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Molecular Cloning of a Gene Selectively Induced by Gamma Interferon from Human Macrophage Cell Line U937

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A cDNA clone encoding a gamma interferon (IFN-γ)-inducible mRNA in human cells of the macrophage lineage was isolated and characterized. The corresponding gene, γ.1, was selectively induced by IFN-γ, responding a hundredfold better to IFN-γ than to IFN-α. The induction was rapid and transient, with maximal mRNA accumulation at about 3 h and decline to the basal level after 48 h. Transcriptional activation could be detected as early as 5 min after IFN-γ stimulation and accounted entirely for the mRNA accumulation. The induction of γ.1 by IFN-γ was cell-type restricted, being seen only in macrophages and endothelial cells. In addition, phorbol ester-induced differentiation of promyelocytic HL-60 cells and promonocytic THP-1 cells rendered the γ.1 gene inducible by IFN-γ. The 1.0-kilobase γ.1 cDNA sequence encoded a small predicted polypeptide of 38 amino acids and had a conserved sequence associated with rapidly turning over mRNAs. In vitro translation of the γ.1 transcript yielded a 4,000-dalton polypeptide.

Interferons (IFNs) constitute a family of polypeptides which exert a wide variety of biological activities in addition to their antiviral activity. They can be divided into two classes. Type I IFNs consist of alpha IFN (IFN-α) and beta IFN (IFN-β) and are generally produced following viral infection, IFN-α being produced predominantly by leukocytes and IFN-β by fibroblasts. Both bind to the same membrane receptor. Type II IFN, or gamma interferon (IFN-γ), shares little structural homology with either IFN-α or IFN-β. This glycoprotein is released primarily from activated T lymphocytes and interacts with a distinct and specific cell membrane receptor. In addition to antiviral activity, IFN-γ exerts unique effects on cells of the immune system (10). One of the principal targets of IFN-γ is cells of the mononuclear phagocyte lineage; indeed, it is the most potent lymphokine for activating these cells (22). IFN-γ augments expression of cell-surface molecules, including all three classes of antigens of the major histocompatibility complex (2, 29, 34) and Fc receptors for immunoglobulin G (11). It enhances cytotoxicity against tumor target cells (15) and induces both oxygen-dependent and -independent antimicrobial states (19, 21). Many of these functions are unique to IFN-γ, since type I IFNs are either inactive or require 100 times more units to induce similar cellular responses. The mechanisms by which this unique polypeptide exerts its pleiotropic biological effects through a single, recently cloned (1) receptor in a given cell type remain intriguing.

It has been established that new mRNA synthesis is required for IFN-γ-inducible cellular responses (31), and several new proteins appear after IFN-γ treatment (35). One approach to understanding the mechanism of IFN-γ action, then, is to identify IFN-γ-inducible genes in the target cell of interest, elucidate how they are regulated, and delineate the cellular response in which they are involved. As predicted from functional studies, interferon-inducible genes can be divided into three groups. One contains genes that are well induced by both types of IFNs, such as 2′-5′-oligoadenylate synthetase (3) and the I-8 gene family (9). A second consists of those that are induced predominantly by type I IFNs, e.g., pIF-1 (14), Mx (28), and 6-16 (13). The last group includes genes induced preferentially by IFN-γ. Thus far, the principal IFN-γ-induced genes studied have been the major histocompatibility class I (34), class II (2), and class III (29) genes; the gene encoding the Fc receptor for immunoglobulin G (11); the IP-10 and IP-30 genes (17, 18); and most recently, the gene encoding the α chain of the chronic granulomatous disease protein (23). We report here the isolation and characterization of a novel IFN-γ-inducible gene, designated γ.1, that is expressed primarily in human macrophages and macrophage cell lines.

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MATERIALS AND METHODS

Interferons. The IFN-γ used was a recombinant human protein synthesized in *Escherichia coli* and generously provided by Genentech (San Francisco, Calif.). It had a specific antiviral titer of 2×10^7 to 4×10^7 U/mg against encephalomyocarditis virus in human lung carcinoma A549 cells. Recombinant IFN-αA was kindly provided by Hoffmann-La Roche Inc. (Nutley, N.J.). The reconstituted stock had an antiviral titer of 3×10^6 U/ml against vesicular stomatitis virus in WISH cells.

Cell culture. The human macrophagelike cell line U937, derived from cells of a patient with histocytic lymphoma (30), was kindly provided by Nancy Hogg (Imperial Cancer Research Fund, London). The cells were grown in Dulbecco modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal calf serum, streptomycin (100 μg/ml), penicillin (100 U/ml), and nonessential amino acids (100 μM). HL-60 cells, derived from a patient with promyelocytic leukemia (7), were obtained from Richard Stanley (Albert Einstein College of Medicine, Bronx, N.Y.). THP-1 cells, derived from a patient with monocytic leukemia (33), were obtained from the American Type Culture Collection (Rockville, Md.). Molt-4 cells, a T-cell line derived from a patient with lymphoblastic leukemia (20), were obtained from J. Minawada (Roswell Park Laboratory, Buffalo, N.Y.). Wil-2 cells, a human B-cell line (26), were obtained from Richard Lerner (Scripps Research Foundation, La Jolla, Calif.). Scl-1 cells, a human keratinocyte line, were obtained from Robert Modlin (University of Southern

California Medical School, Los Angeles, Calif.). HUVE cells, a line of human umbilical cord vein endothelial cells, were prepared by Victor Hatcher (Albert Einstein College of Medicine). Molt-4, THP-1, HL-60, and Scl-1 cells were all grown in RPMI 1640 containing 10% fetal calf serum.

RNA isolation. Nonadherent cells were induced at 5×10^5 to 10×10^5/ml, and adherent cells were induced just before confluence was reached. The cell pellet was dissolved in 4 M guanidinium thiocyanate, and the total RNA was isolated by centrifugation through a 5.7 M CsCl gradient (6). The poly(A)$^+$ RNA of U937 cells was isolated after three rounds of adsorption on an oligo(dT)-cellulose column.

cDNA library construction. U937 cells (5×10^5/ml) were induced with IFN-γ (100 U/ml) for 20 h, and poly(A)$^+$ RNA was isolated. First-strand cDNA was synthesized from 10 μg of poly(A)$^+$ RNA with avian myeloblastosis virus reverse transcriptase, followed by second-strand synthesis with DNA polymerase I. The double-stranded cDNA was blunt-ended with T4 DNA polymerase, internal *Eco*RI sites were methylated, and the cDNA was ligated to *Eco*RI linkers (12). After regeneration of cohesive ends with *Eco*RI and separation from free linkers on Ultragel AcA34 columns, (LKB Instruments, Inc., Rockville, Md.), the cDNA fragments were ligated into the *Eco*RI sites of the bacteriophage cloning vector λ-gt10 (Promega Biotec, Madison, Wis.).

Differential screening of cDNA libraries. About 1,500 phage were plated onto a 150-mm dish, and two carefully marked filter replicas of either nitrocellulose (Scheicher & Schuell, Inc., Keene, N.H.) or Biodyne nylon (Pall Corp.) membranes were lifted from each dish. The filters were treated with 0.5 M NaOH–1.5 M NaCl, neutralized in 0.5 M Tris (pH 7.5), rinsed in 2× SSPE, and vacuum dried. The [^{32}P]cDNA probes were prepared in a reaction mixture similar to that used for first-strand synthesis, except that it contained 100 μCi of [α-^{32}P]dCTP (800 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) without cold dCTP. The "positive" probe was made from poly(A)$^+$ RNA of IFN-γ-induced U937 cells, while the "negative" probe was made from that of untreated cells. Duplicate filters from each dish were hybridized to the positive or negative probes at 3×10^6 cpm/ml in solution containing 50% formamide, 5× SSPE, 5× Denhardt solution, 0.2% sodium dodecyl sulfate, and 100 μg of denatured salmon sperm DNA. After 16 to 18 h at 42°C, the filters were washed twice at room temperature for 30 min per wash with 2× SSPE–0.2% sodium dodecyl sulfate and twice at 50°C in the same buffer. After exposure, the positive and negative filters were superimposed and each spot was compared. Phage clones demonstrating differential hybridization between filters from positive and negative probes were isolated and rescreened twice in a similar way.

RNA analysis. Total RNA (25 μg per sample) was separated by electrophoresis in 1.2% agarose gels containing 1 M formaldehyde and transferred to nylon membranes. After being baked for 2 h, the nylon membranes were hybridized with nick-translated cDNA probes with a specific activity of 2×10^8 to 3×10^8 cpm/μg and washed under conditions of various stringencies.

Nuclear run-on assays. Run-on assays were performed essentially as described in reference 18. For each transcriptional analysis, 10^8 U937 cells were treated for different periods of time with IFN-γ, washed, and lysed, and nuclei were obtained. Transcripts were labeled with [^{32}P]UTP (800 Ci/mmol, Amersham). After being incubated at 30°C for 15 min, the extract was treated successively with DNase I, proteinase K, and phenol-chloroform, and the RNA was precipitated from ethanol. Hybridization was carried out at 37°C, and the filters were washed, treated with RNase A, rewashed, and subjected to autoradiography.

DNA sequencing. The longest γ.1 cDNA was inserted into the *Eco*RI site in the polylinker region of the M13mp18 phage vector. Overlapping clones were generated by using the Cyclone system (International Biotechnologies, Inc.). Dideoxy sequencing of each clone was performed by using a DNA-sequencing kit (International Biotechnologies) and [^{35}S]dATP (Amersham).

In vitro transcription and translation. The γ.1 cDNA was cloned into the *Eco*RI site in the polylinker of pGEM-1 (Promega). The plasmid was digested with *Pvu*II, which linearized it downstream from the polylinker region. SP6 RNA transcripts were synthesized in a solution containing 40 mM Tris (pH 7.5); 6 mM $MgCl_2$; 10 mM dithiothreitol; 10 U of RNasin; 0.4 mM each of ATP, GTP, CTP, and UTP; 10 U of SP6 RNA polymerase; and 1 μg of linearized template. The 25-μl reaction mixture was incubated at 37°C for 1 h. SP6 transcripts were translated in a wheat germ system containing a 40 mM amino acid mixture (without methionine), [^{35}S]methionine (20 μCi; Amersham), and 20 U of RNasin in a total reaction volume of 50 μl and incubated at 27°C for 1 h. A positive-control reaction mixture containing globin mRNA was included in each experiment. Portions of the samples were analyzed by electrophoresis in 15% sodium dodecyl sulfate-polyacrylamide gels, followed by autoradiography.

RESULTS

Differential screening of a cDNA library from IFN-γ-treated U937 cells. The human macrophagelike cell line U937 and primary human monocytes were found to be similarly responsive to IFN-γ in terms of increased major histocompatibility class I expression and production of reactive oxygen intermediates. Therefore, cDNA libraries were prepared in the λ-gt10 vector from poly(A)$^+$ RNA of U937 cells pretreated for 20 h with IFN-γ. The complexity of the recombinant library was 5.0×10^5. To isolate IFN-γ-inducible sequences, about 30,000 phage were screened by differential hybridization with cDNAs from IFN-γ-treated and untreated U937 cells. A novel clone was found that hybridized to an IFN-γ-inducible mRNA with an apparent size of 1.0 kilobase. A full-length cDNA (determined by S1 mapping; data not shown) was cloned into a plasmid, and the insert was isolated, labeled, and employed as a hybridization probe. The corresponding gene was designated γ.1 (IFN-γ-inducible gene 1).

Kinetics of γ.1 induction by IFN-γ. Analysis of the time course of induction of γ.1 (Fig. 1A) showed that an increase in the abundance of the corresponding mRNA transcript could be detected within 20 min and that the maximum accumulation occurred 3 h after IFN-γ treatment. The amount of γ.1 mRNA returned gradually to basal levels over 48 h, while γ-actin mRNA remained relatively constant during the whole period. While there was a very low constitutive level of expression in some experiments, a 15- to 30-fold induction after 3 h was routinely observed after IFN-γ treatment (Fig. 1B).

Preferential induction of γ.1 by IFN-γ. The response of γ.1 mRNA to various doses of IFN-γ and IFN-α (Fig. 2) was strikingly different. While a detectable increase was routinely seen with as little as 0.1 U of IFN-γ per ml, none was seen with as much as 10 U of IFN-α per ml. Half-maximal γ.1 induction was achieved with approximately 3 U of IFN-γ per ml, but more than 300 U/ml was required for IFN-α.

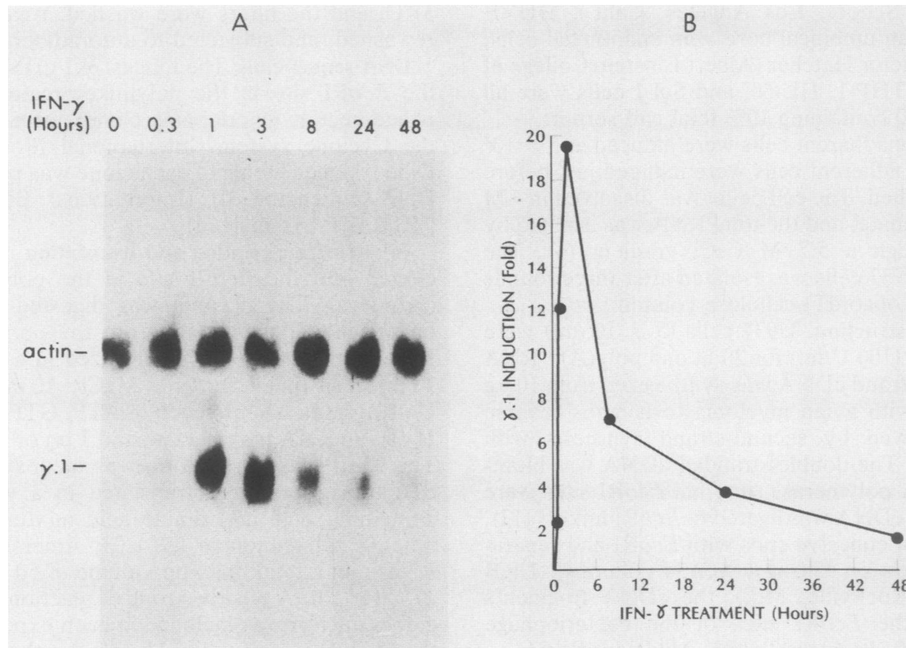

FIG. 1. Kinetics of γ.1 mRNA accumulation in U937 cells in response to IFN-γ. RNA was isolated from U937 cells at various times after treatment with 100 U of IFN-γ per ml. Total RNA was separated by electrophoresis (25 μg per lane), transferred to a nylon membrane, and hybridized to ^{32}P-labeled γ.1 and γ-actin cDNA probes. Densitometric scanning of the autoradiograph (A) was used for generation of the graph (B).

Overall, the induction of γ.1 was 100 times more sensitive to IFN-γ than to IFN-α. This conclusion was confirmed after correcting for molar concentrations of the two IFNs calculated from the specific activity. Tenfold induction of γ.1 required approximately 6 pM IFN-γ dimers, compared with 900 pM IFN-αA monomers.

Activation of γ.1 by IFN-γ is transcriptional. To determine whether the increase in γ.1 mRNA following IFN-γ treatment was dependent on protein synthesis, U937 cells were treated with 50 μg of cycloheximide per ml for various periods in the presence or absence of IFN-γ (Fig. 3). Although the basal level of γ.1 was slightly elevated at 3 h by cycloheximide, the inducibility of γ.1 mRNA by IFN-γ was virtually unaffected by cycloheximide treatment, indicating that the effect of IFN-γ on γ.1 is likely to be direct.

To ascertain directly whether the induction of γ.1 was a primary transcriptional response, the nuclear run-on assay was used. Since initiated transcripts are faithfully elongated

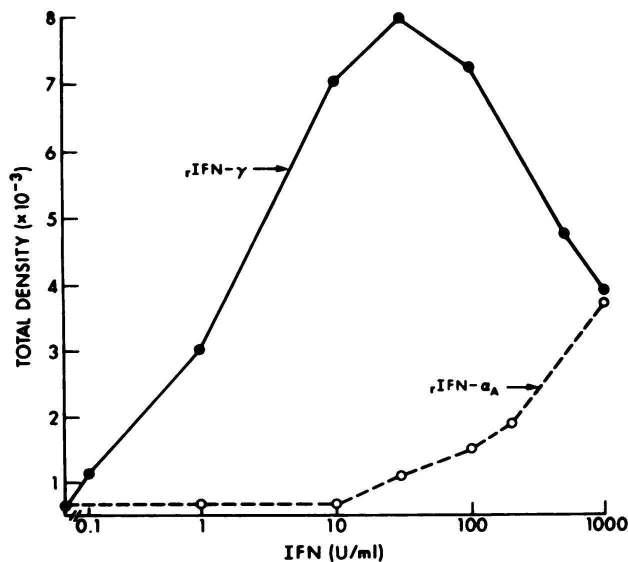

FIG. 2. Densitometric analysis of Northern (RNA) blots of γ.1 mRNA accumulated in response to treatment of U937 cells for 3 h with various concentrations of IFN-γ or IFN-α.

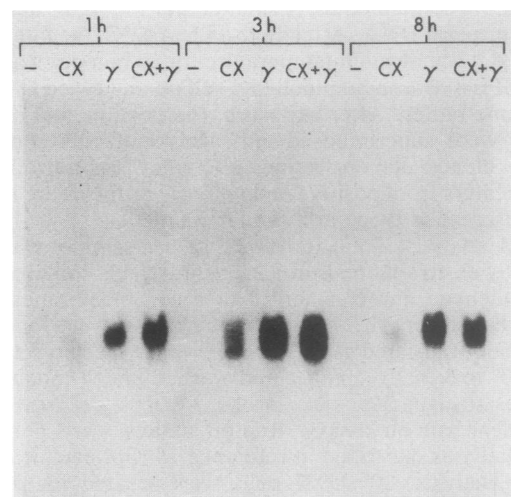

FIG. 3. Effects of cycloheximide treatment of U937 cells on γ.1 mRNA accumulation. RNA was isolated from U937 cells treated for various periods with 50 μg of cycloheximide per ml (CX), 100 U of IFN-γ per ml (γ), 50 μg of cycloheximide per ml plus 100 U of IFN-γ per ml (CX + γ), or medium alone (–). Total RNA was fractionated, transferred, and hybridized to γ.1 cDNA probe.

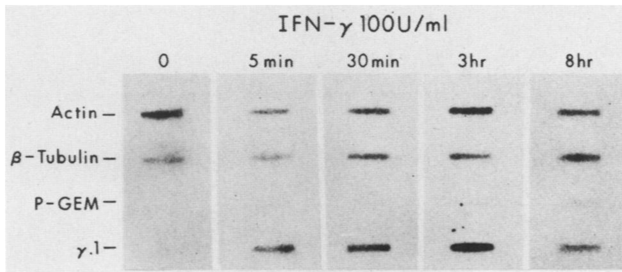

FIG. 4. Kinetics of the transcriptional induction of γ.1 by IFN-γ. U937 cells were incubated with 100 U of IFN-γ per ml for the times indicated. Nuclei were isolated and assayed for transcription by labeling nascent RNA with [α-^{32}P]UTP and hybridizing equal amounts of labeled RNA to DNAs immobilized in slots.

in isolated nuclei, this assay measures the rate of initiation of transcription of a given gene. Figure 4 shows the kinetics of transcriptional activation of γ.1 following IFN-γ treatment. Increased transcription was detected as soon as 5 min, peaked at 3 h, and decreased at 6 to 8 h. Transcription of two housekeeping genes, tubulin and actin, remained relatively constant during the process. Densitometric analysis of the run-on assay data indicated a 20-fold increase in γ.1 transcription at the maximal level. Comparing these results with the kinetics and total steady-state mRNA accumulation results shown in Fig. 1, we infer that the activation of γ.1 by IFN-γ is entirely transcriptional.

Induction of γ.1 by IFN-γ is cell-type restricted. To explore the possibility that γ.1 has some functional significance in mononuclear phagocytes, we examined the inducibility of γ.1 mRNA in different types of primary and cultured human cells (Fig. 5). Induction of γ.1 was seen in cells of the mononuclear phagocyte lineage (i.e., U937 cells), primary human peripheral blood mononuclear cells (PBM), and HL-60 and THP-1 cells but not in B cells (Wil-2), T cells (Molt-4), fibroblastic cells (HeLa), or keratinocytes (Scl-1). Expression of γ.1 was also observed in endothelial cells (HUVE).

Possible differentiation-dependent induction of γ.1. The very low level of γ.1 induction by IFN-γ in HL-60 and

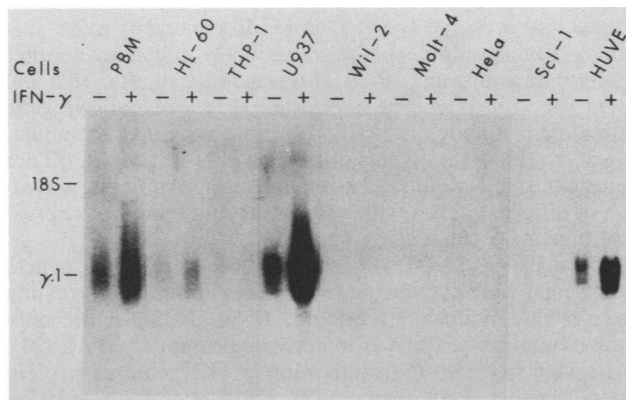

FIG. 5. Cell-type selectivity of IFN-γ induction of γ.1 mRNA. RNA was isolated from cells treated with IFN-γ (100 U/ml) for 3 h. Twenty-five micrograms of total RNA was fractionated per lane, transferred, and hybridized to a ^{32}P-labeled γ.1 cDNA probe. The cell types are indicated at the top: PBM, human peripheral blood mononuclear cells; HL-60, human myelomonocytic line; THP-1, human monocytic line; U937, human monocytic line; Wil-2, human B lymphocytic line; Molt-4, human T lymphocytic line; HeLa, human epithelial line; Scl-1, human keratinocyte line; HUVE, primary human umbilical cord vein endothelial cells.

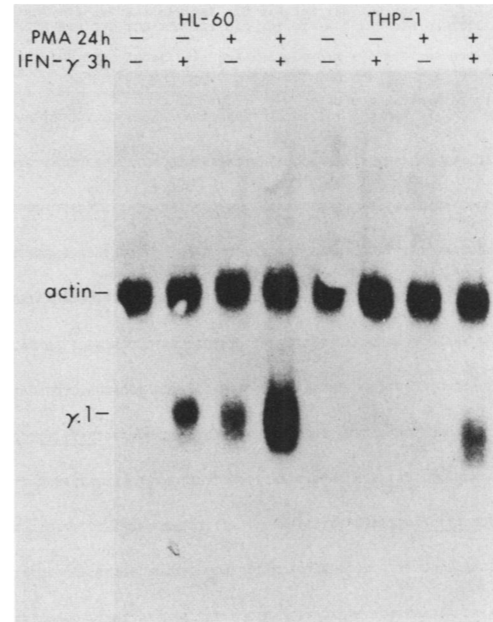

FIG. 6. Inducibility of γ.1 by IFN-γ during differentiation of HL-60 and THP-1 cells. Cells were treated with PMA (10 ng/ml) for 24 h and then with IFN-γ (100 U/ml) for 3 h. RNA was isolated, fractionated, transferred, and probed with γ.1 cDNA.

THP-1 cell lines was puzzling, since they are both thought to be in the mononuclear lineage. Phorbol-12-myristate-13-acetate (PMA) is known to be capable of inducing HL-60 cells and THP-1 cells to differentiate toward the monocyte-macrophage lineage (26, 32). After treatment with PMA for 24 h, the cells display adherence and other characteristics of monocytes and macrophages. Brief pulses of IFN-γ after PMA treatment induced high levels of γ.1 expression in HL-60 cells and moderate levels in THP-1 cells (Fig. 6). This induction was also transcriptional, as indicated by a nuclear run-on experiment on differentiated HL-60 cells (data not shown).

Nucleotide sequence analysis of γ.1 cDNA. The 1-kilobase γ.1 cDNA was cloned into M13mp18 and M13mp19 phage vectors. Overlapping subclones covering both strands were obtained by restriction or deletion (data not shown). The nucleotide sequence, identified in independently isolated sets of phage clones, revealed the longest open reading frame with two adjacent methionine codons at the initiation site. The fidelity of the sequencing data was recently confirmed by sequencing another independent cDNA clone and the genomic γ.1 DNA (data not shown). This open reading frame of 114 base pairs predicts a peptide of 38 amino acids. There is a classical polyadenylation signal, AAUAAA, 13 bases from the start of the poly(A) tail (Fig. 7, underlines). While the 5′ untranslated region is rather short, the 3′ untranslated region is very long. The 3′ half of the cDNA sequence is highly AT-rich. Computer-assisted searches of all eucaryotic DNA sequences in the National Institutes of Health data base failed to reveal any significant homology to previously described mRNA sequences.

In vitro translation of the γ.1 transcript. γ.1 cDNA was cloned into the plasmid pGEM-1, which contains the promoters for the SP6 and T7 polymerases. Since only the ^{32}P-labeled T7 transcript hybridized to γ.1 mRNA from the induced cells (data not shown), the SP6 transcript represented the sense RNA. The sense transcript was translated

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                   Met Gln Ser Met Prc Gln Ser Pro Ala Val Ile Thr Ser Pro
  1 GGTTTATTTTCCAG ATG CAA TCA ATG CCC CAG TCA CCT GCT GTT ATA ACT TCA CCA

    Ile Gly Arg Ser Gln Cys Arg Gly Ser Arg Ala Ile Glu Glu Ser Pro Ala Ala
 58 ATA GGA AGA TCT CAG TGC AGA GGC TCG CGA GCT ATA GAA GAA TCA CCA GCA GCA

    Ser Val Pro Asn Lys Leu OP
112 AGT GTC CCA AAC AAG CTG TGA TGTGAGTTCAGCACACCAACCTTCCCTGGCCTGAAGTTCTTCC

177 TTGTGGAGCAAGGGACAAGCCTCATAAACCTAGAGTCAGAGAGTGCACTATTTAACTTAATGTACAAAGGT

248 TCCCAATGGGAAAACTGAGGCACCAAGGGAAAAAGTGAACCCCAACATCACTCTCCACCTGGGTGCCTATT

319 CAGAACACCCAATTTCTTTAGCTTGAAGTCAGGATGGCTCCACCTGGACACCTATAGGAGCAGTTTGCCCT

390 GGGTTCCCTCCTTCCACCTGCGTTCCTCCTCTAGCTCCCATGGCAGCCCTTTGGTGCAGAATGGGCTGCAC

461 TTCTAGACCAAAACTGCAAAGGAACTTCATCTAACTCTGTCCTCCCTCCCCACAGCTTACAGACCATTGTG

532 GCAAGGAGATCTGTGCTGACCCCAAGCAGAAGTGGGTTCAGGATTCCATGGACCACCTGGACAAGCAAACC

603 CAAACTCCGAAGACTTGAACACTCACTCCACAACCCAAGAATCTGCAGCTAACTTATTTTTCCCTAGCTTT

674 CCCCAGACACCTTGTTTATTTTATTATAATGAATTTTGTTTGTTGATGTGAAACATTATGCCTTAAGTAAT

745 GTTAATTCTTATTTAAGTTATTGATGTTTTAAGTTTATCTTTCATGGTACTAGTGTTTTTTAGATACAGAG

816 ACTTGGGGAAATTGCTTTTCCTCTTGAACCACAGTTCTACCCCTGGGATGTTTTGAGGGTCTTTGCAAGAA

887 TCATTAATACAAAGAATTTTTTTTAACATTCCAATGCATTGCTAAAATATTATTGTGGAAATGAATATTTT

958 GTAACTATTACACCAAATAAATATATTTTTGTACAAAAAAAAAAAAAAAAAAAAA
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FIG. 7. Nucleotide sequence and predicted amino acid sequence of γ.1. Numbers on the left indicate nucleotide positions. The polyadenylation signal and the repeats with high homology to the octameric consensus sequence are underlined.

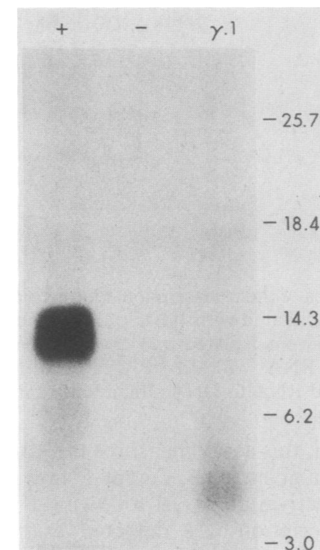

FIG. 8. Analysis of in vitro translation product of γ.1. γ.1 transcripts were synthesized from pGEM γ.1 by SP6 polymerase. Samples of this RNA were incubated in an in vitro wheat germ translation reaction in the presence of [^{35}S]methionine. Symbols: +, positive control with globin mRNA; −, negative control. Low-molecular-weight markers (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) were then prestained.

in a wheat germ system, and the product was analyzed by polyacrylamide gel electrophoresis. The autoradiograph (Fig. 8) shows a 4.0-kilodalton polypeptide, as predicted from the cDNA sequence. The diffuseness of the band may be explained by the small size of the polypeptide and by translation from two slightly different initiation sites.

DISCUSSION

Regulation of gene expression by IFNs is of interest both because of the varied and important biological effects of these molecules in stimulating antiviral, antiproliferative, and immunological responses and because they provide a useful system for studying transcriptional control initiated by a natural ligand-plasma membrane receptor interaction. From studies in many laboratories, approximately 30 cellular genes have now been identified as being transcriptionally activated by various IFNs (25). Because macrophages play a major role in resistance to infection and are activated principally by IFN-γ, it was of interest to analyze genes selectively activated in cells of the mononuclear phagocyte lineage by IFN-γ. It was previously established that the levels of expression of the major histocompatibility antigens, both class I and class II, can be augmented in primary macrophages or macrophage cell lines by IFN-γ, which can enhance the ability of these cells to present antigens to T cells. Two genes, IP-10 and IP-30, have been found to be selectively induced by IFN-γ, and the former has significant sequence homology to platelet proteins, suggesting that it may be involved in inflammatory responses. The isolation and characterization of the novel IFN-γ-inducible gene γ.1 from the human U937 macrophagelike cell line may offer some insights into the regulation of mononuclear phagocytes by this lymphokine.

Although IFN-γ exerts unique effects on macrophages, previously described IFN-γ-regulated genes have been found to be expressed in a wide variety of cells and cell lines. However, in our studies the expression of γ.1 was not detected in B cells, T cells, fibroblasts, or keratinocytes, and it appears to be restricted to mononuclear phagocytes and endothelial cells (Fig. 5). We have a novel opportunity to study mechanisms of cell-type-restricted transcriptional activation by IFN-γ in these important specialized cells of the immune system.

The induction of γ.1 mRNA is very rapid and transient (Fig. 1A). In the present experiments, transcription was detectable within 5 min in nuclear run-on experiments (Fig. 4), and steady-state accumulation was maximal after 3 h (Fig. 1A). In contrast, accumulation of most other IFN-γ-inducible messages occurs more slowly and is more sustained. For example, in the same U937 cell line, another IFN-γ-inducible gene, IP-30 (18), was induced after 18 h and maintained for days. The only other IFN-γ-responsive gene with similar kinetics is IP-10 (17), with maximal accumulation at 5 h. How the signals of a single IFN-γ receptor (4) are transduced and modified to regulate transcriptional activation of different genes with widely varying temporal expression remains a challenging question.

Although γ.1 and IP-10 are both early-response genes, their signal transduction pathways may differ in interesting ways. PMA induces γ.1 but not IP-10. Because the only known function of PMA is to activate protein kinase C (24), this result suggests that activation of γ.1 requires protein kinase C while IP-10 does not (8). Thus, even the regulation of early-response genes is diverse and complex.

Although most of the IFN-induced mRNA accumulation involves transcriptional activation, posttranscriptional regulation seems to be involved as well (9). Even the two genes induced by IFN-γ in U937 cells, IP-30 (18) and IP-10 (17), seem to involve posttranscriptional events. In this respect γ.1 is unique, since its activation by IFN-γ is entirely transcriptional (compare Fig. 1 with Fig. 4).

The relatively long, AT-rich, 3′ untranslated region of γ.1

mRNA is uncommon but has been found in several other transiently induced mRNAs (5), including IP-10 (17). The conserved sequence TTATTTAT is thought to play a role in the high turnover rate of the mRNAs for certain lymphokines, cytokines, and proto-oncogenes (27). The presence of three repeated sequences in the 3′ untranslated region of γ.1 with high homology to this octameric consensus sequence (see Fig. 7) suggests that they confer instability on γ.1 mRNA.

The polypeptide encoded by the longest open reading frame of the γ.1 mRNA, confirmed by in vitro translation, is very small, and its function is currently unknown. It is an intriguing possibility that the variable degree of expression of γ.1 mRNA in primary monocytes and three different transformed macrophagelike cell lines thought to represent different stages in differentiation (i.e., promyelocytic HL-60, promonocytic THP-1, and macrophagelike U937 cells) may correlate with and serve as a useful marker for increasing degrees of macrophage differentiation. In this context, it is possible that the γ.1 protein, which has apparently no signal or transmembrane sequence, or RNA could have a regulatory role in differentiated macrophage function.

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