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Alpha Interferon and Gamma Interferon Stimulate Transcription of a Single Gene through Different Signal Transduction Pathways

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Interferons (IFNs) play a key role in the defense against virus infection and the regulation of cell growth and differentiation, in part through changes in specific gene transcription in target cells. We describe several differences between the signal transduction events that result in transcriptional activation of the human gene coding for a guanylate-binding protein (GBP) by alpha interferon (IFN-α) and gamma interferon (IFN-γ). Activation by IFN-α was rapid, transient, and cycloheximide resistant. Activation by IFN-γ was slower, sustained, and delayed by cycloheximide. IFN-γ led to the formation of a stable intracellular signal which led to continued GBP transcription even if the ligand was withdrawn, whereas IFN-α-induced GBP transcription decayed rapidly if IFN-α was withdrawn. Perturbations of signaling pathways involving classical second messengers (cyclic AMP, Ca^{2+}, protein kinase C) did not induce GBP transcription. However, various kinase inhibitors blocked the transcriptional response to IFN-γ but not IFN-α, suggesting that a specific and possibly novel kinase is involved in gene activation by IFN-γ.

Many polypeptide cytokines (47, 49, 51) and growth factors (2, 12, 16, 24, 25, 31, 36, 37, 43, 44, 50) induce alterations in the expression of specific sets of genes in responsive cells. In some cases, extracellular polypeptides have been shown to induce transcriptional activation of target genes within minutes (2, 12, 16, 23–25, 31, 35–37, 43, 44, 50). Binding of growth factors to their cell surface receptors also results in immediate changes in the cytoplasmic levels of one or more second messengers (Ca^{2+}, cyclic AMP [cAMP], diacylglycerol), which are thought to act through specific protein kinases to produce intracellular responses, including altered transcription (56, 57). The strongest support for the idea that second messengers function physiologically in transcriptional regulation is that agents which artificially perturb intracellular second messenger levels can mimic the effects of growth factors on the transcription of certain genes (22, 26, 31, 55, 56). However, it is difficult to see how changes in the intracellular concentrations of only a few second messengers can generate the specific transcriptional responses that are elicited by the wide variety of ligands that exist in the developing and adult organism.

We have been examining the mechanisms of extracellular ligand-dependent gene activation by studying the events that follow interferon (IFN) treatment of human cells. The two types of IFN (type I or alpha and beta IFNs [IFN-α and -β] and type II or gamma IFN [IFN-γ]) bind to separate cell surface receptors and induce the expression of distinct but overlapping sets of genes (6, 47, 54, 60). We have recently demonstrated that the gene encoding a cytoplasmic guanylate-binding protein (GBP) (7–9) is activated within minutes by either IFN-α or IFN-γ in human fibroblasts (15). In this report, we describe several differences between the signal transduction events leading to the transcriptional response of this gene to IFN-α and IFN-γ in HeLa cells. Neither of these pathways appear to involve known second messengers.

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MATERIALS AND METHODS

Cells and reagents. HeLa S3 cells were obtained from the American Type Culture Collection, (Rockville, Md.) and grown to confluency in monolayer cultures in Dulbecco modified Eagle medium supplemented with 10% calf serum. Human IFN-α was a gift from P. Sorter (Hoffmann-La Roche, Inc., Nutley, N.J.), and human IFN-γ was a gift from D. Vapnek (Amgen). [α-^{32}P]UTP was from Dupont, NEN Research Products (Boston, Mass.). The kinase inhibitors H7, H8, and HA1004 were obtained from Seikagaku, Inc. All other chemicals were from Sigma Chemical Co. (St. Louis, Mo.).

Plasmid DNAs. Probes used in run-on assays were as follows: pGEM, pGEM-1 (Promega Biotec, Madison, Wis.); actin, chicken β-actin *Pst*I cDNA fragment (11) subcloned into pGEM-1; GBP, full-length 2.7-kilobase GBP cDNA cloned into pTZ18R (8); fos, 2.2-kilobase fragment of human c-*fos* gene cloned into pUC19 (pF4 [50]); tubulin, fragment of human β-tubulin pseudogene (64). γ-Actin (used to measure steady-state actin mRNA levels, see below) is a subcloned cDNA fragment of human γ-actin described in reference 17. GT7 (used to measure steady-state GBP mRNA levels) is a 138-base-pair *Hin*dIII-*Xba*I fragment of the GBP cDNA encoding amino acids 244 to 290 cloned into the respective sites of pGEM-1.

Measurement of transcription rate (run-on assay). Isolation of nuclei, elongation reactions in the presence of radiolabeled UTP, and nuclear RNA extraction were performed as described in reference 29 as modified in reference 35. One 150-mm plate (about 3×10^7 cells) of HeLa cells was used per sample and labeled with 200 μCi of [α-^{32}P]UTP. In general, one-half of the labeled RNA was hybridized to 5 μg of the relevant DNAs, which were immobilized on nitrocellulose with a slot-blot manifold. Autoradiographs were scanned, and densitometric units were calculated by subtracting the pGEM signal from the actin and GBP signals on the same filter, dividing the adjusted GBP signal by the adjusted actin signal, and normalizing as described in the figure legends. Although the degree of GBP induction by IFNs (relative to actin transcription) varied somewhat be-

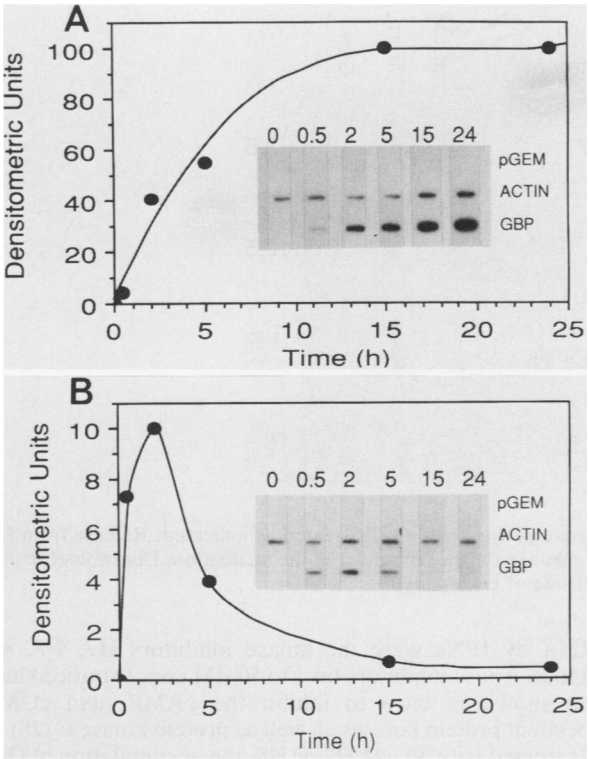

FIG. 1. Time course of induction of GBP transcription in HeLa cells. Run-on assays were performed on HeLa S3 cells treated for the indicated times with 100 U of IFN-γ per ml (A) or 500 U of IFN-α per ml (B). Densitometric units were normalized to the 15-h IFN-γ sample (= 100). Data from which the values were derived are shown in the insets.

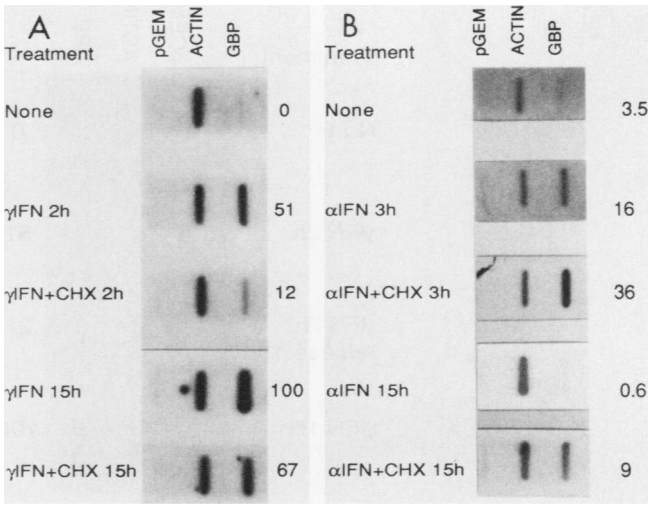

FIG. 2. Effect of cycloheximide (CHX) on induction of GBP transcription in HeLa cells. Run-on assays were performed on cells treated as indicated. Cotreatments with IFN and cycloheximide involved pretreating cells for 10 min with 50 μM cycloheximide followed by cotreatment with 50 μM cycloheximide and 100 U of IFN-γ per ml (A) or 500 U of IFN-α per ml (B) for the times indicated. Densitometric units normalized as described in the legend to Fig. 1 are shown on the right-hand side of each panel.

tween experiments, the time course of induction and the effects of drugs or IFN removal were highly reproducible.

Measurement of mRNA levels. Total RNA was prepared by the guanidinium isothiocyanate method (10). Labeled antisense RNA probes for γ-actin (SP6 RNA polymerase transcript from *Hin*fI-digested γ-actin) and GBP (T7 RNA polymerase transcript from *Hin*dIII-digested GT7) were generated in the presence of [α-^{32}P]UTP. RNA samples (10 μg) were probed simultaneously with labeled antisense γ-actin and GBP RNAs, and the amount of probe protected from T2 RNase digestion was measured (13, 42). Autoradiographs were scanned, and densitometric units were calculated by dividing the GBP signal by the actin signal on the same lane.

RESULTS

Distinct patterns of transcriptional induction of GBP by IFN-α and IFN-γ. Transcription of the GBP gene in HeLa S3 cells was detectable 30 min after IFN-γ addition (Fig. 1A) and continued to increase through 24 h. The maximum transcriptional activation obtained after IFN-γ treatment was 3 to 10 times greater than that after IFN-α treatment. IFN-α induced a much faster rise in GBP transcription, but the peak transcription was reached by 2 h and then declined by 15 h to a level close to that seen in uninduced cells (Fig. 1B). The rise and fall of the IFN-α-dependent transcription was identical to the response described for other interferon-stimulated genes (ISGs) (inducible exclusively by IFN-α) (34) as well as for GBP (15) in fibroblasts.

We tested whether preexisting proteins could mediate the IFN-induced increase of GBP transcription by using the protein synthesis inhibitor cycloheximide. Short (3-h) cotreatment of cells with IFN-α and cycloheximide resulted in a twofold-greater transcription of GBP, and longer exposure to IFN-α in the presence of cycloheximide prevented the normal decline in IFN-α-induced transcription (Fig. 2B), as observed previously in fibroblasts for GBP (15) and ISGs (34, 35). Thus, the proteins responsible for GBP induction by IFN-α preexist in HeLa cells before stimulation, and protein synthesis is required for secondary transcriptional repression.

In contrast, transcriptional induction upon a 2-h exposure to IFN-γ was inhibited to about 25% of control values by cycloheximide (Fig. 2A). However, after 15 h of IFN-γ and cycloheximide, GBP transcription rose to about 67% of the control level, indicating that cycloheximide affected the rate of transcriptional activation much more than its extent. Neither readdition of cycloheximide halfway through the incubation nor doubling the cycloheximide concentration (to 100 μM) altered the result (data not shown). Thus, unlike induction by IFN-α, the maximal rate of induction by IFN-γ requires the participation of cycloheximide-sensitive factors, perhaps proteins that are themselves IFN-γ inducible.

IFN-γ generates a stable intracellular signal. Another difference between the IFN-α and IFN-γ signaling pathways was the stability of the intracellular signals produced by each ligand. This was investigated by measuring GBP transcription after withdrawal of the ligands. Transcription of GBP remained elevated for an extended period after a 2-h exposure of cells to IFN-γ (Fig. 3A, 2-h IFN-γ, 13-h release), although transcription did not continue to increase as it did in the continuous presence of IFN-γ (Fig. 3A, 15-h IFN-γ). Thus, treatment with IFN-γ for 2 h resulted in the formation of a stable signal in the cells which persisted after IFN-γ withdrawal.

In contrast, GBP transcription stimulated by IFN-α was much less stable to withdrawal of the ligand. Upon removal of IFN-α after a 1-h treatment, GBP transcription declined to

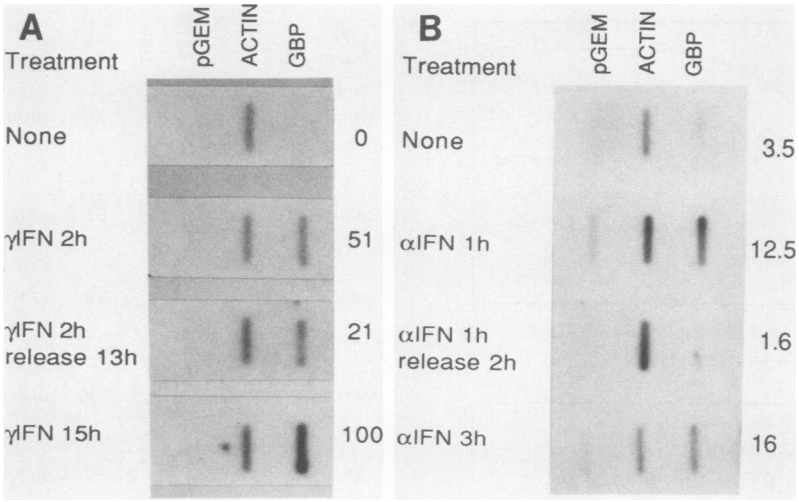

FIG. 3. Effect of IFN withdrawal on GBP transcription. Run-on assays were performed on cells treated as indicated. Release from IFN involved washing the cells once with 20 ml of DME and then adding IFN-free medium for the remainder of the incubation. Densitometric units normalized as described in the legend to Fig. 1 are shown on the right-hand side of each panel.

base line with 2 h (Fig. 3B). This decline was more rapid than the return to basal levels during the normal transcriptional cycle. To assess whether the transcriptional decline following IFN-α removal was due to a protein synthesis-dependent mechanism, as is the case in the continued presence of IFN-α (Fig. 2B), we performed the same experiment in the presence of cycloheximide. Although GBP transcription in the presence of IFN-α and cycloheximide continued for 15 h (Fig. 2B), withdrawal of the IFN-α after 1 h still resulted in a decay of GBP transcription to basal levels within the next 2 h (data not shown). Thus, IFN-α activation of intracellular components leading to GBP transcription is inherently unstable, while the activation brought about by IFN-γ is stable for many hours after IFN-γ removal.

Role of second messengers in GBP induction by IFNs. Effects of IFN treatment on intracellular Ca^{2+} (60), cAMP (45, 59), and diacylglycerol (66, 67) levels, as well as protein kinase C activity (27, 46, 60), have been described in various cell types. In an effort to determine whether pathways involving known second messengers might play a role in the induction of GBP by IFNs, we treated cells for 3 h with various agents known to affect these pathways (Table 1) and measured GBP mRNA levels. The level of γ-actin mRNA was used as a control. None of the treatments resulted in GBP mRNA levels above background (Table 1). This indicates that increased levels of cytosolic Ca^{2+} (row 3) or cAMP (rows 4 to 8) or activation of protein kinase C (row 9) are not sufficient to explain induction by IFNs.

Although not sufficient on their own, it is possible that the known second messengers act in conjunction with other signals produced by IFNs to activate GBP transcription. If this is the case, then artificial perturbation of second messenger levels might prevent normal induction of GBP transcription by IFNs. Cells were treated with IFN-α or IFN-γ together with various agents for 3 h, and the GBP RNA was analyzed as above. Induction of GBP mRNA by IFN-α or IFN-γ was not significantly affected by removal of extracellular calcium (Table 1, row 2) or by artificially raising the intracellular levels of calcium (row 3) or cAMP (rows 4 to 8). Activation of protein kinase C (row 9) and ADP-ribosylation of G proteins (rows 7 and 8) were also without effect. The only agents that significantly affected induction of GBP mRNA by IFNs were the kinase inhibitors H7, H8, and HA1004. These inhibitors (at 1 to 50 μM concentration) have been shown in vitro to inhibit the cAMP- and cGMP-dependent protein kinases as well as protein kinase C (28). In cells treated with 50 μM H7 or H8, the accumulation of GBP mRNA was completely inhibited, while some residual induction (15 to 25% of control) was still visible in the presence of 50 μM HA1004. No changes in the levels of γ-actin mRNA occurred under these conditions.

Transcriptional induction by IFN-γ but not IFN-α is blocked by kinase inhibitors. To determine whether the observed lack of GBP mRNA accumulation reflected inhibition of transcriptional induction or destabilization of GBP RNA after normal induction, we performed run-on transcription assays on cells treated for 2 h with IFN-α or IFN-γ in

TABLE 1. Effect of perturbing classical second messenger signaling pathways on GBP mRNA levels[a]

Agent	Concn	mRNA accumulation		
		Basal	IFN-γ	IFN-α
None		−	+++	+++
EGTA	5 mM	−	+++	+++
A23187	10 μM	−	++	+++
8-Br-cAmp	1 mM	−	+++	++
Dibutyryl cAMP	1 mM	−	++	++
Forskolin	50 μM	−	++	+++
Cholera toxin	1 μg/ml	−	++	+++
Pertussis toxin	0.1 μg/ml	−	+++	++
PMA	0.1 μm/ml	−	+++	+++
H7	50 μM	−	−	−
H8	50 μM	−	−	−
HA1004	50 μM	−	+	+

[a] Cells were pretreated for 10 min with the indicated agents at the final concentrations shown. IFN was then added (100 U of IFN-γ per ml; 500 U of IFN-α per ml) or not added (Basal) for a further 3 h. RNA was extracted and analyzed as described in Materials and Methods. +++, 70 to 100% of the level of GBP RNA present during normal induction; ++, 40 to 70% of the level of GBP RNA present during normal induction; +, 10 to 40% of the level of GBP RNA present during normal induction; −, <10% of the level of GBP RNA present during normal induction. The basal level of GBP RNA is low but somewhat variable. In one experiment in which this level was clearly detectable, the maximum induction observed with any of the above agents was 1.6-fold, whereas on that occasion IFN stimulation was 15- to 20-fold.

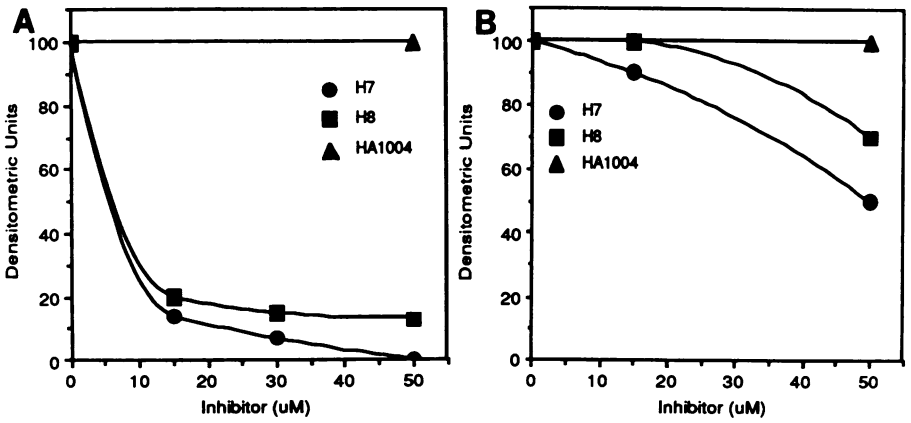

FIG. 4. Effect of the kinase inhibitors H7, H8, and HA1004 on induction of GBP transcription by IFNs. Cells were pretreated for 10 min with the indicated concentrations of the inhibitors and then treated with 100 U of IFN-γ per ml (A) or 500 U of IFN-α per ml (B) for 2 h in the continued presence of the inhibitors. Densitometric units normalized to the signals obtained in the absence of inhibitors are plotted against inhibitor concentration.

the presence of different concentrations of H7, H8, and HA1004. H7 and H8 did inhibit transcriptional induction of GBP by IFN-γ but had almost no effect on induction of transcription by IFN-α (Fig. 4). HA1004 did not affect transcriptional induction by either IFN. Thus, transduction of the signal produced by IFN-γ to activate transcription may well involve the activity of a kinase that is sensitive to H7 and H8, while transduction of the IFN-α signal does not. In addition, the GBP RNA transcribed in response to IFN-α (Fig. 4) does not accumulate in cells exposed to the inhibitors (Table 1), suggesting the involvement of a distinct kinase (also sensitive to HA1004) in a posttranscriptional event.

Inhibition of cellular responses by H7 has been interpreted as evidence for the involvement of protein kinase C in the transduction of a stimulus (5, 19). Although treatment of HeLa cells with phorbol myristate acetate (PMA) (which activates protein kinase C in these cells) did not induce GBP transcription, it remained possible that protein kinase C activity was necessary for IFN-γ induction of GBP and that the inhibition observed with H7 and H8 was due to inhibition of protein kinase C. Transcription of the c-*fos* gene is increased by the protein kinase C agonist PMA in HeLa cells (22). We tested the effects of H7 and H8 on the induction of c-*fos* gene transcription by PMA to determine whether the concentrations of H7 and H8 which inhibited IFN-γ induction of GBP transcription were also sufficient to inhibit a transcriptional induction that was presumably mediated by protein kinase C. A 15-min treatment with PMA (100 ng/ml) caused a 15- to 30-fold induction of c-*fos* transcription; however, this increase was completely unaffected by treatment of cells with 50 μM H7 or H8 (Fig. 5). The same result was obtained if the cells were preincubated with the inhibitors for 2 h before PMA treatment (data not shown), ruling out the trivial explanation that the inhibitors did not have time to enter the cells during the brief incubation with PMA. Thus, c-*fos* induction by PMA in these cells was unaffected by concentrations of the inhibitors which abolished induction of GBP by IFN-γ, suggesting that IFN-γ operates through a kinase much more sensitive to H7 and H8 than protein kinase C.

The inhibition of the transcriptional response to IFN-γ by kinase inhibitors might reflect a role for the kinase either in generating the stable intracellular signal in response to IFN-γ (Fig. 3) or in translating that signal into GBP transcription, or both. To distinguish between these possibilities, we examined the effect of adding H7 to cells which had already been exposed to IFN-γ for 2 h and had thus generated the stable signal. The kinase inhibitor was still able to block GBP transcription (Fig. 6), suggesting that the kinase is necessary for the maintenance or utilization of the stable signal.

2-Aminopurine selectively blocks GBP induction by IFN-γ. Several genes induced by IFNs are also induced to some extent by double-stranded RNA (30, 62), indicating that the signal transduction pathways activated by these agents may have elements in common. In addition, similarities have been noted between the promoter elements conferring responsiveness to these agents (18, 30, 63). Recently, on the basis of inhibition by 2-aminopurine, three groups of investigators (41, 61, 68) have suggested that a kinase is involved in gene induction by double-stranded RNA. We tested the effect of 2-aminopurine on induction of GBP transcription by IFN-α and IFN-γ (Fig. 7). Induction by IFN-γ was completely blocked by the inhibitor, while induction by IFN-α was only two- to threefold decreased when the signal was normalized to actin transcription.

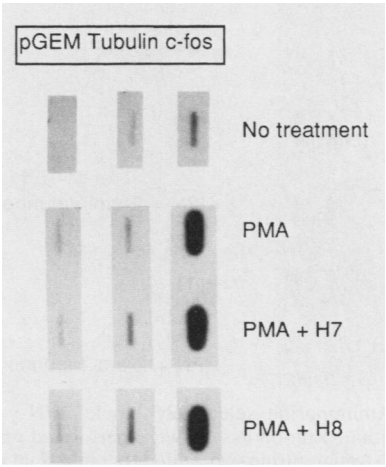

FIG. 5. H7 and H8 do not affect induction of c-*fos* transcription by PMA. Run-on assays were performed on cells pretreated for 10 min with 50 μM H7 or H8 as indicated and then with 100 ng of PMA per ml for 15 min.

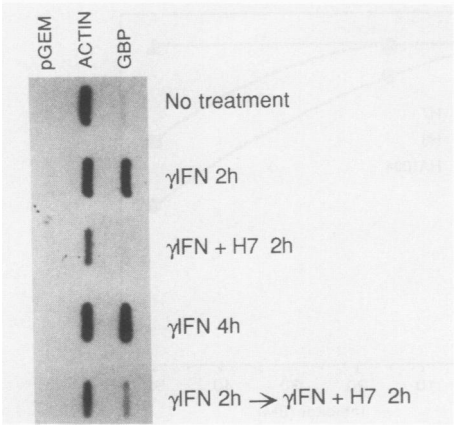

FIG. 6. H7 is able to block IFN-γ-induced GBP transcription even after IFN-γ pretreatment. Run-on assays were performed on cells treated as indicated. IFN-γ was added at 100 U/ml, and H7 was added at 50 μM.

DISCUSSION

Differential regulation of GBP gene by IFN-α and IFN-γ in HeLa cells. IFN-α and IFN-γ were originally named because they induce similar biological effects (antiviral state and slowed growth) in some cells. It is now clear, however, that these two ligands have many distinct, as well as overlapping, effects on cells (e.g., on gene expression [54]). In principle, genes responding to both types of IFN might reflect the existence of shared components between the IFN-α and IFN-γ response pathways. Alternatively, these genes might possess the ability to respond to two completely separate induction pathways. A previous study (6) on the doubly inducible C5-4 gene in fibroblasts described differences (analogous to findings ii and iii below) between the transcriptional induction of this gene by IFN-α and IFN-γ. We found several differences in the pattern of GBP transcription after treatment with the two IFN types, indicative of very different response pathways. (i) Induction by IFN-α is more rapid than induction by IFN-γ. (ii) Induction by IFN-α is followed by transcriptional repression, whereas induction by IFN-γ leads to a sustained response. (iii) IFN-α-induced transcription is increased in the presence of cycloheximide, while IFN-γ-induced transcription is partially inhibited. (iv) IFN-γ-induced transcription is stable to IFN-γ-withdrawal, while IFN-α-induced transcription decays rapidly upon IFN-α withdrawal. (v) Induction by IFN-γ is much more sensitive to various kinase inhibitors than is induction by IFN-α.

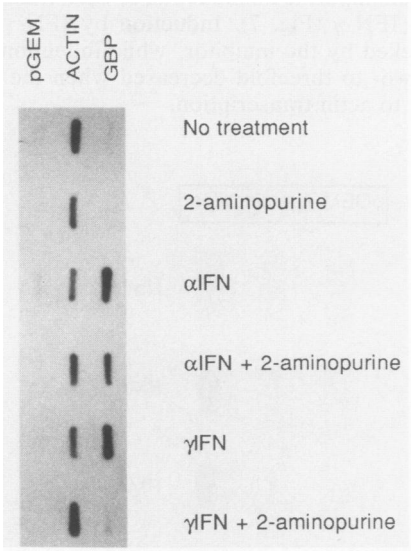

FIG. 7. 2-Aminopurine selectively blocks IFN-γ induction of GBP transcription. Run-on assays were performed on cells treated as indicated. 2-Aminopurine was added to 9 mM final concentration from a 180 mM aqueous stock (boiled to dissolve the 2-aminopurine shortly before use). Cells were pretreated with the inhibitor for 10 min before adding IFN (100 U of IFN-γ per ml, 500 U of IFN-α per ml).

Reid and colleagues (53) recently reported that a single promoter element was responsible for mediating both IFN-α and IFN-γ induction of another doubly inducible gene, 9-27. This element, the interferon-stimulated response element (ISRE), was originally described in a number of genes responding only to IFN-α and has been shown to mediate induction of several of these genes by IFN-α (38, 39, 48, 52). We have recently cloned the promoter of the GBP gene and found that it, too, contains an ISRE. A site-directed deletion mutant missing the central nine bases of the ISRE abolished the IFN inducibility of a GBP promoter construct in transient transfection assays, demonstrating that the ISRE is required for both IFN-α and IFN-γ induction of the GBP gene as well (unpublished data). The pattern of induction of GBP in response to IFN-α in both fibroblasts (15) and HeLa cells (this report) is identical to the pattern of induction of the exclusively IFN-α-responsive ISGs. It is very probable, therefore, that this induction is mediated by the ISRE-binding factor, ISGF-3, which has been implicated in ISG regulation (14, 32, 33, 38). However, ISGF-3 is not induced upon IFN-γ treatment (15), suggesting that another factor(s) must mediate IFN-γ induction of GBP. A detailed mutagenic analysis of this region of the promoter will be required to determine whether these factors recognize the same features of the ISRE as ISGF-3 or a distinct overlapping site. Thus, we believe that IFN-α and IFN-γ employ distinct induction pathways which, at least in the case of GBP, only converge at the target promoter.

An intriguing difference between the induction of GBP by IFN-α and that by IFN-γ was the effect of IFN removal upon GBP transcription. Continued transcription after IFN-α addition required the continued presence of IFN-α at the cell surface. IFN-α removal also caused a rapid decline in the abundance of nuclear ISGF-3 (D. E. Levy and D. J. Lew, unpublished data). GBP transcription declined after a few hours even in the continued presence of IFN-α, but this decline was slower and required protein synthesis, while the decline after IFN-α withdrawal did not. Thus, it seems that there is no stable intracellular signal generated by IFN-α. In contrast, GBP transcription after IFN-γ treatment remained elevated even if IFN-γ was withdrawn. Bound IFN-γ is released within 2 h after washing HeLa cells with fresh medium (58), so it is unlikely that the long-term maintenance of transcription is due to residual bound IFN-γ. The nature of the stable intracellular signal generated by IFN-γ is unknown and could be anything from a stably activated conformation of the IFN-γ receptor to a stable transcription complex on the GBP promoter.

Involvement of classical second messengers in response to IFNs. A battery of agents that perturb classical second messenger signaling pathways were completely ineffective in stimulating GBP expression. Similar experiments (65), including combined treatments with agents perturbing more

than one pathway, also failed to induce another IFN-regulated gene, 2',5'-oligoadenylate synthetase, in mouse cells. These results suggest that these pathways are not sufficient to explain gene regulation by IFNs, although we cannot eliminate the possibility that specific combinations of the second messengers with appropriate subcellular distributions could be responsible for the IFN response.

We addressed the possibility that the classical second messenger pathways, while not sufficient in themselves, play a part in IFN induction by examining the effect of various agents on GBP induction by IFN-α and IFN-γ. With the exception of the kinase inhibitors (discussed below), none of these agents had a greater than twofold effect on GBP mRNA accumulation. In some cases, this small effect proved to be posttranscriptional. Thus, we found no evidence to support a role for classical second messengers in transcriptional induction by IFNs.

IFN-γ induction of GBP transcription requires activity of a kinase. The kinase inhibitors H7, H8, and 2-aminopurine all abolished transcription of GBP in response to IFN-γ, while having relatively little effect on IFN-α-induced transcription. These observations suggest the involvement of a specific protein kinase in the response to IFN-γ. At present, it is unclear whether IFN-γ stimulates a kinase activity as part of a signal transduction pathway or whether the constitutive activity of a kinase is required to allow signal transduction upon IFN-γ stimulation. The inhibitors are able to block transcription even after a stable intracellular signal has been set up by pretreatment with IFN-γ, showing that the kinase is required to transform this signal into GBP transcription. However, the block under these circumstances is not quite as complete as when the inhibitors are present from the beginning, suggesting that inhibition of the kinase may affect generation of the signal as well.

The identity of the kinase(s) remains to be elucidated. It is unlikely that the IFN-γ receptor itself is a kinase, as the sequence of a IFN-γ receptor clone bears no homology to known protein kinases (1). The concentrations of H7 and H8 which abolished transcriptional induction of GBP by IFN-γ had no effect on induction of c-*fos* transcription by the protein kinase C agonist PMA, implicating a kinase other than protein kinase C in the IFN-γ response. These results, combined with those discussed in the previous section, suggest that the kinase involved in the response of GBP to IFN-γ is not linked to the known second messenger pathways.

2-Aminopurine has been shown to inhibit the double-stranded RNA-dependent protein kinase in vitro (21) and also blocks gene induction by double-stranded RNA (41, 61, 68). It is attractive to speculate that 2-aminopurine inhibition of gene induction by IFN-γ and double-stranded RNA reflects the involvement of the same kinase in these two signal transduction pathways. However, 2-aminopurine has pleiotropic effects on cells (68), and further experiments will be required to determine whether the inhibition of a single kinase is responsible for the various effects of 2-aminopurine and whether the double-stranded RNA-dependent kinase is involved in the IFN-γ pathway.

An additional result of the studies with kinase inhibitors was that H7 and H8, and to a lesser extent HA1004, prevented GBP mRNA accumulation in response to IFN-α even though they did not block transcriptional activation of GBP. A recent report (19) on the induction of mRNA accumulation for another gene, γ.1, by IFN-γ in U937 cells also described an inhibition by H7. However, these investigators did not test whether transcriptional or posttranscriptional events were affected.

Cell type and gene specificity of the IFN-γ response. While the pattern of induction of transcription by IFN-α is similar for many genes in several cell types, a much greater variability is observed in the IFN-γ response. The transcriptional response of GBP to IFN-γ described here in HeLa cells differs from that described in fibroblasts (15), where IFN-γ-induced GBP transcription peaked at 2 h and was followed by a slow decline. In addition, transcriptional induction in fibroblasts was resistant to the protein synthesis inhibitor cycloheximide, whereas we showed here that IFN-γ induction of GBP in HeLa cells is significantly delayed by cycloheximide. Whether these differences reflect fluctuations in the abundance of response machinery components between cell types or major differences in the way in which different cells respond to IFN-γ remains an open question.

Great variability is also apparent when the induction patterns of different genes in response to IFN-γ are compared. The speed of the response varies from extremely rapid (induced by 5 min [20]) to very slow (first seen 8 h after IFN-γ addition [3, 4]). The response can be transient (20, 40) or sustained for several days (3, 4, 6, 15). Protein synthesis may be required for induction (3, 4, 6), unnecessary (15, 20), or partially required (this report). These differences are evident even when comparing the response of different genes within one cell type (e.g., GBP versus HLA-DRα [4] in HeLa cells). Therefore, it appears that there are several different ways in which IFN-γ may cause changes in gene expression in any particular cell type.

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