0022-1767/88/1413-1012$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 141, 1012–1017, No. 3, August 1, 1988
Printed in U.S.A.

IFN-γ INHIBITS C-*myc* GENE EXPRESSION BY IMPAIRING THE SPLICING PROCESS IN A COLONY-STIMULATING FACTOR DEPENDENT MURINE MYELOID CELL LINE

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Proliferation of NSF-60.8 cells, a CSF-dependent murine myeloid cell line, is strongly inhibited by murine IFN-γ. Northern analysis of growth arrested, NSF-60.8 cells activated by addition of CSF in the presence or absence of IFN-γ indicated that IFN-γ inhibited the induction of c-*myc* mRNA steady state level by CSF. The effect was observed as early as 30 min after induction and the inhibition was complete after 20 h. IFN-γ did not impair the transcriptional activation of c-*myc* gene, and it had only a slight destabilizing effect on the mature c-*myc* message. Study of the processing steps of c-*myc* mRNA precursor indicated that in the presence of IFN-γ, a putative 3.6-kb splice intermediate accumulated instead of the mature message, suggesting that IFN-γ inhibits the splicing of c-*myc* precursor.

Proliferation of bone marrow cell precursors in vitro is strictly dependent upon the presence of CSF (1, 2). The recent isolation of CSF-dependent myeloid cell lines (3) allows the study of the intracellular events related to growth regulation in myeloid cells.

The c-*myc* gene consists of three exons and two introns, the first exon being untranslated (4). In most systems, activation of the c-*myc* gene is strictly a function of the cell cycle. The expression of c-*myc* gene is universally associated with growth factor directed S phase progression (5–8). When the viral analogue v-*myc* gene is introduced into CSF dependent cell lines, growth factor dependence is abrogated (9). As c-*myc* protein is a nuclear protein and with expression tightly associated with cellular proliferation, the c-*myc* gene is thought to be part of the genetic elements involved in growth regulation. Polypeptides of the IFN family, IFN-α, IFN-β, and IFN-γ, first described for their antiviral activity, have been shown to exert an antiproliferative effect on several cell types (10, 11). In particular, IFN of all classes are known to strongly inhibit the growth of bone marrow hemopoietic precursors in vitro (12–15), suggesting a physiologic role in the control of hemopoietic growth and differentiation (16). IFN are known to modulate gene expression in their target cells, inducing the expression of several genes, such as 2,5 A synthetase, for example (17–21), and enhancing the expression of other genes such as MHC class I Ag (22). IFN also inhibit the expression of a subset of genes related to growth induction. In particular, IFN inhibit the expression of the nuclear proto-oncogene c-*myc* (23–28), in most cases, apparently, by destabilizing the mature message (23, 25). The effects of IFN-γ on gene expression seem to be delayed in time as compared with the other types of IFN (20), although studies have generally been performed on nonsynchronized malignant cells. Here we describe the effect of IFN-γ on a sensitive CSF-dependent murine myeloid cell line, NSF-60.8. The advantage of using such a growth factor dependent cell line is that the cells can be growth arrested by serum and growth factor deprivation. This allowed us to study the effect of IFN-γ on the induction of c-*myc* mRNA by the growth factor rather than on aberrant proto-oncogene expression characteristic of malignant myeloid phenotypes. We show that recombinant murine IFN-γ exerts a strong antiproliferative effect on the NSF 60.8 cells. At the level of nuclear proto-oncogenes induction, IFN-γ inhibits the induction of c-*myc* steady state level of mRNA by CSF. The inhibition seems to be an early event. IFN-γ does not inhibit the transcriptional activation of the c-*myc* gene but rather seems to impair the post-transcriptional splicing process.

MATERIALS AND METHODS

Cell culture and thymidine incorporation assays. NSF-60.8 murine myeloid cells (a kind gift of Dr. Donna Rennick, DNAX Corp. Palo Alto, CA) was maintained in RPMI 1640 (Cellgro, Washington, DC) with standard concentrations of glutamine, antibiotics, and 10% FCS (HyClone Laboratories, Sterile Systems, Inc., Logan, UT) supplemented with 40 ng/ml of r-mu-GM-CSF[2] (provided by Immunex Corp. Seattle, WA).

Proliferation was assayed in microtiter plates (10^4 cells per well in a volume of 0.2 ml) in triplicate, by a 6-h [^{3}H]thymidine (1 μCi/well, 120 Ci/mmole, Amersham Corp., Arlington Heights, IL) pulse after a 24-h period of incubation.

Northern analysis. NSF-60.8 growth was arrested by extensive washing to remove the growth factor followed by a 6-h period in low serum medium (1% FCS) at 10^6 cells per ml. r-mu-GM-CSF (100 ng/ml), in the presence or absence of 100 u/ml of r-mu-IFN-γ (Genentech Corp., South San Francisco, CA) was added as indicated. Cells were harvested after the indicated period of time, washed once in PBS, and resuspended in guanidium-isothyocyanate followed by purification of RNA on a standard cesium chloride gradient (29).

Equal amounts of RNA (20 ug of total RNA) were denatured with 6% formaldehyde for 10 min at 55°C in 1 × MOPS (20 mM morpholine propane sulfonic acid pH 7.1, 5 mM sodium acetate, 1 mM EDTA) and 50% deionized formamide (Fluka Chemical Company, Hauppage, NY), quickly chilled on ice, and size fractionated on 1% agarose gels containing 6% formaldehyde. After soaking of gels in 20 × SSC (1 × SSC = 150 mM sodium chloride, 15 mM trisodium sodium citrate, pH 7), RNA was transferred onto nitrocellulose filters (BA 85, Shleicher Schuell, Keene, NH) by capillary blotting in 20 × SSC using

Received for publication January 29, 1988.
Accepted for publication April 26, 1988.
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[2] Abbreviations used in this paper: r-mu-GM-CSF, recombinant murine granulocyte macrophage CSF; SSC, standard saline citrate.

standard procedures (30). Filters were then baked 2 h under vacuo at 80°C.

All probes used in this study were purified inserts, isolated after appropriate restriction on 1% low-melting-point agarose (Bethesda Research Laboratories, Bethesda, MD), ^{32}P labeled to $2 - 5 \times 10^8$ cpm/μg by primer extension using the Polymeraid labeling kit (PLS) and ^{32}P-dCTP (3000 Ci/mM) (Amersham), and purified on NACS columns (Bethesda Research). The c-*myc* probe was the 1.4-kb *Eco*RI–*Cla*I fragment of the third exon of human c-*myc* (31). The c-*myb* probe was the 0.5-kb *Eco*RI fragment of mouse c-*myb* (32). The mouse c-*myc* intron A probe was a 0.8-kb *Xba*I-*Bam*HI fragment of mouse c-*myc* plasmid (33), kind gift of Dr. Michael Dean. Filters were soaked for 2 to 24 h in prehybridization buffer (5 × SSC, 50% formamide, 1 × Denhardt, Tris pH 7.4 20 mM, 0.2 mg/ml salmon sperm DNA, 5% dextran sulfate) and hybridized with 5 to 10 ng/ml of labeled probes in the same medium for 20 to 48 h at 42°C. Filters were washed several times in 2 × SSC, 0.1% SDS at room temperature, 1 to 3 times 15 min in 0.1 × SSC, 0.1% SDS at 50°C and exposed to Kodak XAR-5 films with Cronex lighting plus intensifying screens (E.I. du Pont de Nemours & Co., Inc., Wilmington, DE) at −70°C for 3 to 48 h. For relative quantities of hybridized radioactive probe, autoradiographs were scanned using a densitometer, and the relative intensities of the bands were estimated by weighing the densitometer profiles. Hybridized radioactive probe was then removed by immersion of the nitrocellulose filters in boiling water. Filters were checked before use with other probes.

Nuclear run off. Nuclear run off experiments were performed essentially as described by Greenberg and Ziff (34) with minor modifications. NSF-60.8 cells were growth arrested and activated by addition of r-mu-GM-CSF in the presence or absence or r-mu-IFN-γ, as described above. At different time points, cells were washed with PBS and lysed in Nonidet P-40 0.5% (v/v), 10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$. Nuclei were isolated by centrifugation (500 rpm, 5 min), resuspended in 50 mM Tris-HCl pH 8.3, 5 mM $MgCl_2$, 0.1 mM EDTA, 40% glycerol (v/v), and frozen down in liquid nitrogen. For run-off experiments, nuclei were thawed and mixed with an equal volume of 5 mM Tris-HCl pH 8, 2.3 mM $MgCl_2$, 140 mM KCl, 0.5 mM each of ribo-ATP, ribo-CTP, and ribo-GTP and containing 0.4 mCi of ^{32}P-UTP (3000 Ci/mMole) (Amersham), and incubated for 20 min at 27°C. RNA were then purified on a guanidium/cesium chloride gradient as described above. ^{32}P-labeled-RNA were treated with 0.2 M NaOH for 10 min on ice, neutralized with 0.24 M acid-free HEPES, and ethanol precipitated. Incorporated radioactive precursor was determined in each sample by TCA precipitation, and equal amounts of counts ($1.5\ 10^6$/ml) were hybridized on nitrocellulose spotted plasmids (prepared as follows: 20 μg of linearized plasmid were denatured by incubation in NaOH 0.2 M at room temperature for 0.5 h, neutralized by addition of 10 volumes of 6 × SSC and spotted onto nitrocellulose filters using a Schleicher Schull Slot Blot device (Minifold II); filters were backed 2 h as described above), in 2 ml of 10 mM Tes pH 7.4, 0.2% SDS, 10 mM EDTA, and 300 mM NaCl, at 65°C for 18 to 24 h. Filters were washed in 2 × SSC at 65°C and treated with 10 μg/ml of RNase A in the same medium for 0.5 h at 37°C. After a last wash in 2 × SSC at room temperature, filters were exposed to XAR-5 Kodak films, as described above.

S1-nuclease protection. S1-nuclease protection analysis was performed essentially as described by Weaver et al. (35) with minor modifications. NSF-60.8 cells were growth arrested and activated by addition of r-mu-GM-CSF in the presence or absence of r-mu-IFN-γ and total RNA were purified on a guanidium/cesium chloride gradient as described above. The mouse c-*myc* *Bam*HI–*Xba*I 0.8-kb fragment (spanning part of intron A) was labeled by filling the recessed end at the *Bam*HI site with Klenow polymerase I large fragment, using ^{32}P-dGTP and ^{32}P-dATP (3000 Ci/mM) (Amersham) to a sp. act. of $2- 3 \times 10^7$ cpm/μg. 5×10^4 cpm of probe were mixed with 20 μg of RNA in 10 μl of 80% formamide, 400 mM NaCl, 40 mM Pipes pH 6.4, 1 mM EDTA, heated at 85°C for 10 min, and immersed in a 64°C water bath for 16 h. Eight hundred units/ml of Nuclease S1 (C. F. Boehringer & Soehne GmbH, Mannheim, W. Germany) in 280 mM NaCl, 30 mM NaAc pH 4.8, 4.5 mM $ZnSO_4$, 20 ng/ml of salmon sperm DNA, were added to each sample and S1 digestion was performed at 32°C for 30 min. S1 digestion products were analyzed on 4% polyacrylamide-7M urea denaturing gels. Labeled protected fragments were visualized by autoradiography as described above. Controls consisting of the probe alone, without addition of cellular RNA, were run in each experiment and did not give any detectable band (not shown).

RESULTS

Antiproliferative effect of IFN-γ on NSF 60.8 cells. NSF 60.8 cell line is a subclone of the murine myeloid cell line NSF 60 (3) and is derived from a retrovirally (Cas-Br-M MuLV) induced murine leukemia. NSF 60.8 is dependent, for its growth, on the presence of CSF, and can be maintained in culture with r-mu-GM-CSF. The effect of r-mu-IFN-γ on r-mu-GM-CSF induced proliferation was assessed. Cells were first deprived of CSF and serum and then allowed to proceed in the cell cycle by addition of r-mu-GM-CSF, in the presence of various amounts of IFN-γ. Proliferation was then assessed by a standard [^3H] thymidine incorporation assay. As shown in Figure 1*A*, IFN-γ inhibited CSF-driven [^3H]thymidine incorporation in a dose-dependent manner when cells were thymidine pulsed after an overnight incubation (20 h), as well as when cultures were pulsed earlier (5 h after the addition of CSF, for 6 h), indicating that the effect of IFN was rapid. Inhibition could not be overcome by increasing the amount of r-mu-GM-CSF (Fig. 1*B*), suggesting that the effect of IFN-γ was intracellular and probably not related to interference with GM-CSF receptor interaction. Because c-*myc* gene expression is heavily implicated as an obligatory event for cell proliferation, we examined the effect of IFN on CSF-directed c-*myc* steady state level.

IFN-γ inhibits the induction of c-myc mRNA by CSF. Growth arrested NSF 60.8 cells were allowed to proceed in the cell cycle by addition of r-mu-GM-CSF in the presence, or absence, of a inhibiting dose of IFN-γ. At different time points, total RNA were extracted and submitted to Northern analysis, using a probe specific for mature c-*myc* message. As shown in Figure 2, growth arrested cells do not express detectable levels of c-*myc* message (time 0). As early as 30 min after induction by CSF, a steady state level of c-*myc* message becomes detectable and increases again after 2 h of incubation, giving a 30-fold increment over nonstimulated cells, as assessed by densitometry scanning of the autoradiographs (data not shown). IFN-γ by itself did not induce c-*myc* message. When added simultaneously with growth factor, IFN inhibited the GM-CSF induced steady state level of c-*myc* mRNA (Fig. 2). The inhibition occurred very rapidly, since it was observed at the first time point studied (0.5 h), and c-*myc* mRNA was virtually undetectable by 20 h after IFN-γ treatment. Relative quantification of c-*myc* mRNA detected by densitometer scanning of the autoradiographs indicated a 60 to 70% inhibition at early time points and a complete inhibition after 20 h (data not shown).

NSF-60.8 expressed a truncated c-*myb* message, as already shown (3), caused by a retroviral insertion in the sixth exon of the c-*myb* gene (3). The steady state level of this truncated c-*myb* mRNA was barely affected by CSF deprivation (time 0) in this particular cell line, probably related to the truncation of the message. A control probe, specific for 18s r-RNA, did not show any modulation (data not shown).

Because steady state level of a given message in eukaryotic cells is a function of the level of transcription, the rate of processing of the precursor message, and the rate of degradation of the mature message, we next investigated the effect of IFN-γ on these different steps of c-*myc* mRNA processing.

IFN-γ does not inhibit the transcriptional activation of c-myc gene. NSF 60.8 cells were growth arrested by CSF deprivation and induced to proliferate by the addition of r-mu-GM-CSF in the presence or absence of r-mu-

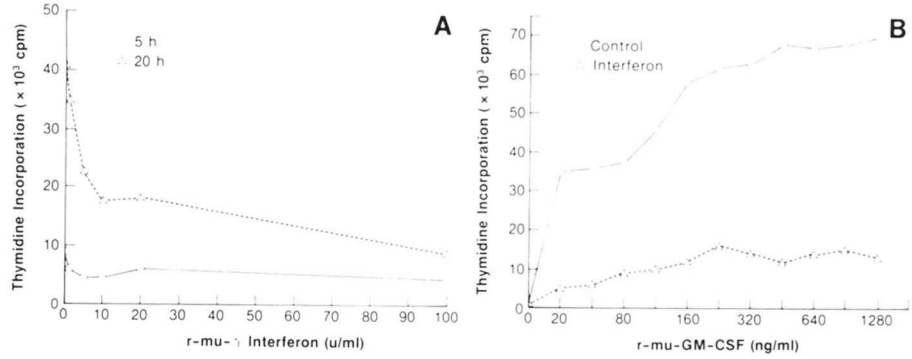

Figure 1. IFN-γ is a strong antiproliferative signal for NSF-60.8 cells. NSF-60.8 cells were growth arrested and stimulated with 40 ng/ml of r-mu-GM-CSF in the presence of various doses of r-mu-IFN-γ (*A*) or with various doses of r-mu-GM-CSF in the presence (△) or absence (○) of 100 u/ml of r-mu-IFN-γ (*B*). (*A*) Cultures were [³H]thymidine pulsed 5 h later (○) or 20 h later (△) for a 6-h period. (*B*) Cultures were pulsed 20 h later.

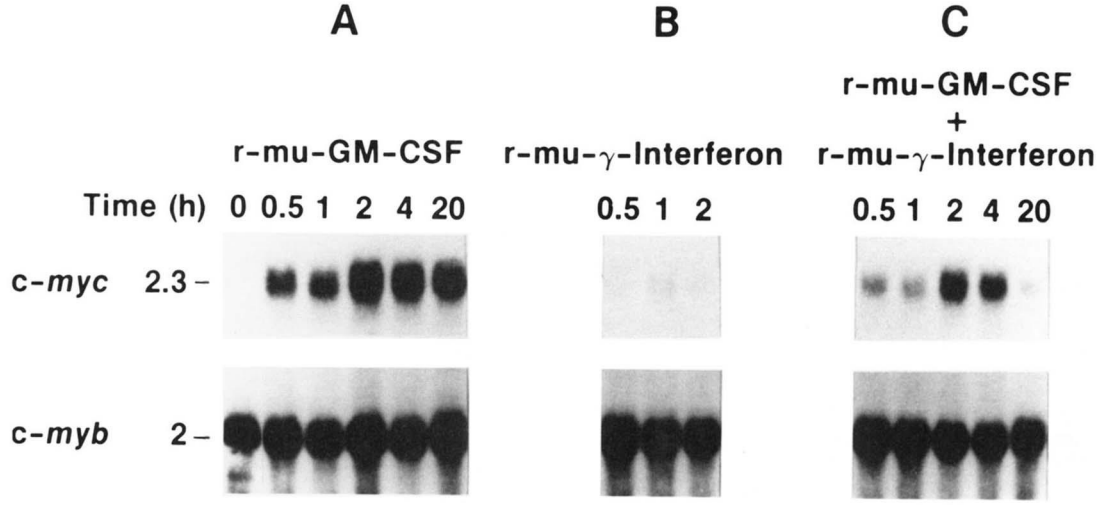

Figure 2. IFN-γ inhibits the CSF-induced steady state level of c-*myc* message. NSF-60.8 cells were growth arrested and stimulated with 100 ng/ml of r-mu-GM-CSF, 100 u/ml of r-mu-IFN-γ, or both, as indicated. At various time points, total RNA were extracted and submitted to Northern analysis using a probe specific for c-*myc* mRNA or for c-*myb* mRNA, as indicated. The size of the message, in kB, is indicated.

IFN-γ. At different time points, nuclei were isolated and ongoing transcriptions allowed to "run on" in vitro, in the presence of ^{32}P-UTP. In vitro transcribed RNA were then purified and detected using nitrocellulose blotted c-*myc* exon 3 or c-*myb* specific probes or a nonspecific plasmid as a control. C-*myc* transcription, activated by the addition of CSF, was not inhibited by r-mu-IFN-γ (Fig. 3), indicating that the inhibitory effect of IFN-γ on c-*myc* steady state accumulation did not occur at the level of the transcriptional activation of the gene. The probe used to assess the level of the transcription corresponds to c-*myc* exon 3. It therefore indicates that the message was normally elongated in the presence of IFN, ruling out the possibility that the elongation of the message is blocked, as has been described in other systems (36). The transcription of c-*myb* was also activated by CSF, indicating that the lack of modulation of steady state level of the message does not result from a constitutive transcriptional activation by the inserted retroviral sequence. Note that the level of transcription of c-*myc* and c-*myb* genes cannot be compared from these results, because the probes used to detect them differ greatly in size.

We next investigated the effect of IFN on the stability of the mature message.

IFN-γ does not significantly reduce the half life of c-myc message in NSF 60.8 cells. Growth arrested NSF 60.8 cells were allowed to proceed in the cell cycle by addition of r-mu-GM-CSF in the presence, or absence of

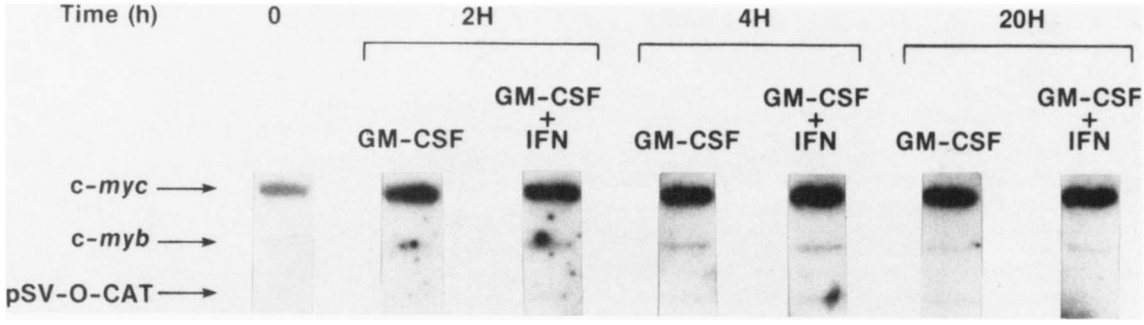

Figure 3. IFN-γ does not inhibit the CSF-induced transcription of c-*myc* gene. NSF-60.8 cells were growth arrested and stimulated with 100 ng/ml of r-mu-GM-CSF in the presence or absence of 100 u/ml of r-mu-IFN-γ, as indicated. At indicated time points, nuclei were isolated and ongoing transcription was allowed to proceed in vitro, in the presence of a radioactive precursor. ^{32}P-labeled RNA were purified and used as a probe on c-*myc*, c-*myb*, or PS-V-O CAT (as a negative control) plasmids.

r-mu-IFN-γ. 4 h later, actinomycin D was added to prevent additional transcription, and RNA were extracted at subsequent time points, and submitted to Northern analysis using a probe specific for c-*myc* exon 3. Several exposures of the Northern blot were scanned using a densitometer, and residual levels of c-*myc* mature message were plotted. Results (Fig. 4), indicated that c-*myc* message was relatively stable in NSF 60.8 cells, with a half life of 1 h 45 min in the absence of IFN. In the presence of IFN, the half life dropped to 1 h 15 min, indicating that destabilization of the mature message may contribute to the inhibitory effect of IFN-γ on the steady state level but cannot account for all of it.

Because the steady state level of a mature message is also a function of the rate of processing of the precursor transcript, we next investigated the effect of IFN-γ on the processing of the mRNA precursor. In fact, IFN-γ has been shown to inhibit the splicing of ribosomal RNA in macrophages (37). We therefore tested the hypothesis that it could have a similar effect on c-*myc* mRNA processing in the myeloid cell line studied here.

Accumulation of c-myc mRNA precursor is enhanced in the presence of IFN-γ. Total RNA, purified at different time points after activation of growth arrested NSF 60.8 cells activated by r-mu-GM-CSF in the presence or absence of IFN-γ, were used to protect a probe allowing the detection of unspliced intermediates and consisting of 0.8-kb fragment of mouse c-*myc* intron A (Fig. 5*A*) against degradation by S1 nuclease. The accumulation of c-*myc* mRNA unspliced precursor was enhanced by the addition of CSF (Fig.5*B*). However, after a short period of time, the steady state level of precursor diminished again. In the presence of IFN-γ the accumulation of the detected precursor was not inhibited, confirming the results obtained by nuclear runoff. In fact, the steady state level of precursor was higher at each time point studied, indicating that, indeed, IFN-γ could inhibit the splicing process of the message. In order to confirm this result, total RNA were also submitted to Northern analysis using the same probe specific for mouse c-*myc* intron A (Fig. 6*A*). Despite nonspecific hybridization caused by the high glucocorticoid content of the probe (62%), the probe hybridized to a ~3.6-kb species (as assessed by comparison with a DNA m.w. marker) on CSF treated cells (Fig. 6*A*). Again, this putative splice intermediate steady state level was higher in the presence of IFN at each time point studied, except after 20 h of incubation. This indicated that, at the early time points, the splicing of this intermediate could be impaired by the presence of IFN. The same Northern blot was probed with a c-*myc* exon 3 fragment (Fig. 6*B*) and showed the pattern of steady state level of mature message in the absence and in the presence of IFN routinely observed.

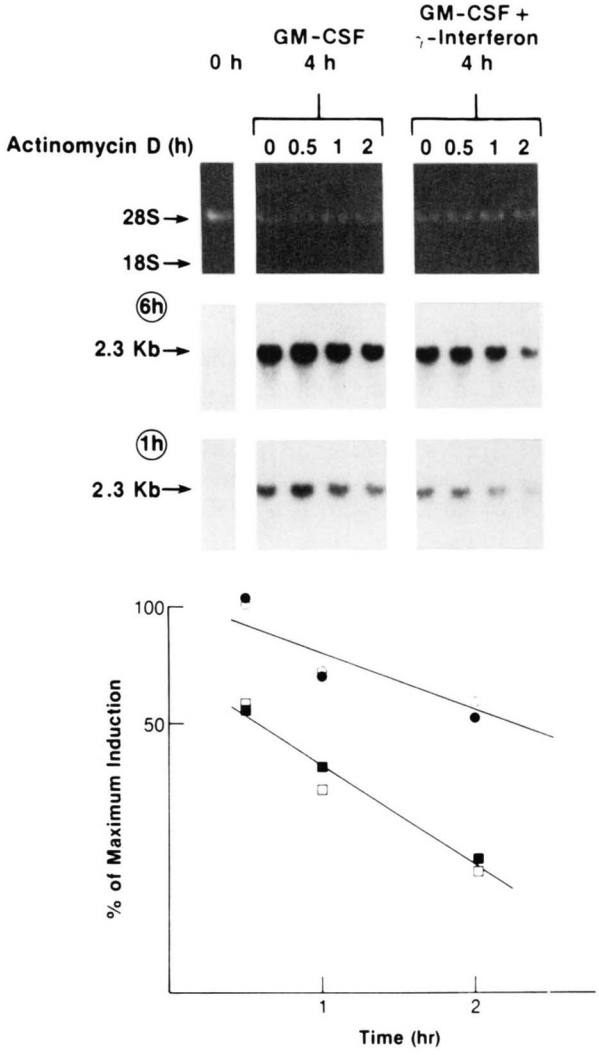

Figure 4. IFN-γ does not significantly destabilize c-*myc* message. Growth arrested NSF-60.8 cells were stimulated for 4 h with 100 ng/ml of r-mu-GM-CSF in the presence or not, as indicated, of 100 u/ml of r-mu-IFN-γ. Actinomycin D was then added, and RNA were isolated after the indicated periods of incubation with actinomycin D and submitted to Northern analysis using a probe specific for c-*myc* message. Several exposures were submitted to densitometric scanning. The figure shows an ethidium bromide staining of the RNA gel (*top*), two exposures of the Northern analysis (1- and 6-h exposures, as indicated), and the result of the quantitative analysis by densitometry scanning (*bottom*). The maximal induction (100%) corresponds to the value obtained with GM-CSF alone, at 30 min after addition of actinomycin D, time of which corresponds to the penetration of the drug.

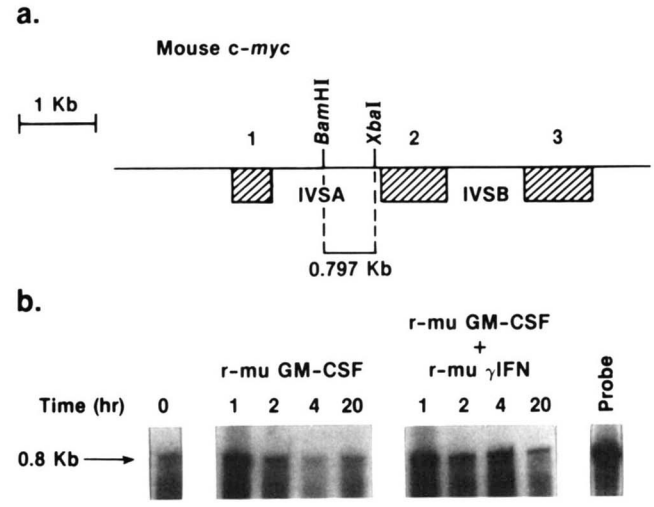

Figure 5. IFN-γ induces an increased accumulation of a putative c-*myc* mRNA precursor. (*a*) Schematic representation of mouse c-*myc* gene, showing the *Bam*HI–*Xba*I fragment used as a probe for S1 analysis. *Boxes* indicate the exons 1, 2, and 3; *IVSA* and *IVSB*, intron A and B. (*b*) RNA were extracted from growth arrested NSF 60.8 cells at various time points after activation by 100 ng/ml of r-mu-GM-CSF in the presence or not, as indicated, of 100 U/ml of r-mu-IFN-γ and used to protect the mouse c-*myc* intron A probe (as shown in a) from S1 nuclease degradation.

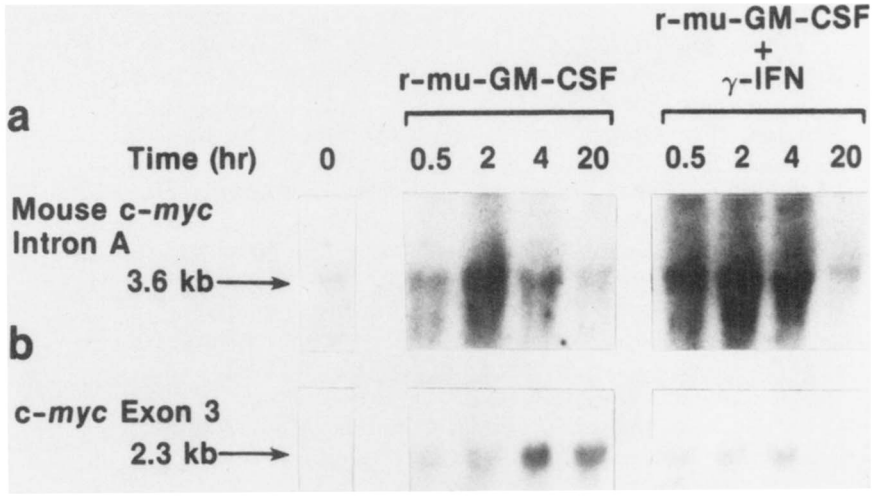

Figure 6. A 3.6-kb transcript expressing a mouse c-*myc* intron A sequence overaccumulates in the presence of IFN-γ. Growth arrested NSF-60.8 cells were stimulated by 100 ng/ml of r-mu-GM-CSF in the presence or not (as indicated) of 100 u/ml of r-mu-IFN-γ. At indicated time points, total RNA were extracted and submitted to Northern analysis using a probe consisting of 0.8 kb of mouse c-*myc* intron A (see Figure 5) (*a*) and a probe specific for c-*myc* exon 3 (*b*). Note that the c-*myc* intron A probe was labeled to a very high sp. act. (8.10^8 cpm/μg).

DISCUSSION

Several lines of evidence indicate that IFN can act as a physiologic growth arrest signal in different tissues. In the hemopoietic system, IFN-γ is known to inhibit the growth of bone marrow precursors (12, 13). In this study, we have taken advantage of the IFN sensitivity of a CSF-dependent murine myeloid cell line to study the effect of IFN-γ on molecular events regulated by the physiologic growth factor. We show that IFN-γ inhibits the entry into S phase and the accumulation of c-*myc* mature message induced by CSF in growth arrested cells. The effect is detectable within 0.5 h. In opposition to other growth inhibitors such as TNF, for example (38), or to IFN-γ in a human cell line (27), IFN-γ does not inhibit the transcriptional initiation or elongation of the c-*myc* gene in this cell line, as assessed by nuclear runoff experiments. The half life of the message was not greatly diminished in the presence of IFN-γ, decreasing from 1 h 45 min to 1 h 15 min. This result is in contrast with what was observed with Daudi cells, a malignant human lymphoblastoid cell line, in which IFN-β significantly destabilized c-*myc* mature message (23, 24). The level of c-*myc* precursor in the CSF dependent NSF.60.8 cells was analyzed by S1 protection or Northern blot analysis. The rate of accumulation of a 3.6-kb species, detected by a mouse c-*myc* intron A probe by both techniques, was significantly enhanced in the presence of IFN. Several arguments favor the hypothesis that this 3.6-kb species is an intermediate splice precursor of c-*myc* message. The steady state level of this species was modulated by the addition of CSF, as assessed by both techniques. The low but detectable amount of the putative precursor in nonstimulated cells is in good correlation with the amount of transcription observed in quiescent cells by nuclear runoff experiments. In the presence of CSF alone, after an initial period of accumulation, both techniques showed a diminution of the steady state level of the putative precursor, exactly as if the rate of splicing of this intermediate was accelerated. Northern analysis showed that, at the same time point, the rate of accumulation of the mature message was enhanced. The high m.w. of this species (~3.6 kb) is in good agreement with a splice intermediate in which intron B (1.7-kb) would have been spliced from the 5.5-kb original precursor. This suggests that intron B is rapidly spliced from the precursor. The resulting splicing intermediate, including only intron A, would be more stable, allowing its detection by S1 protection or on a Northern blot using total RNA. In fact, attempts to detect a precursor in similar experiments using a probe specific for intron B failed to detect any hybridizing species (data not shown), indicating that indeed, the original precursor containing both intron A and B is very unstable.

At early time points, IFN-γ appears to inhibit the splicing process of a 3.6-kb intermediate precursor, or else to stabilize the 3.6-kb intermediate precursor, resulting in accumulation of this precursor instead of the mature message. Such an effect of IFN-γ has been observed on ribosomal RNA in macrophages (37), but to our knowledge it has not as yet been described for modulators of c-*myc* gene expression. After 20 h of incubation, the steady state level of precursor in the presence of IFN-γ was diminished again, as shown by both S1 protection and Northern analysis. At this time, because no effect is observed on transcription, the principal action of IFN could be destabilization of the message.

The effect of IFN-γ seen here on a growth-factor-dependent cell lines provides a useful model in which to study the molecular consequences of two opposite physiologic regulators of cell growth. As the molecular action of CSF on target tissues becomes unveiled, so do the opportunities to test the biochemical basis of antiproliferative signals within an approximate physiologic model. The ability of a potent growth inhibitor such as IFN-γ to regulate the steady state level of c-*myc* mRNA may be a key function to IFN-γ antiproliferative action in normal and malignant growth. High level of c-*myc* mRNA appears to be a universal characteristic of rapidly growing malignant cells and a nuclear consensus event associated with growth factor stimulation. It is of interest to note that c-*myc* gene expression seems to be regulated at each level of the processing of the message, initiation of the transcription (27, 38), elongation of the precursor chain (36), processing of the precursor into mature message (this study), and stability of the mature message (23–25). This tight regulation again suggests a fundamental role for c-*myc* protein in the process of cell growth.

Acknowledgments. The authors are grateful to Dr. Michael Dean for the kind gift of the mouse c-*myc* probe

and for very helpful discussion, and to Dr. Joost Oppenheim and Dan Longo for comment on the manuscript.

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