MOLECULAR AND CELLULAR BIOLOGY, Oct. 1988, p. 4289–4294
0270-7306/88/104289-06$02.00/0

Vol. 8, No. 10

Gene Induction by Interferons and Double-Stranded RNA: Selective Inhibition by 2-Aminopurine

RAJ K. TIWARI, JYOTIRMOY KUSARI, RAKESH KUMAR, AND GANES C. SEN†*

Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Received 26 May 1988/Accepted 27 July 1988

Transcription of several interferon-inducible human genes is also induced by double-stranded RNA. The nature and the mechanism of action of signals generated by interferons and by double-stranded RNA which mediate the induction of these genes are under investigation. Here we report that 2-aminopurine, a known inhibitor of protein kinases, could selectively block this induction process. Induction of mRNAs 561 and 6-16 in HeLaM cells by double-stranded RNA was completely inhibited by 10 mM 2-aminopurine, whereas cellular protein and RNA syntheses as well as the induction of metallothionein mRNA by $CdCl_2$ were unaffected by this inhibitor. In addition, 2-aminopurine blocked the induction of the same two mRNAs and of mRNAs 2-5(A) synthetase, 2A, and 1-8 by alpha interferon and of mRNAs 2A and 1-8 by γ interferon in HeLaM cells. The observed inhibition was at the level of transcription, and for establishing efficient inhibition, the 2-aminopurine treatment had to begin at early stages of interferon treatment. In GM2767 cells, 2-aminopurine inhibited induction of mRNAs 561 and 6-16 by double-stranded RNA but not by alpha interferon. These results suggest that double-stranded RNA-induced signal 2 is distinct from the interferon-α-induced signal 2 (R. K. Tiwari, J. Kusari, and G. C. Sen, EMBO J. 6:3373–3378, 1987) and that 2-aminopurine can block the former but not the latter. Moreover, it appeared that 2-aminopurine could block the production of signal 1 by interferons. This was confirmed by experiments in which we separately tested the effects of 2-aminopurine on signal 1 and signal 2 production by interferons in HeLaM cells. Although no direct experimental evidence is available as yet, our results are consistent with the hypothesis that the functioning of a protein kinase activity may be necessary for transcriptional induction of genes by double-stranded RNA and for gene induction by interferons in those cells in which signal 1 production is needed.

Interferons (IFNs) have a variety of biological actions, including the ability to render cells resistant to viral infection (1, 19, 25, 35). All of these actions of IFNs are mediated by the products of IFN-inducible genes (29). The regulation of expression of many of these genes is being studied in various laboratories (3, 8, 17, 20, 24, 30, 32, 34, 36, 40).

We have been studying the regulation of expression of several IFN-α-inducible genes in HeLaM cells (14–16, 38; G. C. Sen, R. K. Tiwari, R. Kumar, and J. Kusari, *In* H. Moses, P. Lengyel, and C. Stiles (ed.), *Proceedings of the UCLA Symposium on Growth Inhibitory and Cytotoxic Polypeptides*, in press). These studies have revealed unexpected complexities in the regulatory processes. The HeLaM cells are unusual in the sense that ongoing protein synthesis is required for induction by IFN-α of all the mRNAs that have been tested. We have proposed a working model for induction of these genes which can accommodate all our experimental observations. IFN-α produces a signal, signal 1, which enhances the synthesis of a mRNA encoding a putative protein, protein X; IFN-α also produces signal 2, whose functioning does not need continued protein synthesis. Protein X and signal 2 together are needed for transcriptional induction of the various IFN-inducible mRNAs for which hybridization probes are available. In other cells, production of signal 2 is sufficient for induction of these genes presumably because these cells have protein X as a constitutive protein; consequently, ongoing protein synthesis is not needed for IFN-α-induced gene transcription in these cells.

Our recent studies (38) have shown that signals which are functionally equivalent to IFN-α-induced signal 1 or signal 2 can be produced by other agents as well. IFN-γ can produce signal 1 but not signal 2. Similarly, epidermal growth factor or platelet-derived growth factor can produce signal 1 but not signal 2. On the other hand, double-stranded RNA (dsRNA) in the form of polyinosinic acid-polycytidylic acid [poly(I) · poly(C)] or of vesicular stomatitis virus defective interfering particle 011 (VSV DI-011) can produce signal 2, leading to induction of IFN-inducible genes, such as 561 and 6-16. Signal 1 produced by any agent can functionally complement signal 2 produced by another agent in HeLaM cells.

The revelation that at least some of the IFN-inducible genes can be directly induced by dsRNA as well has been unexpected (38, 39, 43; Sen et al., in press). It has brought the fields of study of regulation of IFN-inducible genes and that of IFN genes themselves closer. It is apparent now that there are remarkable similarities between the two processes. IFN genes, like some of the IFN-inducible genes, are transcriptionally induced by dsRNA or by virus infection (41). Moreover, both induction processes exhibit the priming effect. For IFN biosynthesis, it has long been known that dsRNA cannot induce IFN in certain cell lines unless they have been pretreated or 'primed' with a low dose of IFN (37). The priming is unnecessary for other lines. Protein synthesis is necessary for manifestation of the priming effect, and the putative priming protein is *trans*-acting and dominant in human-mouse cell hybrids (6). Our observations about the requirements for IFN-inducible gene expression in HeLaM cells parallel the above description. Characteristics of signal 1 production are very similar to those needed for priming of induction of IFN-β_1 gene, and signal 2 generated

* Corresponding author.
† Permanent address: Department of Molecular Biology, Cleveland Clinic Foundation, Cleveland OH 44106.

by dsRNA can induce either the IFN-β_1 gene or the 561 and 6-16 genes, which were originally identified as IFN-inducible genes.

cis-acting sequences necessary for inducibility of IFN genes have been analyzed extensively (9, 10, 13, 27). Recently, several groups have defined the corresponding sequences necessary for imparting IFN inducibility to a gene (2, 4, 19a, 26, 28, 31). Further analyses of these *cis*-acting sequences are being carried out now. Examination of the primary sequences of the regulatory regions of the IFN-β_1 gene and those of 561, 6-16, and other IFN-inducible genes revealed the presence of sequence motifs of striking similarity (26, 39; Sen et al., in press). However, it remains to be shown that these common sequence motifs are indeed necessary and sufficient for induction of all of these genes by dsRNA. Moreover, the contribution of these motifs toward inducibility by IFN remains to be evaluated.

There are *trans*-acting protein factors which bind to the above-mentioned *cis*-acting sequences. For IFN-inducible genes, the presence of several such proteins has been suggested (4, 19a, 26, 31). In the case of the IFN-β_1 gene, the interactions between the *trans*-acting factors and the regulatory elements have been studied more extensively. There is evidence for binding of both positive and negative regulatory proteins to defined regions of this gene (13).

It is presumed that some signals generated by dsRNA cause alterations in the binding characteristics of these regulatory proteins to the target genes, thereby inducing their transcription. The nature and the mode of transmission of these putative signals are however unknown. Marcus (21) proposed that cellular proteins which have high affinity for dsRNA may participate in this process. Two such proteins have been described in the IFN system: 2′-5′-oligoadenylate synthetase and a dsRNA-dependent protein kinase (19, 25, 35). This protein kinase seems to be an attractive candidate for dsRNA-mediated signal transduction: it is present constitutively in detectable quantities in many cell lines. Moreover, protein phosphorylation is a common biochemical mechanism by which functions of many regulatory proteins are modulated in nature.

Here we report the results of a series of experiments which are consistent with the notion of participation of a protein kinase in dsRNA-mediated signal transduction. 2-Aminopurine (2AP), a known inhibitor of the dsRNA-dependent protein kinase (5, 7, 12, 18), selectively inhibited induction of genes 561 and 6-16 by dsRNA. These results are completely consistent with recent reports that induction of IFN by dsRNA is also inhibited by 2AP (23, 42; P. I. Marcus and M. J. Sekellick, Abstr. VIIth Int. Congr. Virol., p. 198). In addition, our experiments demonstrate that 2AP can completely abrogate gene induction by IFNs in those cells in which signal 1 production is necessary for this process.

MATERIALS AND METHODS

Materials. Cells and their culture conditions and the sources of interferons, VSV DI-011, and the cDNA clones have been described before (14, 16, 22, 38). 2AP was purchased from Sigma Chemical Co., St. Louis, Mo. Stock solutions (150 mM) were prepared in phosphate-buffered saline containing glacial acetic acid (200:1, vol/vol). For dissolving the drug, it was necessary to heat the solution to 60°C and to shake it periodically. After dissolution, the stock was aliquoted and kept frozen. Each aliquot was thawed, heated, and mixed just before use (23).

Methods. Procedures for IFN treatment and virus infection were described before (38). Cytoplasmic RNA was isolated from frozen cells which were disrupted with nonionic detergent, followed by removal of nuclei, digestion with proteinase K, and extraction with phenol-chloroform (14). Three twofold serial dilutions of these RNA samples were used for dot blot analysis (14). Probes were gel-purified inserts of various cDNA clones which were radiolabeled by nick translation before hybridization. For quantitation of autoradiograms by densitometric scanning, care was taken to ensure linearity of response as described before (14). Nuclear run-on transcription assays were performed by procedures described before (15). The cDNA clones used as probes were linearized before immobilization on filters. A plasmid DNA without insert was used as a negative control.

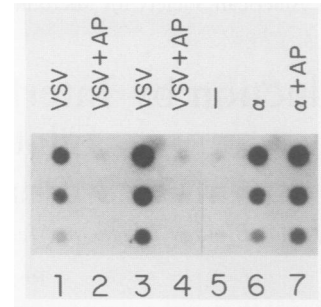

FIG. 1. Effects of 2AP on induction of mRNA 561 in GM2767 cells. Confluent monolayers of GM2767 cells were treated with 500 U of IFN-α per ml or with 10 mM 2AP and infected with VSV DI-011. After 6 h, the cells were harvested, cytoplasmic RNA was extracted, and the levels of mRNA 561 were estimated by dot blot analysis. RNA samples (8, 4, and 2 μg from top to bottom) were analyzed. Lanes: 1 and 2, infection at a multiplicity of infection of 10; 3, and 4, infection at a multiplicity of infection of 100; 5, untreated.

RESULTS

2AP blocked gene induction by dsRNA. As a means of supplying dsRNA to the cells, infection with the VSV DI-011, which has a dsRNA genome, is very effective (22, 38). Several IFN-inducible mRNAs are induced well in human fibroblasts (GM2767) upon infection with VSV DI-011. We have reported (38) that such induction does not need either ongoing protein synthesis or pretreatment with IFN. Induction of mRNA 561 by VSV DI-011 infection was completely blocked by including 10 mM 2AP in the culture medium (Fig. 1). The same was true for induction of mRNA 6-16 (data not shown). However, 2AP did not block induction of these mRNAs by IFN-α in GM2767 cells (Fig. 1, lanes 6 and 7 and data not shown). Under the conditions of this experiment, 2AP did not interfere with penetration and replication of VSV in these cells (23; data not shown). These results suggest that the signals produced by dsRNA and by IFN-α, which are responsible for mRNA 561 induction, are different in their sensitivities to the inhibitory action of 2AP.

Induction of mRNA 561 in HeLaM cells needs production of two signals (14, 38), and dsRNA can provide only signal 2. When signal 1 was provided by IFN-γ, VSV DI-011 (22, 38) could induce mRNA 561 effectively in these cells (Fig. 2, lane 4), and this induction was completely blocked by 2AP if it was present throughout the induction process (lane 3). The induction process was also inhibited severely if 2AP was present during either signal 1 production by IFN-γ (lane 5) or signal 2 production by dsRNA (lane 6).

Induction of gene expression by IFNs was blocked by 2AP in HeLaM cells. Results shown in Fig. 2 suggest that 2AP

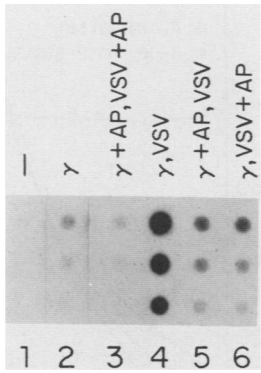

FIG. 2. Effects of 2AP on induction of mRNA 561 in HeLaM cells by VSV DI-011. mRNA 561 levels were determined as described in the legend to Fig. 1. Cells were treated with 500 U of IFN-γ per ml and with 10 mM 2AP for 6 h or infected for 6 h with VSV DI-011 at a multiplicity of infection of 100. Lanes: 1, no treatment, 2, IFN-γ; 3, IFN-γ and 2AP followed by VSV and 2AP; 4, IFN-γ followed by VSV; 5, IFN-γ and 2AP followed by VSV; 6, IFN-γ followed by VSV and 2AP.

blocks induction of genes by IFN in HeLaM cells. Indeed, this was the case for all mRNAs tested, including 561, 6-16, 2-5(A) synthetase, 1-8, and 2A. 2AP completely blocked induction of mRNAs which are rapidly inducible by IFN-α in these cells, e.g., mRNA 561 (Fig. 3, lanes 1 to 3), mRNA 6-16, and mRNA 2-5(A) synthetase (data not shown). Slower induction of mRNAs 1-8 and 2A by IFN-α was also blocked by 2AP (Fig. 3, lanes 4, 5, and 8). Induction of the latter two mRNAs by IFN-γ was blocked by 2AP as well (lanes 6, 7, and 8).

Characteristics of 2AP-mediated inhibition of induction. The extent of inhibition by 2AP was dependent on the concentration of the inhibitor in the culture medium. A low level of inhibition (about 20%) was observed even with 0.1 mM 2AP (data not shown). But for attaining the maximum inhibition, a high 2AP concentration (5 to 10 mM) was

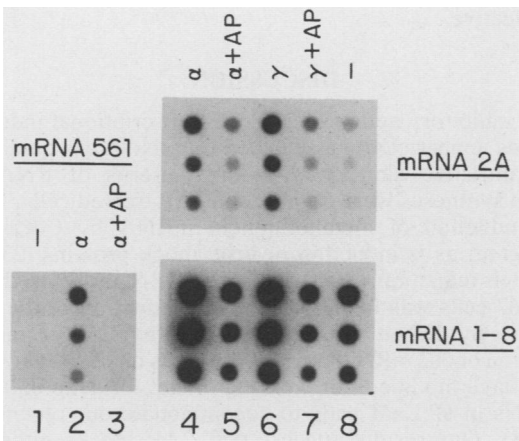

FIG. 3. Effects of 2AP on induction of mRNAs 561, 2A, and 1-8 by IFN-α and IFN-γ in HeLaM cells. IFN-α or IFN-γ (500 U per ml) and 10 mM 2-aminopurine were used as indicated. In lanes 1 to 3, induction of mRNA 561 was measured; cells were untreated (lane 1) or treated with IFN-α for 6 h (lane 2) or IFN-α and 2AP for 6 h (lane 3). In lanes 4 to 8, induction of mRNA 2A (top) and of mRNA 1-8 (bottom) was measured; cells were treated with IFN-α for 18 h (lane 4), IFN-α and 2AP for 18 h (lane 5), IFN-γ for 18 h (lane 6), or IFN-γ and 2AP for 18 h (lane 7) or left untreated (lane 8).

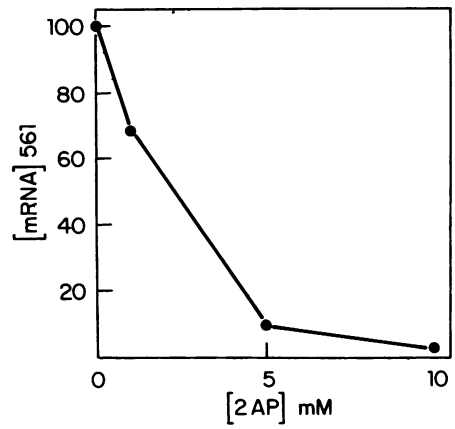

FIG. 4. Effects of increasing concentrations of 2AP on induction of mRNA 561 in HeLaM cells by IFN-α. Cells were treated with 500 U of IFN-α per ml and the indicated doses of 2AP for 6 h. Dilutions of stock 2AP were prepared in the same buffer as the stock in such a way that each plate received the same volume of the buffer. mRNA 561 levels were estimated by dot blot analyses, the autoradiograms were quantitated by densitometric scanning, and the level of mRNA 561 in cells treated with IFN-α alone was assigned an arbitrary value of 100.

necessary (Fig. 4). This concentration is in complete agreement with the optimum concentration of 2AP needed in other systems (12, 23, 42).

At the highest concentration tested (10 mM), 2AP did not exert any gross adverse effects on the cells. Cellular protein synthesis, total RNA synthesis, and poly(A)$^+$ RNA synthesis were unimpaired in 2AP-treated cells. These were tested by pulse-labeling 2AP-treated cells with [^{35}S]methionine or with [^{3}H]uridine (data not shown). Moreover, induction of metallothionein mRNA by $CdCl_2$ was unaffected by 2AP, demonstrating that all inducible mRNAs were not affected by this drug (data not shown). These data and the observations that 2AP did not inhibit induction of mRNA 561 by IFN-α in GM2767 cells (Fig. 1) and of mRNA 202 (32) by IFN-β in mouse L929 cells (data not shown) indicate that the observed effect of 2AP in HeLaM cells is selective and cell specific.

Production of signal 1 by IFN-α was blocked by 2AP. According to our working model, the only known special characteristic of HeLaM cells which is relevant to gene induction by IFNs is that they need production of signal 1, which leads to synthesis of the putative protein, protein X. For this reason, induction of mRNA 561 and of other mRNAs by IFN-α in HeLaM cells needs ongoing protein synthesis. This need is observed only at the beginning of the induction process, when, presumably, protein X is being synthesized.

The inhibition of mRNA 561 induction by 2AP was most marked during the early stage of the induction process (Fig. 5). In the experiment shown in Fig. 5, 2AP was added at various times after IFN addition, cells were harvested after 6 h, and the levels of mRNA 561 were estimated. When 2AP was added between 0 and 3 h after IFN treatment, the induction was completely blocked. These results suggest that 2AP might be blocking induction or functioning of signal 1.

The experiment shown in Fig. 6 demonstrates that production of signal 1 but not of signal 2 in HeLaM cells was inhibited by 2AP. When 2AP was present during IFN-α treatment, little mRNA 561 was induced (lanes 4 and 5).

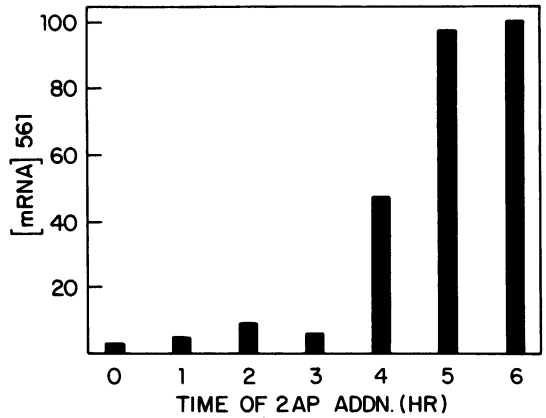

FIG. 5. Effects on mRNA 561 induction of addition of 2AP at different time points after the beginning of IFN-α treatment. HeLaM cells were treated with 500 U of IFN-α per ml for 6 h. 2AP (10 mM) was added to the culture medium at the indicated time after IFN-α treatment began. Levels of mRNA 561 were measured as described in the legend to Fig. 4.

Similarly, if 2AP was present during pretreatment with IFN-γ, during which protein X is synthesized, mRNA 561 was induced poorly (lane 3). However, the presence of 2AP during signal 2 production by IFN-α did not inhibit induction of mRNA 561 (lane 6). The above results suggest that signal 1 production by either IFN-α or IFN-γ in HeLaM cells is blocked by 2AP but signal 2 production by IFN is not blocked. As a result 2AP is ineffective in blocking mRNA induction by IFN-α in cells, such as GM2767, in which signal 2 production is sufficient.

2AP-mediated block was at the level of transcription. In all the experiments described so far, induction of different mRNAs was assayed by measuring their steady-state cytoplasmic levels. To examine whether the block mediated by 2AP is at the level of transcription or at the level of posttranscription processing or of turnover, the experiment shown in Fig. 7 was done. The rates of transcription of four

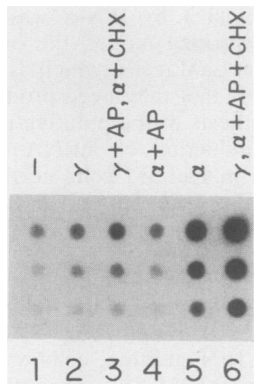

FIG. 6. Effects of 2AP on the production of signal 1 and signal 2 by IFNs in HeLaM cells. Signal production was measured by the level of induction of mRNA 561. IFN (500 U per ml), 2AP (10 mM), and cycloheximide (50 μg per ml) were used as indicated. Lanes: 1, no treatment; 2, treatment with IFN-γ for 6 h; 3, treatment with IFN-γ and 2AP for 6 h followed by treatment with IFN-α and cycloheximide for 6 h; 4, treatment with IFN-α and 2AP for 6 h; 5, treatment with IFN-α for 6 h; 6, treatment with IFN-γ for 6 h followed by treatment with IFN-α, 2AP, and cycloheximide for 6 h.

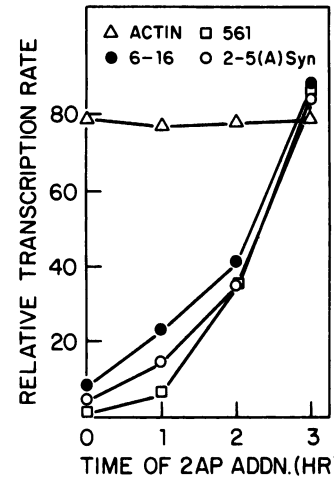

FIG. 7. Effects of 2AP on the rates of transcription of mRNAs in IFN-α-treated cells. The rates of transcription of different mRNAs were measured by nuclear run-on transcription assays as described previously (15). The relative rates of transcription are presented in an arbitrary scale; the absolute values can be compared for the same mRNA only. HeLaM cells were treated with 500 U of IFN-α per ml for 3 h, cells were harvested, and the nuclei were isolated for the assays. In addition, cells were treated with 10 mM 2AP at the indicated times after IFN-α treatment began.

mRNAs, actin, 561, 2-5(A) synthetase, and 6-16, were measured by using nuclei isolated from cells that had been treated with IFN-α for 3 h and with 2AP for different lengths of time. 2AP treatment did not affect the rate of transcription of actin mRNA, but transcription of the other three mRNAs was strongly inhibited by it. The 3-h time point was chosen because the rates of transcription of the induced mRNAs peak at this time (15). Although adding 2AP at the time of IFN-α addition had the most profound inhibitory effect on transcription of these mRNAs, addition of the drug 1 or 2 h after IFN addition still had considerable effect on the rates of transcription. The lack of effect on actin mRNA transcription strengthens the view that the observed effects of 2AP are selective.

DISCUSSION

The inhibitory action of 2AP on transcriptional induction of genes appears to be a selective one. No gross qualitative or quantitative alterations in the patterns of RNA and protein syntheses were observed in 2AP-treated cells. Moreover induction of metallothionein mRNA by $CdCl_2$ was unaffected, as is induction of heat shock proteins (23, 42). Although induction of 561 and 6-16 mRNAs by dsRNA in GM2767 cells was inhibited by 2AP, induction of the same mRNAs by IFN-α was not affected by 2AP. Similarly, induction of 202 mRNA in L929 cells was unaffected by 2AP.

We have modified our working model for gene induction by IFNs in HeLaM cells to accommodate our recent data (Fig. 8). Our results suggest that 2AP blocks either the production or the function of signal 2 elicited by dsRNA. Sensitivity to 2AP distinguishes signal 2 produced by dsRNA from that produced by IFN-α. There were hints from our previous studies (38), e.g., desensitization characteristics, that the signals generated by the two agents, although functionally equivalent, may not be the same. It should be mentioned here that although in most of our experiments 2AP did not affect signal 2 generation by IFN-α, in a few

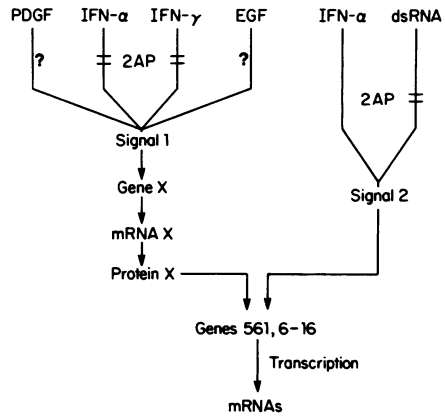

FIG. 8. Schematic presentation of our current understanding of the processes involved in induction of mRNAs 561 and 6-16 in HeLaM cells. Symbols: ⊣, inhibition of the process by 2AP; ?, effect not tested. PDGF, Platelet-derived growth factor; EGF, epidermal growth factor.

experiments it was affected slightly. The experimental parameters responsible for these observed variations have not been identified as yet.

In contrast to IFN-mediated signal 2, signal 1 generated by IFN-α or IFN-γ was blocked by 2AP. We do not know whether 2AP blocked the generation of signal 1 or the synthesis of protein X in response to this signal. However, it is clear that the blockage is beyond the stage of IFN-receptor interactions since signal 2 generation was unaffected by 2AP. Signal 1 generated by IFN-γ was equally affected by 2AP. It remains to be seen whether the same is true for generation of this signal by the growth factors. It is relevant to note in this regard that 2AP blocks induction of c-*fos* and c-*myc* genes by both growth factor and virus infection (42). Although Marcus and Sekellick (23) reported that the inhibitory effect of 2AP on induction of IFN is readily reversible, we observed that mRNA 561 was not inducible by IFN-α in 2AP-treated HeLaM cells which had not been in contact with 2AP for as long as 24 h before IFN treatment (data not shown). We have demonstrated that the production of signal 1 by IFNs, which is necessary for induction of many IFN-inducible genes in HeLaM cells, is blocked by 2AP. Since this effect is very similar to the priming effect in IFN biosynthesis, it is tempting to speculate that the priming of IFN-β_1 gene induction could also be inhibited by 2AP.

The mechanism of 2AP-mediated inhibition of gene induction remains to be investigated. The only enzymes whose actions are known to be inhibited by 2AP are the dsRNA-dependent protein kinase and the heme-regulated protein kinase (5, 7, 12, 18). The known physiological substrate of both kinases is the peptide chain initiation factor eIF-2. The observed stimulatory effect of 2AP on specific protein synthesis in vivo (12) might be mediated by prevention of phosphorylation and of the resultant inactivation of eIF-2. There is no evidence, however, that the state of eIF-2 phosphorylation has any role in the action of 2AP on gene induction. Even though 2AP affects the function of certain protein kinases, those of others are not affected, as evidenced by its lack of effect on the phosphorylation state of cellular proteins in general (42). One piece of information argues against an involvement of the dsRNA-dependent protein kinase in dsRNA-mediated gene induction. GM2767 and other human fibroblasts have very low levels of the dsRNA-dependent protein kinase even after IFN treatment (11; unpublished data), but nonetheless genes 561 and 6-16 were strongly induced by dsRNA in these cells. To test the possible involvement of protein kinases in induction of these genes, a logical beginning would be to examine the effect of 2AP on the phosphorylation states of various *trans*-acting proteins which bind to the regulatory sequences of these genes.

ACKNOWLEDGMENTS

We thank Philip I. Marcus for VSV DI-011 and for helpful discussion and Jaklin Levi for secretarial assistance.

This study was supported in part by Public Health Service grant AI-22510 and by core grant CA-08748 from the National Institutes of Health. J.K. was supported by a fellowship from the Exxon Corp.

LITERATURE CITED

1. **Baglioni, C., and T. W. Nilsen.** 1983. Mechanisms of antiviral action of interferon. Interferon **5**:23–42.
2. **Benech, P., M. Vigneron, D. Peretz, M. Revel, and J. Chebath.** 1987. Interferon-responsive regulatory elements in the promoter of the human 2′,5′-oligo(A) synthetase gene. Mol. Cell. Biol. **7**: 4498–4504.
3. **Caplen, H. C., and S. L. Gupta.** 1988. Differential regulation of a cellular gene by human interferon-γ and interferon-α. J. Biol. Chem. **263**:332–339.
4. **Cohen, B., D. Peretz, D. Vaiman, P. Benech, and J. Chebath.** 1988. Enhancer-like interferon responsive sequences of the human and murine (2′-5′) oligoadenylate synthetase gene promoters. EMBO J. **7**:1411–1419.
5. **De Benedetti, A., and C. Baglioni.** 1983. Phosphorylation of initiation factor eIF-2α, binding of mRNA to 48S complexes, and its reutilization in initiation of protein synthesis. J. Biol. Chem. **258**:14556–14562.
6. **Enoch, T., K. Zinn, and T. Maniatis.** 1986. Activation of the human β-interferon gene requires an interferon-inducible factor. Mol. Cell. Biol. **6**:801–810.
7. **Farrell, P. J., K. Balkow, T. Hunt, R. J. Jackson, and H. Trachsel.** 1977. Phosphorylation of initiation factor eIF-2 and the control of reticulocyte protein synthesis. Cell **11**:187–200.
8. **Friedman, R. L., S. P. Manly, M. McMahon, I. M. Kerr, and G. R. Stark.** 1984. Transcriptional and post-transcriptional regulation of interferon-induced gene expression in human cells. Cell **38**:745–755.
9. **Fujita, T., H. Shibuya, H. Hotta, K. Yamanishi, and T. Taniguchi.** 1987. Interferon-β gene regulation: tandemly repeated sequences of a synthetic 6 bp oligomer function as a virus-inducible enhancer. Cell **49**:357–367.
10. **Goodbourn, S., and T. Maniatis.** 1988. Overlapping positive and negative regulatory domains of the human β-interferon gene. Proc. Natl. Acad. Sci. USA **85**:1447–1451.
11. **Holmes, S. L., and S. L. Gupta.** 1982. Interferon action in human fibroblasts: induction of 2′,5′-oligoadenylate synthetase in the absence of detectable protein kinase activity. Arch. Virol. **72**: 137–142.
12. **Kaufman, R. J., and P. Murtha.** 1987. Translational control mediated by eucaryotic initiation factor-2 is restricted to specific mRNAs in transfected cells. Mol. Cell. Biol. **7**:1568–1571.
13. **Keller, A. D., and T. Maniatis.** 1988. Identification of an inducible factor that binds to a positive regulatory element of the human β-interferon gene. Proc. Natl. Acad. Sci. USA **85**: 3309–3313.
14. **Kusari, J., and G. C. Sen.** 1986. Regulation of synthesis and turnover of an interferon-inducible mRNA. Mol. Cell. Biol. **6**: 2062–2067.
15. **Kusari, J., and G. C. Sen.** 1987. Transcriptional analyses of interferon-inducible mRNAs. Mol. Cell. Biol. **7**:528–531.
16. **Kusari, J., R. K. Tiwari, R. Kumar, and G. C. Sen.** 1987. Expression of interferon-inducible genes in RD-114 cells. J. Virol. **61**:1524–1531.
17. **Larner, A. C., G. Jonak, Y.S.-E. Cheng, B. Korant, E. Knight, and J. E. Darnell.** 1984. Transcriptional induction of two genes

in human cells by β-interferon. Proc. Natl. Acad. Sci. USA **81:** 6733–6737.

18. **Legon, S., A. Brayley, T. Hunt, and R. J. Jackson.** 1974. The effect of cyclic AMP and related compounds on the control of protein synthesis in reticulocyte lysates. Biochem. Biophys. Res. Commun. **56:**745–752.
19. **Lengyel, P.** 1982. Biochemistry of interferons and their actions. Annu. Rev. Biochem. **51:**251–282.

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

20. **Luster, A. D., J. C. Unkeless, and J. V. Ravetch.** 1985. γ-interferon transcriptionally regulates an early response gene containing homology to platelet proteins. Nature (London) **315:** 672–676.
21. **Marcus, P. I.** 1984. Interferon induction by viruses: double-stranded RNA as the common proximal inducer molecule, p. 113–175. *In* R. M. Friedman (ed.), Interferon: mechanisms of production and action, vol. 3. Elsevier Biomedical Press, Amsterdam.
22. **Marcus, P., and M. J. Sekellick.** 1977. Defective interfering particles with covalently linked [+/−]RNA induce interferon. Nature (London) **266:**815–819.
23. **Marcus, P. I., and M. J. Sekellick.** 1988. Interferon induction by viruses. XVI. 2-Aminopurine blocks selectively and reversibly an early stage in interferon induction. J. Gen. Virol. **69:**1637–1645.
24. **Merlin, G., J. Chebath, P. Benech, R. Metz, and M. Revel.** 1983. Molecular cloning and sequence of partial cDNA for interferon induced (2′-5′) oligo(A)synthetase mRNA from human cells. Proc. Natl. Acad. Sci. USA **80:**4904–4908.
25. **Pestka, S., J. A. Langer, K. C. Zoon, and C. E. Samuel.** 1987. Interferons and their actions. Annu. Rev. Biochem. **56:**727–767.
26. **Porter, A. C. G., Y. Chernajovsky, T. C. Dale, C. S. Gilbert, G. R. Stark, and I. M. Kerr.** 1988. Interferon response element of the human gene 6-16. EMBO J. **7:**85–92.
27. **Ragg, H., and C. Weissmann.** 1983. Not more than 117 base pairs of 5′-flanking sequence are required for inducible expression of a human IFN-α gene. Nature (London) **303:**439–442.
28. **Reich, N., B. Evans, D. Levy, D. Fahey, E. Knight, Jr., and D. E. Darnell, Jr.** 1987. Interferon-induced transcription of a gene encoding a 15-kDa protein depends on an upstream enhancer element. Proc. Natl. Acad. Sci. USA **84:**6394–6398.
29. **Revel, M., and J. Chebath.** 1986. Interferon-activated genes. Trends Biochem Sci. **11:**166–170.
30. **Rubin, B. Y., S. L. Anderson, R. M. Lunn, G. R. Hellerman, N. K. Richardson, and L. J. Smith.** 1988. Production of a monoclonal antibody directed against an interferon-induced 56,000-dalton protein and its use in the study of this protein. J. Virol. **62:**1875–1880.
31. **Rutherford, M. N., G. E. Hannigan, and B. R. G. Williams.** 1988. Interferon-induced binding of nuclear factors to promoter elements of the 2-5A synthetase gene. EMBO J. **7:**751–759.
32. **Samanta, H., J. P. Dougherty, M. E. Brawner, H. Schmidt, and P. Lengyel.** 1982. Interferon action: cloning of cDNA segments complementary to messenger RNAs induced by interferons. UCLA Symp. Mol. Cell. Biol. **25:**59–72.
33. **Samanta, H., D. A. Engel, H. M. Chao, A. Thakur, M. A. Garcia-Blanco, and P. Lengyel.** 1986. Interferons as gene activators: cloning of the 5′ terminus and the control segment of an interferon activated gene. J. Biol. Chem. **261:**11849–11858.
34. **Saunders, M. E., D. R. Gewert, M. E. Tugwell, M. McMahon, and B. R. G. Williams.** 1985. Human 2-5A synthetase: characterization of a novel cDNA and corresponding gene structure. EMBO J. **4:**1761–1768.
35. **Sen, G. C.** 1984. Biochemical pathways in interferon action. Pharmacol. Ther. **24:**235–257.
36. **Staeheli, P., P. Danielson, O. Haller, and J. G. Sutcliffe.** 1986. Transcriptional activation of the mouse *Mx* gene by type I interferon. Mol. Cell. Biol. **6:**4770–4774.
37. **Stewart, W. E., II.** 1981. The interferon system, 2nd ed., Springer-Verlag, Vienna.
38. **Tiwari, R. K., J. Kusari, and G. C. Sen.** 1987. Functional equivalents of interferon-mediated signals needed for induction of an mRNA can also be generated by double-stranded RNA and growth factors. EMBO J. **6:**3373–3378.
39. **Wathelet, M. G., I. M. Clauss, C. B. Nols, J. Content, and G. A. Huez.** 1987. New inducers revealed by the promoter sequence analysis of two interferon-activated human genes. Eur. J. Biochem. **169:**313–321.
40. **Wathelet, M. G., S. Moutschen, P. Defilippi, A. Cravador, M. Collet, G. Huez, and J. Content.** 1986. Molecular cloning: full-length sequence and preliminary characterization of a 56kDa protein induced by human interferons. Eur. J. Biochem. **155:**1–7.
41. **Weissmann, C., and H. Weber.** 1986. The interferon genes. Prog. Nucleic Acid Res. **33:**251–300.
42. **Zinn, K., A. Keller, L.-A. Whittemore, and T. Maniatis.** 1988. 2-Aminopurine selectively inhibits the induction of β-interferon, c-fos, and c-myc gene expression. Science **240:**210–213.
43. **Zullo, J. N., B. H. Cochran, A. S. Huang, and C. D. Stiles.** 1985. Platelet-derived growth factor and double-stranded ribonucleic acids stimulate expression of the same genes in 3T3 cells. Cell **43:**793–800.