

Rapid enhancement of β_2 -interferon/B-cell differentiation factor BSF-2 gene expression in human fibroblasts by diacylglycerols and the calcium ionophore A23187

(1,2-dioctanoylglycerol/1-oleoyl-2-acetylglycerol/tumor necrosis factor/interleukin 1/antiviral effects)

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ABSTRACT The expression in human fibroblasts of the β_2 -interferon (IFN- β_2) gene, which is now recognized to be identical to the gene encoding B-cell differentiation factor BSF-2, is enhanced by several cytokines that affect cell growth (tumor necrosis factor, interleukin 1, platelet-derived growth factor, and β_1 -interferon). We have examined the possibility that IFN- β_2 gene expression is regulated through activation, by diacylglycerol, of the protein kinase C pathway. The synthetic diacylglycerols 1,2-dioctanoylglycerol (diC₈) and 1-oleoyl-2-acetylglycerol strongly enhanced IFN- β_2 , but not IFN- β_1 , gene expression in human fibroblasts (FS-4 strain). An increase in IFN- β_2 mRNA level was detected within 15 min after addition of diC₈ (290 μ M) to FS-4 cells and was maximal approximately 20 hr later. An increase in IFN- β_2 gene transcription was detected within 5 min of addition of diC₈, and the rate of transcription was near-maximal by 15–30 min. The enhancement of IFN- β_2 gene expression by diC₈, interleukin 1, or tumor necrosis factor was not prevented by H8, a preferential inhibitor of cAMP- and cGMP-dependent protein kinases, but was blocked by H7, an inhibitor of protein kinase C as well as of cyclic nucleotide-dependent protein kinases. diC₈ was found to protect FS-4 cells from the cytopathic effect of vesicular stomatitis virus; this protection was blocked by polyclonal or monoclonal antibodies that neutralize IFN- β , suggesting that the antiviral effect was due to the secretion of IFN- β_2 by the diC₈-treated fibroblasts. The calcium ionophore A23187 (1–10 μ M) also elicited an increase in the level of IFN- β_2 mRNA in FS-4 fibroblasts; appropriate combinations of A23187 and diC₈ had at least an additive effect in enhancing IFN- β_2 mRNA levels. These results show that protein kinase C-activating or [Ca²⁺]-elevating agents rapidly increase the expression of the IFN- β_2 gene in human fibroblasts.

Human β_2 -interferon (IFN- β_2) (1–6) is a cellular regulatory molecule with diverse cell- and tissue-dependent functions that include antiviral activity, inhibition of fibroblast proliferation, enhancement of histocompatibility antigen expression and of immunoglobulin secretion, and stimulation of growth of certain hybridoma cell lines (reviewed in ref. 6). It is now recognized that the genes encoding the B-cell differentiation factor BSF-2 (7) and the hybridoma growth factor HGF (J. Van Snick and colleagues as cited in ref. 8) are identical to the IFN- β_2 gene (reviewed in ref. 6). IFN- β_2 is a glycoprotein of apparent molecular weight 21,000 (4), derived from a gene located on human chromosome 7 (9).

Human IFN- β_2 mRNA is expressed constitutively, or appears to be so expressed, in fibroblasts, in monocytes, in certain T-cell lines, in T24 bladder carcinoma cells, and in tumor tissue and cultured cells from certain cardiac myxomas and a uterine carcinoma (reviewed in ref. 6; ref. 10). The

present studies were stimulated by the observations that the expression of IFN- β_2 in human fibroblasts, but not that of IFN- β_1 , is markedly stimulated by tumor necrosis factor (TNF), interleukin 1 α and 1 β (IL-1 α and -1 β), platelet-derived growth factor, bovine serum, and other interferons, but not by epidermal growth factor (refs. 4, 5, 11–13; M. Kohase and P.B.S., unpublished data). These findings suggested that the enhancement of expression of the IFN- β_2 gene may be mediated through activation of the diacylglycerol/protein kinase C and/or the inositol trisphosphate/Ca²⁺ signal-transduction pathways known to be activated by some of the cytokines that enhance IFN- β_2 expression (refs. 14–16; reviewed in refs. 17 and 18). This possible mechanism could also help explain the enhancement of IFN- β_2 expression by phytohemagglutinin (19) and concanavalin A (J. Van Snick and colleagues as cited in ref. 8) in lymphocytes. It is already known that phorbol 12-myristate 13-acetate, which activates protein kinase C, enhances IFN- β_2 expression in lymphocytes (7).

We have used synthetic diacylglycerols (ref. 20; reviewed in ref. 21) as messenger molecules to investigate whether the activation of the protein kinase C pathway is involved in the expression of the IFN- β_2 gene in fibroblasts. We have also examined the effect of the calcium ionophore A23187 (ref. 22; reviewed in refs. 17 and 18) on IFN- β_2 gene expression. Our results show that protein kinase C-activating or [Ca²⁺]-elevating agents rapidly and markedly increase the expression of the IFN- β_2 gene in human fibroblasts and that the effect of the two together is at least additive. The IFN- β_2 gene lends itself well to a detailed analysis of the role of signal-transduction mechanisms in regulating its expression in response to a wide range of cytokines that affect cell growth.

MATERIALS AND METHODS

The human foreskin diploid fibroblast strain FS-4 was obtained from J. Vilček (New York University School of Medicine, New York); procedures for its growth in cell culture and for the IFN- β_2 mRNA induction experiments have been described (1, 5, 12, 13). In brief, FS-4 cells were grown to confluence in T-175 Falcon flasks in Eagle's minimal essential medium supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum (GIBCO). The cultures were refed at weekly intervals, and confluent cultures were used for experiments 6–8 days after the last medium change. Concentrated solutions of the experimental reagents were added directly to the spent medium in the cultures. 1,2-Dioctanoylglycerol (diC₈) was purchased from Avanti Polar

Abbreviations: diC₈, 1,2-dioctanoylglycerol; H7, 1-(5-isoquinoline-sulfonyl)-2-methylpiperazine dihydrochloride; H8, N-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride; IFN, interferon; IL-1, interleukin 1; OAG, 1-oleoyl-2-acetylglycerol; TNF, tumor necrosis factor; VSV, vesicular stomatitis virus.

Lipids, 1-oleoyl-2-acetyl-glycerol (OAG) was purchased from Sigma, and the calcium ionophore A23187 was purchased from Calbiochem-Behring. These chemicals were dissolved in concentrated form in dimethyl sulfoxide prior to use. The protein kinase inhibitors H7 [1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride] and H8 [*N*-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride] were purchased from Seikagaku America (St. Petersburg, FL). Recombinant *Escherichia coli*-derived human IL-1 α (specific activity 3×10^7 units/mg) was a gift from Hoffmann-La Roche (Nutley, NJ), and recombinant *E. coli*-derived human TNF (specific activity 4.8×10^7 units/mg) was a gift from the Suntory Institute for Biomedical Research (Osaka, Japan). Bovine polyclonal neutralizing antiserum to human IFN- β was a gift from J. Vilček, murine monoclonal neutralizing antibodies to human IFN- α and - β were purchased from Boehringer Mannheim, and murine monoclonal neutralizing antibody to human IFN- γ was purchased from Interferon Sciences (New Brunswick, NJ). The Indiana strain of vesicular stomatitis virus (VSV) was used to evaluate antiviral effects.

Procedures for isolation of poly(A)⁺ RNA from FS-4 cells (approximate RNA yield 1 μ g per T-175 culture), electrophoresis of the RNA in 1% agarose gels containing 10 mM methylmercury(II) hydroxide, transfer to aminobenzyl-oxymethyl cellulose paper (ABM paper; Pharmacia P-L Biochemicals), and hybridization with full-length human IFN- β_1 (pD19; ref. 23) and - β_2 (p β_2 .24; ref. 5) cDNA clones have been described (5, 12, 13, 23). [α -³²P]dCTP purchased from New England Nuclear was used for nick-translation (New England Nuclear kit) of plasmid DNA; autoradiography was usually carried out overnight at -70°C, using Kodak XAR-5 film and Quanta III DuPont intensifying screens. Nuclear run-on transcription assays using FS-4 cell nuclei and [α -³²P]UTP (New England Nuclear) were carried out essentially as described (24–26) except that separated DNA strands (in appropriate bacteriophage M13 single-stranded DNA preparations) derived from both the 5' flanking and the coding regions of the IFN- β_2 gene were bound to the nitrocellulose paper.

RESULTS

Enhancement of IFN- β_2 mRNA Levels in FS-4 Cells by diC₈ and OAG. Fig. 1 illustrates an experiment in which confluent FS-4 cell sheets were treated with high concentrations ($\geq 250 \mu$ M) of diC₈ and OAG for 6 hr. There was a marked enhancement of IFN- β_2 mRNA levels in cells treated with either diC₈ (lanes 2 and 3) or OAG (lanes 4 and 5). No hybridization was observed when this blot was reprobed with IFN- β_1 cDNA (data not shown). We chose to use diC₈ in most of our subsequent experiments because OAG appeared to be deleterious to FS-4 cells in experiments lasting several days. Subsequent illustrations of RNA blot-hybridization experiments depict only the 1.3-kilobase IFN- β_2 mRNA region of autoradiograms.

A 6-hr exposure of FS-4 cells to diC₈ at a concentration as low as 3 μ M increased the level of IFN- β_2 mRNA (Fig. 2A, lane 2). Maximal enhancement of IFN- β_2 mRNA levels occurred at diC₈ concentrations in the 145–290 μ M range (Fig. 2B, lanes 4 and 5). Therefore, we used diC₈ at 290 μ M in many of our experiments.

The increase in IFN- β_2 mRNA levels in response to diC₈ (290 μ M) was rapid and sustained (Fig. 3). An increase in IFN- β_2 mRNA was detected as early as 15 min after diC₈ addition (Fig. 3A, lane 2). IFN- β_2 mRNA levels then increased markedly to a maximum reached at approximately 20 hr and slowly declined thereafter (Fig. 3B and C).

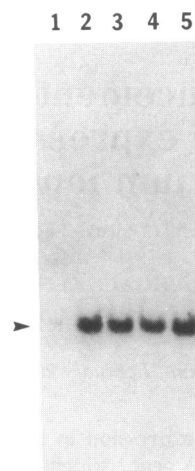


FIG. 1. Diacylglycerols enhance IFN- β_2 mRNA levels in human fibroblasts. FS-4 cell cultures in T-175 flasks were treated with diC₈ or OAG at various concentrations (one flask per group) in 10 ml of spent medium for 6 hr, and the content of IFN- β_2 mRNA was assayed by blot hybridization using a full-length IFN- β_2 cDNA probe (p β_2 .24). Lanes: 1, control culture treated with 25 μ l of dimethyl sulfoxide (Me₂SO); 2, diC₈, 290 μ M in 10 μ l of Me₂SO; 3, diC₈, 725 μ M in 25 μ l of Me₂SO; 4, OAG, 250 μ M in 10 μ l of Me₂SO; 5, OAG, 625 μ M in 25 μ l of Me₂SO.

No hybridization was detected in the blots shown in Figs. 2 and 3 when they were probed with a full-length IFN- β_1 cDNA clone (data not shown).

Rapid Increase in IFN- β_2 Transcription in diC₈-Treated FS-4 Cells. The rapid and marked increase in IFN- β_2 mRNA levels in diC₈-treated FS-4 cells was due to a rapid enhancement of IFN- β_2 gene transcription, as measured by nuclear run-on transcription assays. Transcription across the IFN- β_2 gene along the correct coding strand was increased within 5 min of exposure of FS-4 cells to diC₈ (Fig. 4, strip 5), and the rate of transcription was near-maximal by 15–30 min. Furthermore, the increase in transcription occurred in the presence of cycloheximide (compare strips 2 and 7), suggesting that the enhancing effect of diC₈ on IFN- β_2 gene transcription is mediated via preexisting regulatory factors. An increase in transcription across the IFN- β_2 gene was also detected within 5 min of addition of OAG (250 μ M) to FS-4 cells (data not shown). The calcium ionophore A23187 also increased IFN- β_2 gene transcription in the presence of cycloheximide (Fig. 4, strip 8).

Protein Kinase Inhibitor H7, but Not H8, Blocks the Expression of IFN- β_2 mRNA. The compound H8 is a preferential inhibitor of cAMP- and cGMP-dependent protein kinases, whereas the compound H7 is an inhibitor of protein kinase C as well as of cyclic nucleotide-dependent protein kinases (27). The increased expression of IFN- β_2 mRNA in FS-4 cells treated with 290 μ M diC₈ was blocked by simultaneous exposure to 100 μ M H7 (Fig. 5, lane 5). In contrast, 100 μ M H8 was much less effective in inhibiting diC₈-triggered

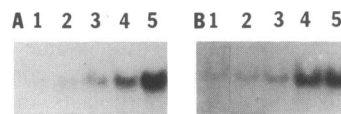


FIG. 2. Dependence of the enhancement of IFN- β_2 mRNA levels on diC₈ concentration. FS-4 cell cultures were treated with diC₈ at various concentrations (one flask per group) for 6 hr, and IFN- β_2 mRNA content was assayed by blot hybridization. The region of the blots containing the IFN- β_2 mRNA is illustrated. (A) Lane 1: control. Lanes 2–5: diC₈ (3, 15, 30, and 60 μ M, respectively). (B) Lane 1: control. Lanes 2–5: diC₈ (30, 60, 145, and 290 μ M, respectively).

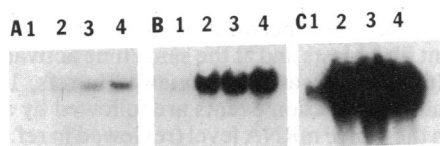


FIG. 3. Time course of the accumulation of IFN- β_2 mRNA in diC $_8$ -treated fibroblasts. FS-4 cell cultures were treated with diC $_8$ at 290 μ M, and the IFN- β_2 mRNA content was assayed by blot hybridization at various times thereafter (one flask per group). (A) Lane 1: control. Lanes 2-4: diC $_8$ (15, 30, and 45 min). (B) Lane 1: control. Lanes 2-4: diC $_8$ (1, 2, and 4 hr). (C) Lane 1: control. Lanes 2-4: diC $_8$ (6, 20, and 27 hr).

accumulation of IFN- β_2 mRNA (lane 6). Although exposure of FS-4 cells to H7 at 50 or 100 μ M for approximately 3 hr inhibited the overall rate of cellular RNA synthesis by approximately 65% and 75%, respectively (measured by a 10-min [3 H]uridine pulse, data not shown), the H7 suppression of IFN- β_2 mRNA levels in diC $_8$ -treated FS-4 cells (Fig. 5) far exceeded this level of inhibition. There was no detectable alteration in the rate of cellular protein synthesis (as measured by a 10-min [3 H]leucine pulse) in cells treated with H7 for approximately 3 hr at concentrations as high as 100 μ M. The observations summarized in Fig. 5 are consistent with the hypothesis that protein kinase C activation is responsible for the enhanced levels of IFN- β_2 mRNA seen in diC $_8$ -treated FS-4 cells.

Comparison of lanes 1, 3, and 4 in Fig. 5 shows that the constitutive expression of IFN- β_2 mRNA in FS-4 cells was also reduced by H7 but not by H8, suggesting that the apparent constitutive expression of this mRNA may also be dependent on protein kinase C activation.

The enhanced expression of IFN- β_2 mRNA in IL-1 α - or TNF-treated fibroblasts was also blocked preferentially by H7 but not by H8 (Fig. 6), again suggesting the involvement of protein kinase C in the enhancement of IFN- β_2 gene transcription in IL-1 α - or TNF-treated FS-4 cells (refs. 12 and 13; Z.W., L. T. May, and P.B.S., unpublished data).

Enhancement of IFN- β_2 mRNA Levels by the Calcium Ionophore A23187. FS-4 cells exposed to the calcium ionophore A23187 (1 μ M) for 6 hr were found to contain elevated levels of IFN- β_2 mRNA (Fig. 7, lane 4); the maximal increase obtained when this compound was used alone was observed with 10 μ M A23187 (Fig. 7, lane 5). A combination of diC $_8$ and A23187 led to at least additive and possibly synergistic

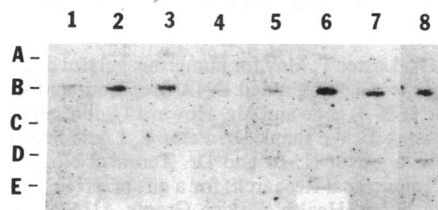


FIG. 4. Rapid enhancement of IFN- β_2 gene transcription in fibroblasts treated with diC $_8$ or A23187. FS-4 cell cultures were treated with diC $_8$ (290 μ M) or A23187 (10 μ M) in the absence or presence of cycloheximide (50 μ g/ml), the nuclei were isolated at different times thereafter (one flask per group), and transcription across the IFN- β_2 gene was monitored by the nuclear run-on assay (24-26). The 32 P-labeled RNA was hybridized to the following IFN- β_2 M13 single-stranded DNA preparations bound to strips of nitrocellulose paper; 100-200 ng per slot: (+)-strand *Rsa* I-*Xba* I coding region (slots A); (-)-strand *Rsa* I-*Xba* I coding region (slots B); (+)-strand *Bam*HI-*Xho* I 5' flanking region (slots C); (-)-strand *Bam*HI-*Xho* I 5' flanking region (slots D); pBR322 (slots E). Cells not treated with cycloheximide: control (strip 1); diC $_8$, 30 min (strip 2); diC $_8$, 60 min (strip 3). Cells treated with cycloheximide: no other additions, 60 min incubation (strip 4); diC $_8$, 5 min (strip 5); diC $_8$, 15 min (strip 6); diC $_8$, 30 min (strip 7); A23187, 60 min (strip 8).

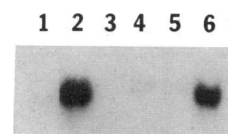


FIG. 5. Enhancement of IFN- β_2 mRNA content by diC $_8$ is markedly inhibited by the protein kinase inhibitor H7 but not by H8. FS-4 cell cultures were treated with diC $_8$ (290 μ M), H7 (100 μ M), and H8 (100 μ M) for 4 hr in various combinations (one culture per group), and the IFN- β_2 mRNA content then was assayed by blot hybridization. Lanes: 1, control; 2, diC $_8$; 3, H7; 4, H8; 5, diC $_8$ plus H7; 6, diC $_8$ plus H8.

enhancement of IFN- β_2 mRNA levels (Fig. 7, lanes 6-8). Thus, protein kinase C-activating and [Ca $^{2+}$]-elevating agents, separately and together, enhance IFN- β_2 gene expression in human fibroblasts.

Antiviral Effect of diC $_8$ in FS-4 Cell Cultures. IFN- β_2 expression following diC $_8$ stimulation led to the development of an antiviral state in FS-4 cells. The addition of diC $_8$ in the decreasing concentration range from 145 to 2 μ M protected FS-4 cell sheets from the cytopathic effect of VSV (Fig. 8). This protection could be blocked by addition of polyclonal or monoclonal neutralizing antibodies to IFN- β together with diC $_8$ but not by monoclonal neutralizing antibodies to IFN- α or - γ (Fig. 8). The extent of cell damage in the VSV controls could be enhanced further by treating cells with monoclonal neutralizing antibody to IFN- β prior to the VSV challenge (Fig. 8), an observation consistent with the constitutive, low-level expression of IFN- β_2 in FS-4 cells. The high sensitivity of the interferon assay used is attested to by the fact that the reference IFN- β preparation elicited an antiviral effect at 0.08-0.16 international unit/ml.

DISCUSSION

Human IFN- β_2 has emerged as a cellular regulatory molecule whose expression and actions suggest that it plays a number of important physiological roles. It is expressed in both lymphoid and nonlymphoid cells in response to several different cytokines. We have examined the response of the IFN- β_2 gene in human fibroblasts to the activation of the diacylglycerol/protein kinase C or the inositol trisphosphate/Ca $^{2+}$ signal-transduction pathways. The two pathways were separately activated by using either synthetic, cell-permeant diacylglycerols (diC $_8$ or OAG) or a calcium ionophore (A23187). Activation of either or both of the signal-transduction pathways rapidly and strongly enhanced IFN- β_2 gene expression. Enhanced expression of IFN- β_2 mRNA in diC $_8$ -treated monocytes has also been observed in preliminary experiments (D. C. Helfgott and P.B.S., unpublished data). The exquisite sensitivity of IFN- β_2 gene expression to activation of the diacylglycerol/protein kinase C and the inositol trisphosphate/Ca $^{2+}$ signal-transduction pathways, when either one or both are activated, is likely to be a factor

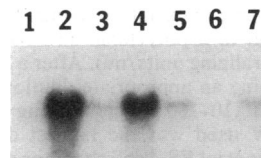


FIG. 6. Enhancement of IFN- β_2 mRNA content by IL-1 α and TNF is efficiently inhibited by the protein kinase inhibitor H7 but not by H8. FS-4 cell cultures were treated with IL-1 α (1 ng/ml) or TNF (30 ng/ml), alone or together with H7 (50 μ M) or H8 (50 μ M) for 6 hr in various combinations (one culture per group), and the IFN- β_2 mRNA content then was assayed by blot hybridization. Lanes: 1, control; 2, IL-1 α ; 3, IL-1 α plus H7; 4, IL-1 α plus H8; 5, TNF; 6, TNF plus H7; 7, TNF plus H8.

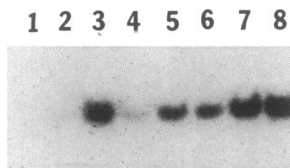


FIG. 7. Enhancement of IFN- β_2 mRNA content by the calcium ionophore A23187 in the absence or presence of diC $_8$. FS-4 cell cultures were treated with A23187 or diC $_8$ at various concentrations in different combinations for 6 hr (one culture per group), and the IFN- β_2 mRNA content then was assayed by blot hybridization. Lanes: 1, control; 2, diC $_8$ (30 μ M); 3, diC $_8$ (290 μ M); 4, A23187 (1 μ M); 5, A23187 (10 μ M); 6, diC $_8$ (30 μ M) plus A23187 (1 μ M); 7, diC $_8$ (30 μ M) plus A23187 (10 μ M); 8, diC $_8$ (290 μ M) plus A23187 (1 μ M).

in the enhancement of transcription across this gene by a number of different cytokines.

One and the same cytokine can activate more than one signal-transduction pathway. Platelet-derived growth factor

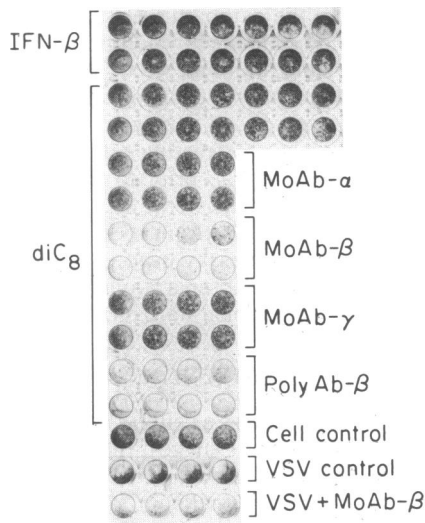


FIG. 8. Antiviral activity of diC $_8$ in human fibroblasts. FS-4 cells were plated in 96-well microtiter plates in Eagle's minimal essential medium with 5% heat-inactivated fetal bovine serum (100 μ l per well) to produce dense monolayers (cells from one confluent T-175 flask were distributed into one 96-well plate). The spent medium was discarded 4 days later and replaced with 100- μ l aliquots of fresh medium containing duplicate sets of serial 2-fold dilutions (from left to right in the figure) of IFN- β or of diC $_8$ in the presence or absence of anti-IFN antibodies as indicated. First two rows at top: IFN- β at decreasing concentrations in the range from 5 to 0.08 international units/ml. [An appropriately calibrated laboratory reference standard of poly(I)-poly(C)- and cycloheximide-induced FS-4 cell IFN- β was used]. Rows 3 and 4 from top: diC $_8$ at decreasing concentrations in the range from 145 to 2 μ M. Rows 5–12 from top: diC $_8$ at decreasing concentrations in the range 145 to 18 μ M in the simultaneous presence of a constant concentration of monoclonal antibody (MoAb, \approx 300 neutralizing units/ml) to human IFN- α , - β , or - γ or a constant concentration of a polyclonal antibody to human IFN- β (Poly Ab- β , \approx 500 neutralizing units/ml). After a 24-hr incubation, 50 μ l of medium containing an appropriate dilution of the VSV stock was added to each well (10–100 plaque-forming units per well). The concentration of VSV used was the highest dilution that caused \geq 75% destruction of control FS-4 cell sheets as determined 4–5 days later in additional experiments. Also shown are appropriate control wells representing uninfected cell sheets, cells exposed to VSV alone, and those exposed to VSV after a 24-hr incubation with the anti-IFN- β monoclonal antibody. In the experiment illustrated, the FS-4 cell monolayers were fixed with ethanol 4 days after the addition of VSV and then stained with Giemsa solution (Fisher). All cell cultures shown in this composite figure were contained in the same 96-well microtiter plate. (See refs. 1, 12, 13, and 28 for a description of key aspects of this highly sensitive interferon assay.)

rapidly increases the concentration of free intracellular Ca $^{2+}$ in quiescent fibroblasts and at the same time activates protein kinase C (refs. 15, 29, and 30; reviewed in refs. 17 and 18). These signal-transduction events are followed by a transient increase in the *c-myc* mRNA level (reviewed in ref. 30). It has also been shown that one and the same gene, as exemplified by the *c-myc* gene, can be activated via each of the two distinct second-messenger pathways. At concentrations lower than 2 μ M, calcium ionophores (A23187 and ionomycin) increase intracellular [Ca $^{2+}$] without stimulating protein kinase C, while the protein kinase C activators OAG and phorbol 12-myristate 13-acetate stimulate protein kinase C without increasing intracellular [Ca $^{2+}$] (30). The Ca $^{2+}$ ionophores and protein kinase C-activating agents both increase *c-myc* mRNA levels when used separately, and when the ionophores and kinase activators are used simultaneously, their effects are additive (30). The human IFN- β_2 gene not only responds to each of these two second-messenger pathways but does so with a sustained enhancement of gene expression. In contrast, the enhanced expression of some genes, as exemplified by expression of the interleukin 2 gene in human tonsillar lymphocytes, appears to require the synergistic activation of both second-messenger pathways (31).

Although both TNF and IL-1 appear to enhance IFN- β_2 gene expression via activation of protein kinase C (Fig. 6), it is likely that the signal-transduction pathways used by these two cytokines differ in some respects. The enhancing effect of IL-1 on IFN- β_2 gene transcription, but not that of TNF, is decreased by cycloheximide, suggesting that newly synthesized protein mediates most of the increase in transcription in response to IL-1 but not that in response to TNF (Z. W., L. T. May, and P. B. S., unpublished data). Furthermore, transcription of the IFN- β_2 gene is sustained at a high level for at least 14 hr in IL-1-treated fibroblasts, whereas the increase is transient following TNF addition and declines by 6 hr (Z. W., L. T. May, and P. B. S., unpublished data).

Molecular biological tools are now available to dissect the mechanism of IFN- β_2 gene regulation at the chromosomal level. Critical aspects of the cytokine interactions to which IFN- β_2 gene expression is subject can be defined by establishing the specific signal-transduction mechanisms involved in the activation of the IFN- β_2 gene in fibroblasts by different cytokines. The induction of IFN- β_2 gene expression by growth factors is an experimental system that is well-suited to detailed studies of the mechanisms by which signals are transduced from the cell surface to the nucleus.

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