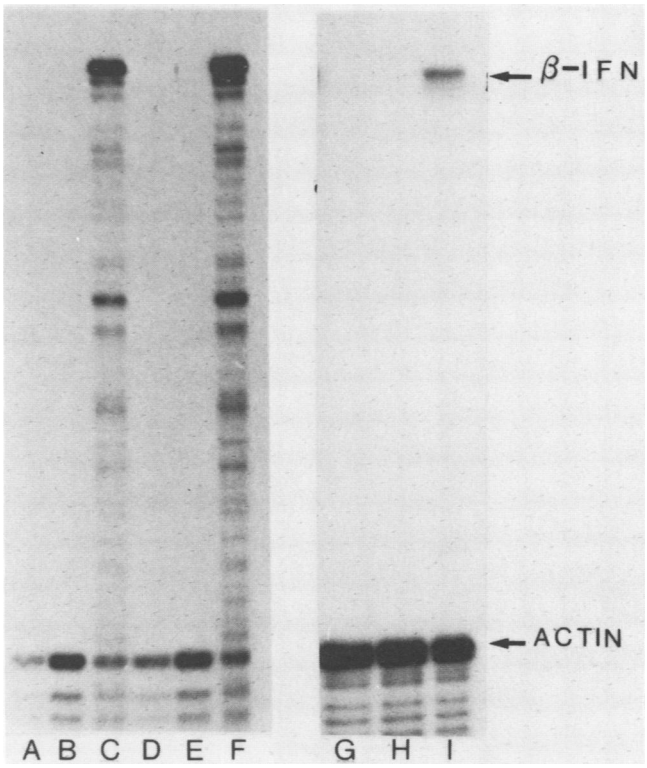


FIG. 1. Expression of the β-IFN gene in HeLa, 143 tk⁻, and MG-63 cells. Cellular RNA (5 μg) isolated from cells induced with poly(I)-poly(C), virus, or CHX was analyzed by quantitative RNase mapping (51) with a human β-IFN 5′ probe and an actin probe. Lanes: A, B, and C, poly(I)-poly(C)-induced HeLa, 143 tk⁻, and MG-63 cells, respectively; D, E, and F, virus-induced HeLa, 143 tk⁻, and MG-63 cells respectively; G, H, and I, CHX-induced HeLa, 143 tk⁻, and MG-63 cells, respectively. With a 20-fold-longer exposure a signal is visible in lanes A, B, D, and E.

Cloned β-IFN genes are not expressed when introduced into HeLa or 143 tk⁻ cells by stable cotransformation. The lack of β-IFN induction in HeLa and 143 tk⁻ cells could be due to a defect in the β-IFN gene or to quantitative or qualitative differences in the cellular factors required for β-IFN gene regulation. Cloned β-IFN genes are correctly regulated when transfected into a variety of established cell lines (7, 24, 36, 51).

To test the possibility that 143 tk⁻ or HeLa cells produce the factors necessary for induction but carry inactive β-IFN genes, we studied the expression of cloned β-IFN genes stably introduced into these cell lines by using a linked neomycin resistance gene as a selectable marker. A synthetic DNA linker was inserted into the coding sequence of the cloned gene to allow discrimination between β-IFN transcripts derived from the endogenous and transfected genes. Neither the endogenous or the transfected β-IFN genes were transcribed in pools of neomycin (G418)-resistant HeLa (Fig. 2, lanes A and B) or 143 tk⁻ (Fig. 2,

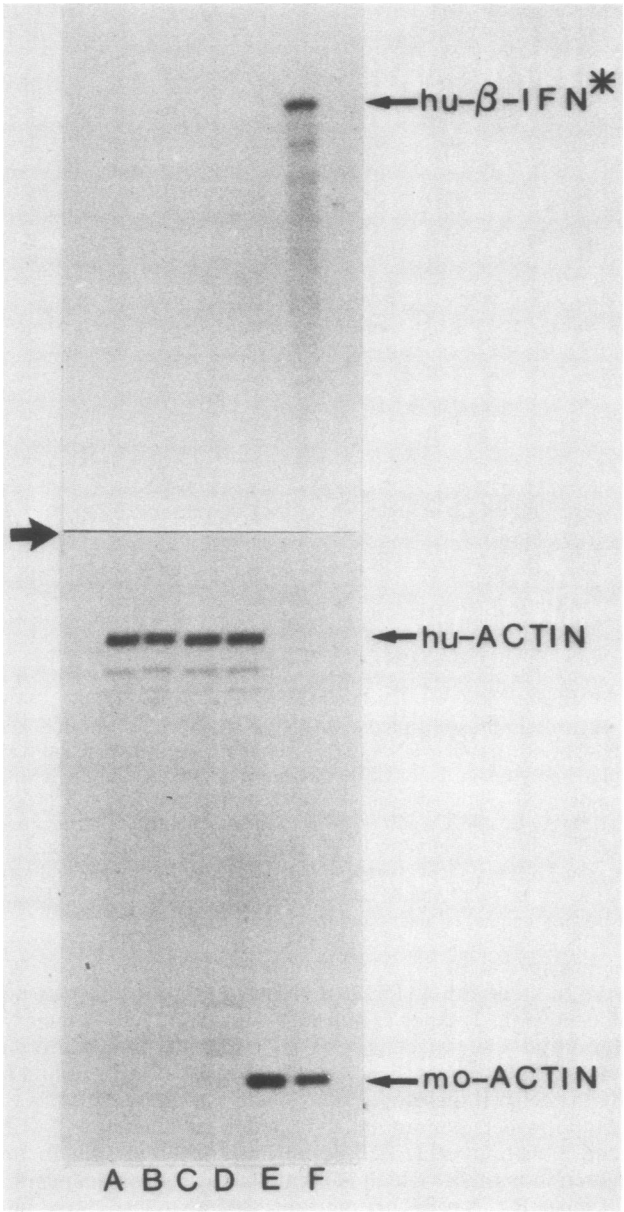


FIG. 2. Expression of cloned human β-IFN genes transfected into HeLa, 143 tk⁻, and C-127 cells. Cellular RNA was isolated from untreated and poly(I)-poly(C)-induced pools of G418-resistant transformants obtained by transfecting cells with the plasmid pβ-IFN*NEO which contains a marked human β-IFN gene linked to a neomycin resistance gene (see text). The RNA was analyzed as described in the legend to Fig. 1. Transcripts from the transfected human β-IFN gene were detected with a marked probe so that they could be distinguished from the endogenous gene. This probe does not hybridize to mouse β-IFN transcripts (50). Both mouse (mo) and human (hu) actin transcripts could be detected with a human actin probe, although the mouse transcript generates a smaller protected fragment. To conveniently show all three bands, the middle portion of the autoradiogram is not shown. The arrow marks the boundary between the upper and lower parts of the gel. Lanes: A and B, HeLa cells, uninduced and induced, respectively; C and D, 143 tk⁻ cells, uninduced and induced, respectively; E and F, C-127 cells, uninduced and induced, respectively. With a 20-fold-longer exposure a signal is visible in lanes B and D.

lanes C and D) cells in the presence or absence of inducers. In contrast, the cloned β-IFN gene was expressed and correctly regulated when transfected into mouse C-127 cells (Fig. 2, lanes E and F), a highly inducible fibroblast cell line (20, 50). The human β-IFN probe does not hybridize to mouse β-IFN mRNA under these conditions (51). Genomic DNA blotting experiments showed that the pools of transformed HeLa, 143 tk⁻ and C127 cells contained approximately equal numbers of unrearranged transfected β-IFN genes (data not shown). Since HeLa and 143 tk⁻ cells fail to express transfected β-IFN genes that are expressed in inducible cells, we conclude that these cell lines have a *trans*-acting deficiency in β-IFN induction.

Activation of β-IFN genes in HeLa and 143 tk⁻ cells by fusion to highly inducible cells. The *trans*-acting deficiency in β-IFN induction of HeLa and 143 tk⁻ cells could be due to the absence of cellular factors required for induction or to the presence of factors that block induction. To distinguish between these possibilities, we analyzed induction of the human β-IFN gene in interspecific heterokaryons formed by fusing HeLa or 143 tk⁻ cells to mouse C-127 cells. The cells were fused by treatment with PEG as described by Schneiderman et al. (38) and then induced 0 to 48 h later. 143 tk⁻ cells fused to mouse C-127 cells produced 5 to 20-fold more human β-IFN mRNA upon induction compared with 143 tk⁻ cells treated with PEG in the absence of C-127 cells. This stimulation was observed when either poly(I)-poly(C) (Fig. 3, compare lanes C and B) or virus (Fig. 3, compare lanes E and D) was used as the inducer. Similarly, HeLa cells fused to C-127 cells produced 10- to 20-fold more human β-IFN mRNA after induction with poly(I)-poly(C) (Fig. 3, lane H) or virus (Fig. 3, lane J) than HeLa cells treated with PEG and induced in the absence of C-127 cells (Fig. 3, lanes G and I).

Enhanced induction of the human β-IFN genes in heterokaryons was also observed when CHX was used as an inducer (data not shown), although the amounts of β-IFN produced were somewhat lower, perhaps because CHX is a relatively inefficient inducer compared with poly(I)-poly(C) or virus (Fig. 1) (K. Zinn and S. Goodbourn, unpublished observations). β-IFN mRNA was not detected in the absence of inducers (Fig. 3, lanes A and F), and the stimulation of human β-IFN mRNA production required fusion since unfused mixtures of C-127 and HeLa or 143 tk⁻ cells did not produce significant quantities of human β-IFN mRNA (data not shown). Induction of the human β-IFN gene was stimulated to the same extent in heterokaryons induced between 0 and 48 h after fusion, and stimulation of induction was observed even when protein synthesis inhibitors were added immediately after fusion (data not shown). An identical stimulation of human β-IFN mRNA induction was observed when HeLa or 143 tk⁻ cells were fused to Ehrlich ascites tumor cells, another highly inducible mouse cell line (data not shown).

We conclude that HeLa and 143 tk⁻ cells are poorly inducible because they lack a cellular factor required for β-IFN gene activation. This factor can be provided in *trans* by highly inducible mouse cells in the heterokaryons. It seems likely that both human cell lines lack the same factor since β-IFN genes in HeLa-143 tk⁻ heterokaryons are not inducible (data not shown).

Expression of β-IFN mRNA in individual heterokaryons. Since we did not select for heterokaryons after fusion, the RNA analyzed in the above experiments was isolated from a mixture of homokaryons, heterokaryons, and unfused cells. To show that the β-IFN transcripts detected are synthesized

in interspecific heterokaryons, we used in situ hybridization to cellular mRNA (5, 13, 16) to study β-IFN mRNA expression in individual HeLa-C-127 and 143 tk⁻-C-127 heterokaryons induced with poly(I)-poly(C). To identify heterokaryons, cells were stained with Hoechst 33258, a fluorescent DNA-binding dye with a preferential affinity for AT-rich sequences. Mouse nuclei stained in this fashion contain bright dots, which correspond to AT-rich satellite DNA sequences. Human nuclei, which do not contain these sequences, stain uniformly (3). Figures 4A and B show the results of a typical in situ hybridization experiment with a ^{32}P-labeled probe complementary to human β-IFN mRNA. Panel A and panel B show the same field photographed without and with dark-field illumination, respectively. In the center of each panel is a 143 tk⁻-C-127 heterokaryon which contains three mouse nuclei and one human nucleus. The human nucleus is indicated by an arrow. This nucleus is covered with autoradiographic grains in panel B, indicating that the heterokayon is expressing human β-IFN mRNA. An unfused human cell immediately above this heterokaryon clearly does not express human β-IFN mRNA. Hybridization to the human β-IFN mRNA probe is not observed unless the heterokaryons are induced with poly(I)-poly(C) or virus (data not shown). The human β-IFN probe did not hybridize to cellular RNA in induced mouse cells, indicating that this probe is specific for human β-IFN mRNA under the conditions used in our experiments. In situ hybridization experiments with either HeLa-C-127 or 143 tk⁻-C-127 heterokaryons showed that over 90% of the cells that expressed human β-IFN mRNA contained both mouse and human nuclei. Thus, these studies show that expression of significant amounts of β-IFN mRNA by HeLa or 143 tk⁻ cells requires fusion to inducible cells. This confirms that a factor or factors in highly inducible cells can complement the deficiency of the poorly inducible human cells.

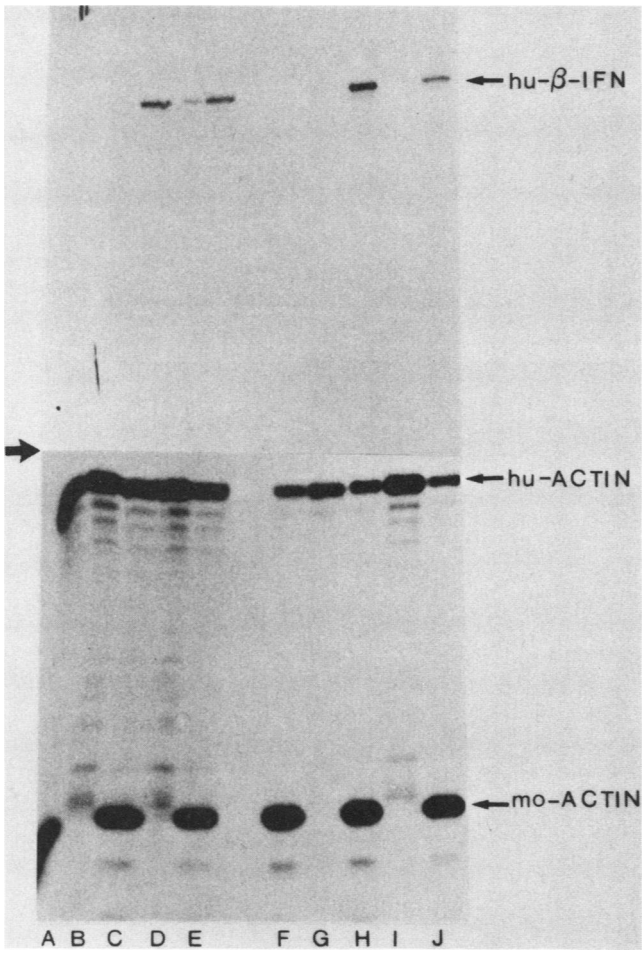
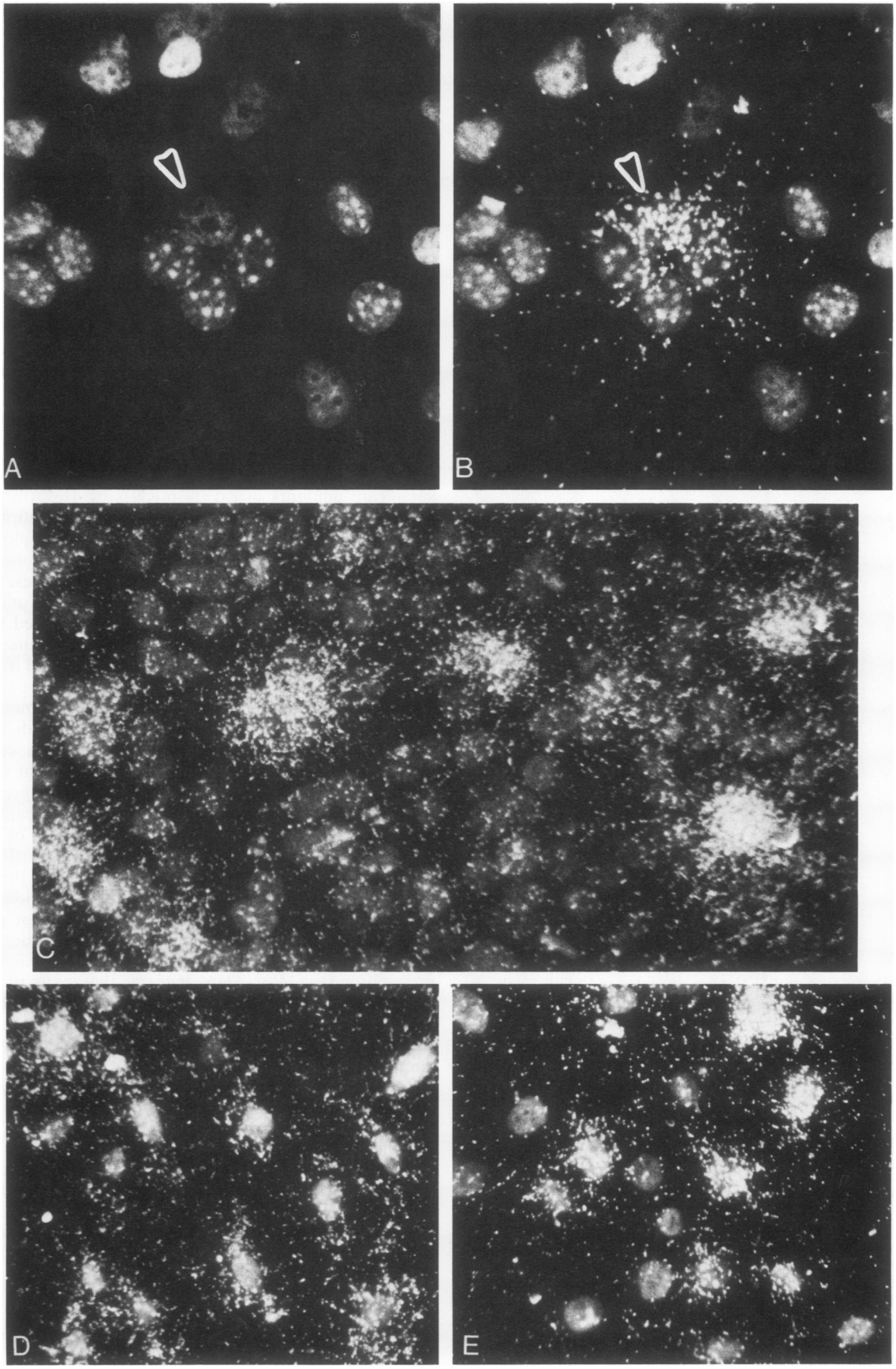


FIG. 3. Expression of β-IFN genes in HeLa and 143 tk⁻ cells is stimulated by fusion to C-127 cells. Cellular RNA was isolated from induced and uninduced PEG-treated cells and analyzed by RNase mapping with the human (hu) β-IFN 5′ probe and a human actin probe. Human actin mRNA protects the entire 145-bp actin probe and is present in all the samples. Mouse (mo) actin message protects only 65 bp of the probe and is present only when C-127 cells were included in the fusion mixture. Fusions were carried out as described in Materials and Methods. Lanes: A, 143 tk⁻ plus C-127 cells, PEG treated but not induced; B, 143 tk⁻ cells alone, treated with PEG and induced with poly(I)-poly(C); C, 143 tk⁻ cells plus C-127 cells, treated with PEG and induced with poly(I)-poly(C); D and E, same as lanes B and C, respectively, except the induction is done with virus; F, HeLa and C-127 cells treated with PEG but not induced; G, HeLa cells alone, treated with PEG and induced with poly(I)-poly(C); H, HeLa plus C-127 cells, treated with PEG and induced with poly(I)-poly(C); I and J, same as lanes G and H, respectively, except using virus as the inducer. In lanes A, C, E, F, and H 10 μg of cellular RNA was analyzed; in lanes B, D, G, I, and J 50 μg of cellular RNA was analyzed. The part of the autoradiogram shown below the arrow was exposed for 6 h; the part above the arrow was exposed for 18 h.

Although 30 to 60% of the human nuclei in these samples were found in cells that contained both mouse and human nuclei, the in situ hybridization studies revealed that less than 10% of these heterokaryons expressed human β-IFN mRNA. Similarly, a mouse β-IFN probe detected transcripts in only 10% of the mouse cells in these samples (Fig. 4C). The fact that only a small number of the mouse cells produced β-IFN upon induction was not a result of fusion since an equally small fraction of these cells expressed β-IFN mRNA in the absence of PEG treatment (data not shown). We also observed heterogeneous expression of β-IFN mRNA in populations of induced MG-63 cells. In this case, a somewhat higher proportion (25 to 30%) of the cells expressed β-IFN mRNA (Fig. 4E). This proportion is the same whether virus or poly(I)-poly(C) is used as the inducer (data not shown). The low frequency of hybridization to β-IFN mRNA probes is not due to a failure of probe to penetrate some cells, since a human actin probe detected roughly equal amounts of actin RNA in all of the cells in our preparation (Fig. 4D). Thus, it seems that many of the cells in highly inducible cell populations do not express β-IFN in response to induction. This explains why only a fraction of the HeLa-C-127 and 143 tk⁻-C-127 heterokaryons express human β-IFN mRNA. Heterogeneity in the expression of α- and β-IFN in response to induction has also been reported in mouse C-243 cells (49). Variation in the responses of individual cells to inducers may be a general property of the IFN system.

β-IFN gene induction in HeLa and 143 tk⁻ cells requires an IFN-inducible factor. Treatment of certain cell lines with IFN before induction with poly(I)-poly(C) or virus can lead to an increase in the amount of IFN subsequently produced, an effect known as priming (see reference 42 for a review). To determine whether the induction of the β-IFN gene in HeLa and 143 tk⁻ cells could be increased by priming, we pretreated these cells with α or β-IFN and then induced the cells with virus. Priming of HeLa or 143 tk⁻ cells with IFN resulted in a 20 to 50-fold increase in the level of induced β-IFN mRNA (Fig. 5, lanes, B, D, E, and F; Fig. 6, lanes B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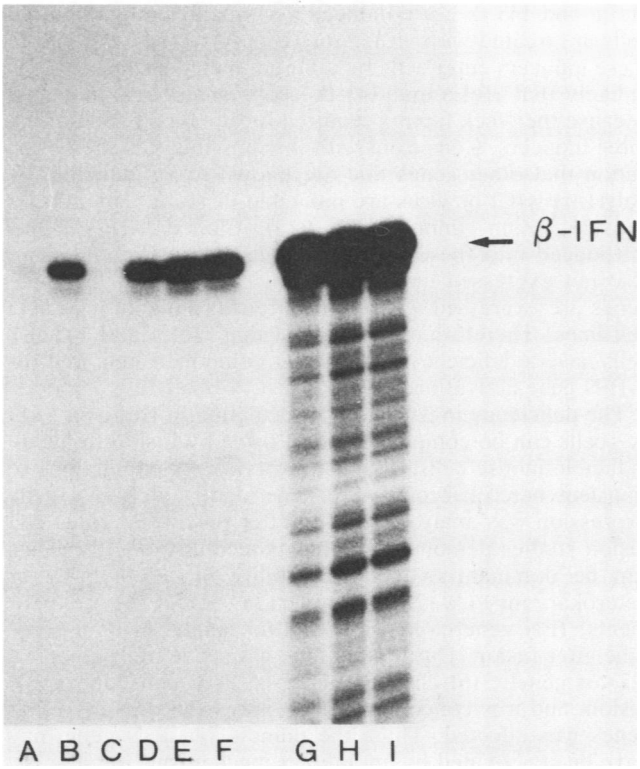

FIG. 5. Priming of HeLa and 143 tk⁻ cells with IFN enhances induction of the β-IFN gene. RNA was isolated from untreated or IFN-primed cells induced with poly(I)-poly(C) or virus and analyzed by quantitative RNAase mapping with the 5′ human β-IFN probe. Lanes: A, 143 tk⁻ cells, no IFN pretreatment; B, 143 tk⁻ cells pretreated with 250 U of β-IFN per ml, C, HeLa cells, no IFN pretreatment; D and E, HeLa cells pretreated with 250 U of β-IFN per ml (each sample is from a separate experiment); F, HeLa cells pretreated with 250 U of α-IFN per ml; G and H, MG-63 cells, no IFN pretreatment; I, MG-63 cells pretreated with 250 U of β-IFN per ml. All samples except lane G come from virally induced cells; the sample in lane G comes from poly(I)-poly(C)-induced cells. Lanes A to F, 20 μg of RNA; lanes G, H, and I, 10 μg of RNA.

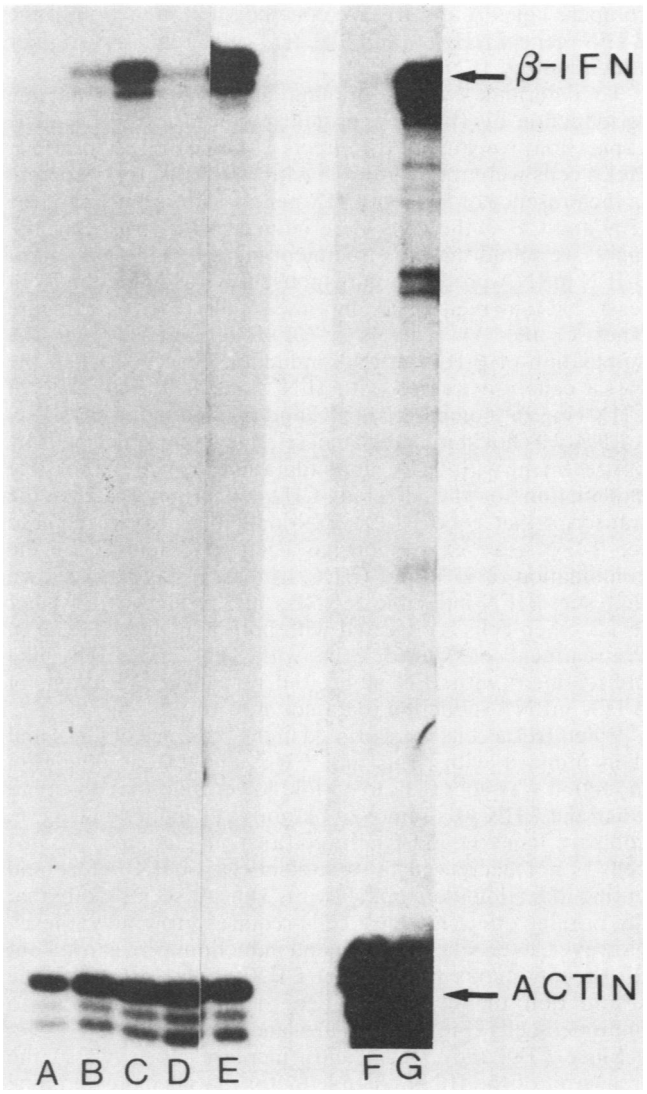

FIG. 6. Effect of CHX on priming. RNA was isolated from virus-induced cells after the indicated pretreatments, and 20 μg of each sample was hybridized to the actin and 5′ human β-IFN probe. Lanes: A, HeLa cells, no IFN pretreatment; B, HeLa cells pretreated with 500 U of α-IFN per ml for 6 h; C, HeLa cells pretreated with 500 U of α-IFN per ml plus 50 μg of CHX per ml for 6 h; D, same as sample C except induction was done in the presence of 50 μg of CHX per ml such that the cells were kept in CHX continuously throughout IFN pretreatment and induction; E, MG-63 cells pretreated with 500 U of α-IFN per ml plus 50 μg of CHX per ml F and G, 20-fold-longer exposure of lanes A and B.

and G) compared with induction without priming (Fig. 5, lanes A and C; Fig. 6, lanes A and F). Priming with IFN also caused a large increase in induction (~100-fold for 143 tk⁻ cells and ~15-fold for HeLa cells) when poly(I)-poly(C) was used as the inducer (data not shown). However, even with priming, the level of β-IFN mRNA produced by HeLa and 143 tk⁻ cells is still 10 to 20-fold lower than that produced by MG-63 cells (Fig. 5, compare lanes G to I with lanes B and D to F). MG-63 cells primed with IFN (Fig. 5, lane I) did not produce more β-IFN mRNA than unprimed MG-63 cells (Fig. 5, lanes G and H). Induction of β-IFN in mouse C-127 cells is also not significantly affected by priming (K. Zinn and S. Goodbourn, unpublished observations). Priming does not stimulate expression of the β-IFN gene in uninduced cells (data not shown). The priming effect required about 6 h to develop fully at 500 U of IFN per ml; at <25 U/ml it did not reach maximal levels even after 24 h (data not shown). Priming had no effect on γ-actin mRNA levels (Fig. 6,

FIG. 4. Detection of specific mRNAs in individual cells by in situ hybridization. 143 tk⁻-C-127 heterokaryon preparations and unfused MG-63 cells were induced with virus, fixed after 4 h, and then hybridized with the appropriate ^{32}P-labeled probes as described in Materials and Methods. Nuclei were stained with Hoechst 33258. Mouse nuclei stained in this fashion contain bright dots, while human nuclei stain uniformly. Cells were visualized by fluorescence microscopy (A) or a combination of dark-field and fluorescence microscopy (B to E); the bright spots in panels B to E are autoradiographic grains. Panels A and B show the same field photographed in the absence and presence, respectively, of dark-field illumination. In the center of panel A is a heterokaryon; the human nucleus is indicated by an arrow. This nucleus can be seen to be covered with autoradiographic grains in panel B. Panel C shows a heterokaryon preparation hybridized to a mouse β-IFN probe. Panel D shows MG-63 cells hybridized with an actin probe; panel E shows MG-63 cells hybridized to a human β-IFN probe.

compare lanes A and B). We conclude that induction of the β-IFN gene in HeLa and 143 tk$^-$ cells requires a factor which is itself IFN inducible.

To determine wheter priming of HeLa cells was due to induction by IFN of a protein required for β-IFN gene expression, we studied the effects of CHX on IFN priming. HeLa cells were treated for 6 h with 500 Units of IFN per ml in the presence of 50 μg of CHX per ml. After removal of the IFN and CHX, the cells were induced with virus. Surprisingly, we found that this treatment increased the amount of β-IFN mRNA produced in response to virus induction by at least 200-fold relative to unprimed cells (Fig. 6, compare lanes C and A). This is a ~10-fold increase over the stimulation of β-IFN mRNA induction observed when the HeLa cells are treated with IFN alone in the absence of CHX (Fig. 6, compare lanes C and B). Induction of β-IFN mRNA was not stimulated at all relative to unprimed cells by pretreatment with CHX alone (data not shown). A possible explanation for the effect of CHX on priming is that the transcript that encodes the IFN-inducible protein required for β-IFN gene expression has been superinduced by the combination of IFN and CHX. In fact, it has been shown that some IFN-inducible mRNAs are produced at higher levels when cells are treated with both IFN and CHX (17). Pretreatment of MG-63 cells with IFN and CHX, like pretreatment with IFN alone, did not affect the extent of β-IFN mRNA induction (data not shown).

When HeLa cells were primed in the presence of CHX and then induced with virus and CHX, the extent of β-IFN induction was about 10- to 15-fold lower than that observed when the CHX was removed before virus induction (Fig. 6, compare lanes D and C). In contrast, induction of MG-63 cells is not decreased by treatment with CHX before and during induction (data not shown). Thus, it seems likely that the priming effect in HeLa cells requires protein synthesis. However, even when priming and induction were carried out in the continuous presence of CHX, there was still some stimulation of the β-IFN gene expression relative to unprimed cells (Fig. 6, compare lanes D and A).

Since CHX may significantly increase the levels of the transcript of the IFN-inducible factor that mediates priming (see above), residual priming could result even if only a small amount of the protein is translated, which could occur if inhibition is less than 100% complete. In addition, the β-IFN transcript itself is superinducible (10, 39), and thus CHX may incidentally increase the amount of β-IFN mRNA synthesized even as it blocks a large part of the priming effect. It is also possible that some part of the priming effect is mediated by a preexisting factor which is modified in response to IFN treatment rather than being synthesized de novo. However, this would be unprecedented (see reference 32 for a review) and furthermore is not compatible with the fact that the priming effect takes a relatively long time (~6 h) to develop (43; unpublished data).

We conclude that untreated HeLa and 143 tk$^-$ cells respond poorly to inducers because they lack a factor required for β-IFN gene induction. The expression of this factor can be induced by IFN and superinduced by IFN and CHX. When the factor is superinduced, the previously uninducible HeLa cells synthesize almost as much β-IFN mRNA upon induction as the highly inducible MG-63 cells (Fig. 6, compare lanes C and E).

DISCUSSION

HeLa and 143 tk$^-$ cells lack an activator required for β-IFN gene induction. The β-IFN genes in two human cell lines, HeLa and 143 tk$^-$, are induced very inefficiently when the cells are treated with virus, poly(I)-poly(C), or CHX. Since these inducers enter cells by different mechanisms, it seems unlikely that HeLa and 143 tk$^-$ cells are poorly inducible because they lack factors required for uptake of each of the three inducers. Consistent with this possibility is the observation that other genes that are known to be inducible by poly(I)-poly(C) or virus are inducible in HeLa and 143 tk$^-$ cells (K. Zinn, unpublished data). Cloned β-IFN genes introduced into these cells by stable cotransformation are also not expressed upon induction, even though the same genes are expressed when introduced into highly inducible cell lines. Therefore, we conclude that HeLa and 143 tk$^-$ cells have a deficiency in a cellular component required for β-IFN gene activation.

The deficiency in β-IFN gene regulation in HeLa and 143 tk$^-$ cells can be complemented in *trans* by fusion to highly inducible mouse cells. In these experiments populations of transient heterokaryons were generated, and β-IFN gene expression was analyzed shortly after fusion. Previous cell fusion studies demonstrated that inducibility of IFN genes can be dominant over uninducibility in cell hybrids or heterokaryons (6, 9, 22). However, in each of these experiments, IFN gene expression was not analyzed until some time after fusion. During this time extensive morphological (22) or genetic (6) changes occurred as a consequence of fusion, and it is conceivable that expression of many other genes was affected. Thus, the uninducible IFN gene may have been activated by an indirect mechanism. In contrast, in the transient heterokaryons we studied, there must be factors present in the inducible mouse cells before fusion that can complement the deficiency in β-IFN gene regulation in HeLa and 143 tk$^-$ cells. It seems likely that both the uninducible cell lines lack a common regulatory factor, since the deficiency in β-IFN induction is not complemented in either cell when they are fused to each other.

Heterogeneity of β-IFN mRNA expression in inducible cell populations after induction with virus or poly(I)-poly(C). In situ hybridization experiments showed that only 10% of the human-mouse heterokaryons express human β-IFN mRNA after induction. When we investigated the expression of the β-IFN gene in populations of unfused highly inducible cells, we found that an average of only 10% of mouse C-127 cells and 25% of human MG-63 cells synthesize β-IFN mRNA in response to induction. Roughly the same fraction of cells was observed to express β-IFN message whether poly(I)-poly(C) or virus was used as the inducer. Zawatsky et al. (49) have also observed heterogeneous expression of α- and β-IFN mRNA in virus-induced mouse C-243 cells. They have shown that this heterogeneity in the response of cells to IFN inducers is not due to genetic variation within the populations, since 30 subclones derived from the initial cell line all produced IFN upon induction. The fact that heterogeneity in the response of inducible cells has now been observed in three cell lines in response to different inducers suggests that this may be a general feature of the IFN system. An interesting possibility is that IFN mRNA induction may only be possible during certain parts of the cell cycle. We are currently investigating this possibility.

IFN induces a factor required for β-IFN gene induction in HeLa and 143 tk$^-$ cells. The cell fusion experiments presented here demonstrate that HeLa and 143 tk$^-$ cells are poorly inducible because they lack a factor required for efficient β-IFN gene induction. This factor can be provided in *trans* by highly inducible mouse cells in heterokaryons. The deficiency in β-IFN induction can also be overcome by

treating HeLa and 143 tk$^-$ cells with IFN before induction with virus or poly(I)-poly(C). It therefore seems likely that IFN induces the expression of an activating factor required for expression of the β-IFN gene. Since the priming effect seems to require protein synthesis, it seems likely that IFN induces a protein that is required for expression of the β-IFN gene. IFN has been shown to induce the expression of a number of genes, and many of these genes can be superinduced if protein synthesis inhibitors are added at the time of induction (17, 31). Since priming of HeLa and 143 tk$^-$ cells by IFN can be further augmented by inhibitors of protein synthesis, we conclude that the IFN-inducible factor required for β-IFN gene activation in these cells is superinducible. We do not know whether the IFN-inducible factor is the same as the factor provided by the inducible cells in the heterokaryons.

IFN priming has been observed in many different cell lines (see reference 42, for a review). As with HeLa and 143 tk$^-$ cells, priming in other cell lines can develop in as little as 6 h (43), requires protein synthesis (18), and increases IFN production by increasing IFN mRNA levels (15, 18, 19, 35). The difference between these previous studies and the priming of HeLa and 143 tk$^-$ cells is the magnitude of the effect. With most cell lines, priming augments IFN gene induction about 3- to 20-fold, in contrast to the ~200-fold effect we observed with HeLa and 143 tk$^-$ cells. This quantitative difference can be explained by the fact that we superinduced the expression of the activator by priming the cells with a combination of IFN and CHX.

In contrast to HeLa and 143 tk$^-$ cells, human MG-63 and mouse C-127 cells did not respond significantly to priming. These observations are consistent with the fact that other highly inducible cell lines do not produce higher levels of IFN as a result of priming (25, 44). Priming may fail to stimulate IFN induction in highly inducible cells because the IFN-inducible activator is already present at a level that does not limit the extent of induction. In contrast, β-IFN induction is significantly stimulated by priming in HeLa and 143 tk$^-$ cells because this activator is present at relatively low levels in the absence of IFN treatment. On the basis of these observations we propose that the observed differences in IFN gene inducibility among cell lines are due to differences in the basal level of this IFN-inducible activating factor.

The basal level of this activating factor may also vary in vivo. If this is the case, the ability of IFN to regulate the expression of the β-IFN gene could have important physiological consequences. Cells that constitutively express high levels of the activating factor and are therefore highly inducible by virus could constitute a primary defense against virus infection. The IFN produced by such cells could then prime other cells that are not directly inducible because they express low levels of the activating factor. These primed cells would then be able to produce more IFN in response to subsequent rounds of virus infection. In addition, IFN might be produced more rapidly by these cells, since priming can accelerate the kinetics of induction. The existence of these two types of cells (and perhaps cells with intermediate properties) would allow amplification of the response of cells to viral induction as an infection spreads. However, high levels of IFN would not be produced in response to minor infections since many of the cells would not be induced in the absence of exogenous IFN. This might be advantageous since IFN has antigrowth properties (reviewed in reference 42), and production of more IFN than is necessary to combat a virus infection could be detrimental.

In conclusion, induction of the β-IFN gene by poly(I)-poly(C) or virus requires an activating factor that can itself be induced by IFN. We propose that HeLa and 143 tk$^-$ cells express low levels of this factor, while higher levels of this factor are expressed by inducible cells such as the MG-63 and C-127 lines. It should be possible to exploit the differences among these cell lines to isolate the gene that encodes this activating factor.

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LITERATURE CITED

1. **Abreu, S. L., F. C. Bancroft, and W. E. Stewart II.** 1979. Interferon priming: effects on interferon messenger RNA. J. Biol. Chem. **254**:4114–4118.
2. **Billiau, A., V. G. Edy, H. Heremans, J. van Damme, J. Desmyter, J. A. Georgiades, and P. deSomer.** 1977. Human interferon: mass production in a newly established cell line, MG-63. Antimicrob. Agents Chemother. **12**:11–15.
3. **Blau, H. M., C.-P. Chiu, and C. Webster.** 1983. Cytoplasmic activation of human nuclear genes in stable heterokaryons. Cell **32**:1171–1180.
4. **Bouhnik, J., D. Cassio, E. Coezy, P. Corvol, and M. C. Weiss.** 1983. Angiotensinogen production by rat hepatoma cells in culture and analysis of its regulation by techniques of somatic cell genetics. J. Cell Biol. **97**:549–555.
5. **Brigati, D. J., D. Myerson, H. Leary, B. Spalholz, S. Travis, C. Fong, G. D. Hsiung, and D. C. Ward.** 1983. Detection of viral genomes in cultured cells and paraffin-embedded tissue sections using biotin labeled hybridization probes. Virology **126**:32–50.
6. **Burke, D. C., T. Ege, and N. R. Ringertz.** 1980. Production of chick interferon by reactivating chick erythrocytes. J. Gen. Virol. **50**:437–440.
7. **Canaani, D., and P. Berg.** 1982. Regulated expression of human interferon β1 gene after transduction into cultured mouse and rabbit cells. Proc. Natl. Acad. Sci. USA **79**:5166–5170.
8. **Cantell, K., and K. Paucker.** 1963. Studies on viral interference in two lines of HeLa cells. Virology **19**:81–87.
9. **Carver, D. H., D. S. Y. Seto, and B. R. Migeon.** 1968. Interferon production and action in mouse, hamster, and somatic hybrid mouse-hamster cells. Science **160**:558–559.
10. **Cavalieri, R. L., E. A. Havell, J. Bilcek, and S. Pestka.** 1977. Induction and decay of human fibroblast interferon mRNA. Proc. Natl. Acad. Sci. USA **74**:4415–4419.
11. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**:5294–5299.
12. **Cochran, B. H., J. Zullo, I. M. Verma, and C. D. Stiles.** 1984. Expression of the c-fos gene is stimulated by platelet-derived growth factor. Science **226**:1080–1082.
13. **Coghlan, J. P., J. D. Penschom, and P. Hudson.** 1984. Hybridization histochemistry: use of recombinant DNA for tissue localizations of specific mRNA populations. J. Clin. Exp. Hypertens. **6**:63–78.
14. **Content, J., L. De Wit, J. Tavernier, and W. Fiers.** 1984. Human fibroblast interferon RNA transcripts of different sizes in poly(I)-poly(C) induced cells. Nucleic Acids Res. **11**:2627–2638.
15. **Content, J., M. I. Johnston, L. De Wit, J. De Maeyer-Guignard, and E. De Clercq.** 1980. Kinetics and distribution of interferon mRNA in interferon-primed and unprimed mouse L-929 cells. Biochem. Biophys. Res. Commun. **96**:415–424.
16. **Cox, K. H., D. DeLeon, L. M. Angerer, and R. C. Angerer.** 1983.

Detection of mRNAs in sea urchin embryos by in situ hybridization using assymetric probes. Dev. Biol. **101**:485–502.
17. **Friedman, R. L., S. P. Manly, M. McMahon, I. M. Kerr, and G. Stark.** 1984. Transcriptional and posttranscriptional regulation of interferon-induced gene expression in human cells. Cell **38**:745–755.
18. **Fujita, T., and S. Kohno.** 1981. Studies on interferon priming: cellular response to viral and nonviral inducers and requirement for protein synthesis. Virology **112**:62–69.
19. **Fujita, T., S. Sakura, and S. Kohno.** 1979. Priming increases the amount of interferon mRNA in poly(rI)-poly(rC) treated L cells. J. Gen. Virol. **45**:301–308.
20. **Goodbourn, S., K. Zinn, and T. Maniatis.** 1985. Human β-interferon gene expression is regulated by an inducible enhancer element. Cell **41**:509–520.
21. **Grodzicker, T., and D. F. Klessig.** 1980. Expression of unselected adenovirus genes in human cells co-transformed with the HSV-1 tk gene and adenovirus 2 DNA. Cell **21**:453–463.
22. **Guggenheim, M. A., R. M. Friedman, and A. S. Rabson.** 1967. Interferon: production by chick erythrocytes activated by cell fusion. Science **159**:542–543.
23. **Gunning, P., P. Ponte, H. Okayama, J. Engel, H. Blau, and L. Kedes.** 1983. Isolation and characterization of full-length cDNA clones for human alpha-, beta-, and gamma-actin mRNAs: skeletal but not cytoplasmic actins have an amino-terminal cysteine that is subsequently removed. Mol. Cell. Biol. **3**:787–795.
24. **Hauser, H., G. Gross, W. Burns, H.-K. Hochkeppel, U. Mayr, and J. Collins.** 1982. Inducibility of human β-interferon gene in mouse L-cell clones. Nature (London) **297**:650–654.
25. **Havell, E. A., and J. Vilcek.** 1972. Production of high-titered interferon in cultures of human diploid cells. Antimicrob. Agents Chemother. **2**:476–484.
26. **Higashi, Y., Y. Sokawa, Y. Watanabe, Y. Kawade, S. Ohno, E. Takaoga, and T. Taniguchi.** 1983. Structure and expression of a cloned cDNA for mouse interferon-beta. J. Biol. Chem. **258**:9522–9529.
27. **Hiscott, J., K. Cantell, and C. Weissmann.** 1984. Differential expression of human interferon genes. Nucleic Acids Res. **12**:3727–3745.
28. **Kelly, K., B. H. Cochran, C. D. Stiles, and P. Leder.** 1983. Cell-cycle specific regulation of the c-myc gene by lymphocyte mitogens and platelet-derived growth factor. Cell **35**:603–610.
29. **Kelly, K. A., and P. M. Pitha.** 1985. Characterization of a mouse interferon gene locus. II. Differential expression of α-interferon genes. Nucleic Acids Res. **13**:825–839.
30. **Kilary, A. M., and R. E. K. Fournier.** 1984. A genetic analysis of extinction: trans-dominant loci regulate expression of liver-specific traits in hepatoma hybrid cells. Cell **38**:523–534.
31. **Larner, A. C., G. Jonak, Y. S. Cheng, B. Korant, E. Knight, and J. E. Darnell, Jr.** 1984. Transcriptional induction of two genes in human cells by beta interferon. Proc. Natl. Acad. Sci. USA **81**:6733–6737.
32. **Lengyel, P.** 1982. Biochemistry of interferons and their actions. Annu. Rev. Biochem. **51**:251–282.
33. **Maroteaux, L., C. Kahana, Y. Mory, T. Groner, and M. Revel.** 1983. Sequences involved in the regulated expression of the human interferon-β1 gene in recombinant SV40 DNA vectors replicating in monkey cells. EMBO J. **2**:325–332.
34. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **12**:7035–7056.
35. **Nir, U., B. Cohen, L. Chen, and M. Revel.** 1984. A human IFN β1 gene deleted of promoter sequences upstream from the TATA box is controlled post-transcriptionally by dsRNA. Nucleic Acids Res. **12**:6979–6993.
36. **Ohno, S., and T. Taniguchi.** 1983. The 5′ flanking sequence of human interferon-β1 gene is responsible for viral induction of transcription. Nucleic Acids Res. **11**:5403–5412.
37. **Raj, N. B. K., and P. M. Pitha.** 1983. Two levels of regulation of β-interferon gene expression in human cells. Proc. Natl. Acad. Sci. USA **80**:3923–3927.
38. **Schneiderman, S., J. L. Farver, and R. Baserga.** 1979. A simple method for decreasing the toxicity of polyethylene-glycol in mammalian cell hybridization. Somatic Cell Genet. **5**:263–269.
39. **Seghal, P. B., B. Dobberstein, and I. Tamm.** 1977. Interferon mRNA content of human fibroblasts during induction, shutoff and superinduction of interferon production. Proc. Natl. Acad. Sci. USA **74**:3409–3412.
40. **Southern, P., and P. Berg.** 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV-40 early region promoter. J. Mol. Appl. Genet. **1**:327–341.
41. **Stein, G., and R. Yanishevsky.** 1979. Autoradiography. Methods Enzymol. **43**:279–292.
42. **Stewart, W. E., II.** 1979. The interferon system. Springer-Verlag, New York.
43. **Stewart, W. E., II, L. B. Gosser, and R. Z. Lockart, Jr.** 1971. Priming: a nonantiviral function of interferon. J. Virol. **7**:792–801.
44. **Stewart, W. E., II, L. B. Gosser, and R. Z. Lockart, Jr.** 1972. The effect of priming with interferon on interferon production by two lines of L cells. J. Gen. Virol. **15**:85–87.
45. **Tan, Y. H., and W. Berthold.** 1977. A mechanism for induction and regulation of human interferon genetic expresiosn. J. Gen. Virol. **34**:401–412.
46. **Tomkins, G. M., B. Levinson, J. Baxter, and L. Dethlefsen.** 1972. Further evidence for posttranscriptional control of inducible tyrosine amino transferase synthesis in cultured hepatoma cells. Nature (London) New Biol. **239**:9–14.
47. **Wigler, M., R. Sweet, G.-K. Sim, B. Wold, A. Pellicer, E. Lacy, T. Maniatis, S. Silverstein, and R. Axel.** 1979. Transformation of mammalian cells with genes from procaryotes and eucaryotes. Cell **16**:777–785.
48. **Yamamoto, K., U. Gehring, M. Stampfer, and C. H. Sibley.** 1976. Genetic approaches to steroid hormone action. Recent Prog. Horm. Res. **32**:3–32.
49. **Zawatsky, R., E. De Maeyer, and J. De Maeyer-Guignard.** 1985. Identification of individual interferon-producing cells by in situ hybridization. Proc. Natl. Acad. Sci. USA **82**:1136–1140.
50. **Zinn, K., D. DiMaio, and T. Maniatis.** 1983. Identification of two distinct regulatory regions adjacent to the human β-interferon gene. Cell **34**:865–879.
51. **Zinn, K., P. Mellon, M. Ptashne, and T. Maniatis.** 1982. Regulated expression of an extrachromosomal human β-interferon gene in mouse cells. Proc. Natl. Acad. Sci. USA **79**:4897–4901.
52. **Zwarthoff, E. C., A. T. A. Mooren, and J. Trapman.** 1985. Organization, structure and expression of murine interferon alpha genes. Nucleic Acids Res. **13**:791–804.