Inhibition of transcription and translation of globin messenger RNA in dimethyl sulfoxide-stimulated Friend erythroleukemic cells treated with interferon

(differentiation of Friend cells/complementary [³H]DNA-RNA hybridization/globin synthesis)

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ABSTRACT The addition of appropriate doses of interferon (IF) to cultures of Friend erythroleukemic cells inhibits dimethyl sulfoxide (Me₂SO)-stimulated erythroid differentiation. In this study, the synthesis of heme, hemoglobin, and globin mRNA in Me₂SO-stimulated cultures, with or without IF added, was compared. Although the hemoglobin content in Me₂SO+IF-treated cultures was reduced 6- to 9-fold compared to that of cultures treated with Me₂SO alone, there was less than a 2-fold decrease in the amount of heme accumulated.

Globin mRNA, although unchanged in size or base sequence, was reduced in content in the Me₂SO+IF cultures. The level of reduction of globin mRNA was insufficient to account for the lack of globin synthesis. Thus, it appears that IF may operate on two levels—one involving the transcription of globin mRNA and the other involving its translation.

Friend erythroleukemic cells (FLC) undergo erythroid differentiation accompanied by the synthesis of heme and hemoglobin (Hb), the accumulation of globin messenger RNA (mRNA), and erythrocyte-specific membrane changes upon treatment with dimethyl sulfoxide (Me₂SO) (1-6) and other polar solvents (7, 8). Although the mechanism of action is still unknown, evidence suggests that induction of differentiation by these agents may involve their interaction with the cell membrane (9-12). However, stimulation of erythroid differentiation also occurs upon treatment of FLC with structurally unrelated compounds such as butyric acid (13), purine analogues (14), and hemin (15).

The inhibitory effects of interferon (IF) on cell multiplication and functions are well known (16-18). Although there are claims that the factor(s) responsible for the anticellular effect(s) could be physically separated from those that affect antiviral activity (19), evidence by Stewart *et al.* (20) has shown that both activities of IF preparations are indeed intrinsic properties of the covalent structure of interferons. Both antiviral and anticellular activities of exogenous IF are seemingly mediated by interactions with as yet unidentified membrane receptors (21, 22). Sepharose-bound IF is apparently as active as unbound IF (23) and IF treatment of L cells *in vitro* increases their agglutinability by concanavalin A (24).

Because both Me₂SO and IF appear to affect cell membrane functions, we have been studying the effects of combined treatment upon growth and differentiation in the Friend system to monitor the expression of one gene and the synthesis of its specific protein (globin). We have shown that appropriate doses of IF markedly inhibit FLC growth both *in vivo* (25) and *in vitro* (26), as well as Hb synthesis in Me₂SO-stimulated cultures (26). Virus production is also inhibited, although accumulation of viral antigens is increased (27).

The data presented here indicate that Me₂SO-induced FLC treated with IF contain lower amounts of globin mRNA than cells stimulated by Me₂SO alone. In spite of the accumulation of appreciable residual globin mRNA, little globin synthesis is detected. Thus, the evidence suggests that IF affects both the transcription and translation of globin mRNA in these cells.

MATERIALS AND METHODS

Cells. Friend leukemia cells, clone 745A, obtained from C. Friend (New York), were grown in Dulbecco's medium supplemented with 15% fetal calf serum (Eurobio, Paris). The cells were routinely seeded at 10⁵/ml, and portions were incubated simultaneously as follows: (a) untreated cells growing in regular medium (*control*); (b) cells cultured in presence of 1.5% (vol/vol) Me₂SO (*Me₂SO*); (c) cells seeded with 1.5% Me₂SO and given 24 hr later (day 1) 1000 units/ml of mouse IF (*Me₂SO* + *IF*). Incubation was carried out at 37° until day 5.

Interferon. Several preparations of IF, generously provided by I. Gresser (Institut des Recherches Scientifiques sur le Cancer, Villejuif, France) and F. Dianzani (Istituto di Microbiologia, University of Turin, Italy), were used in the present studies. Procedures for IF production, partial purification, and titration have been described (28). IF dosages employed here are given in mouse interferon units, which equal 4 mouse interferon reference standard units.

Globin Antiserum. Rabbits were inoculated with DBA/2 mouse globin (2 mg/week for 3 weeks) extracted according to Rossi-Fanelli *et al.* (29) from gel-analyzed purified Hb. That only one class of antibodies was present was ascertained by immunodiffusion tests against globin itself, and against crude and purified Hb preparations (data not shown).

Preparation of RNAs. Total cell RNAs were prepared according to Preisler *et al.* (30) from pellets of 10 to 50 × 10⁶ cells and stored at -20° until used. Nuclear and cytoplasmic fractions were obtained by subcellular fractionation according to Penman (31), and RNAs were extracted using the phenol/chloroform/isoamyl alcohol technique.

Assay of Globin mRNA Content. (a) *Preparation of [³H]cDNA:* RNA-dependent DNA polymerase of avian myeloblastosis virus was kindly supplied by J. Beard, Life Sciences, Inc., St. Petersburg, FL, through the Office of Program Resources and Logistics, National Cancer Institute, Bethesda, MD. [³H]dCTP (20-30 Ci/mmol) was purchased from the Radiochemical Centre (Amersham). (dT)₁₂₋₁₈ and oligo(dT)-cellulose were obtained from Collaborative Research. *Aspergillus oryzae* S₁ nuclease was obtained from Miles Laboratories and unlabeled deoxynucleotides from Sigma Chemical Co. Mouse globin mRNA was extracted from reticulocytes of mice made anemic by phenylhydrazine administration, and purified by the pro-

Abbreviations: IF, interferon; FLC, Friend leukemic cells; Me₂SO, dimethyl sulfoxide; cDNA, DNA complementary to RNA.

FIG. 1. Kinetics of (A) Hb and (B) heme synthesis and (C) growth in Me_2SO -induced 745A cells or Me_2SO -induced 745A cells given IF on day 1. Cells were counted with a hemocytometer in 0.05% trypan blue. Hemoglobin was measured according to Crosby *et al.* (58). Percentages of benzidine-positive cells were determined according to Orkin *et al.* (59) and are given in parentheses. ^{59}Fe citrate (specific activity 4 mCi/mg of Fe), incubated with fetal calf serum for 30 min at room temperature, was added to the medium at the final concentration of $0.1 \mu\text{Ci}/\text{ml}$ per 10^5 cells. At the intervals indicated, cell samples were washed carefully and lysed in 3.0 ml of H_2O , and heme was extracted according to Scher *et al.* (3). Radioactivities of samples were measured in a Packard well-type γ counter with an efficiency of 10%. $\square$, untreated 745A cells; $\bullet$, Me_2SO -induced 745A cells; $\circ$, Me_2SO +IF-treated 745A cells.

cedure of Forget *et al.* (32). Full length ^3H -labeled DNA complementary to globin RNA (^3H]cDNA), as verified by 3.5% sodium dodecyl sulfate/polyacrylamide gel electrophoresis, was obtained by incubating globin mRNA with avian myeloblastosis virus RNA-dependent DNA polymerase, ^3H]dCTP, and actinomycin D at 37° for 2 hr according to Forget *et al.* (32). After alkaline hydrolysis and Sephadex G-50 gel filtration, ^3H]cDNA (specific activity, 10^7 cpm/ μg) was purified by alkaline sucrose density gradient centrifugation; fractions corresponding to 9–10 S were pooled, neutralized, ethanol-precipitated, and resuspended in water.

(b) ^3H]cDNA-RNA hybridization: All hybridizations were performed in 0.2 M sodium phosphate buffer (pH 6.8) and 0.5% sodium dodecyl sulfate, containing 1000 cpm cDNA and different amounts of RNAs in a final volume of 15 μl . Reaction mixtures placed in sealed capillary tubes were heated at 100° for 10 min, and then incubated for 43 hr at 68° . The amount of ^3H]cDNA hybridized was determined by S_1 nuclease digestion, diluting each sample into 2 ml 0.1 M sodium acetate (pH 4.5), 1 mM ZnSO_4 , and calf-thymus DNA at $10 \mu\text{g}/\text{ml}$ (heat denatured) (Sigma). Digestion was carried out for 40 min at 45° , then trichloroacetic acid-precipitable radioactivity of samples was determined. Background radioactivity (always less than 3%) was measured by S_1 digestion of an RNA-free hybridization mixture. Glassware was treated with a silane and heated at 180° for 3 hr.

Preparation of Carboxymethyl-Cellulose Columns. The procedure described by Clegg *et al.* (33) was followed, employing Whatman CM-52 columns.

Elution was carried out with a linear gradient (200 ml), 5–30 mM sodium phosphate (pH 6.9) in 8 M urea and 0.05 M 2-mercaptoethanol. The gradient was made in an LKB system.

RESULTS

Effect of IF Treatment upon Hb and Heme Production. Treatment of Me_2SO -stimulated 745A cells with 1000 units/ml of IF causes a 6- to 9-fold inhibition of Hb synthesis on day 5, as shown in Fig. 1A, whereas the amounts of ^{59}Fe incorporated into heme are only slightly lower than in cultures stimulated by Me_2SO alone (Fig. 1B). This effect occurs under conditions where FLC double more than twice (Fig. 1C), fulfilling the requirement of one cycle of DNA synthesis in the presence of Me_2SO (34–36) for differentiation, which does not seem to be

FIG. 2. Mouse globin ^3H]cDNA-RNA hybridization curves. Hybridization was allowed to proceed, as described in *Materials and Methods*, for 43 hr at 68° . 1000 cpm (0.1 ng) of ^3H]cDNA was added per reaction mixture. A blank reaction mixture gave a value of 30 cpm. (A) Mouse reticulocyte globin mRNA. (Under our experimental conditions, this preparation contains equal amounts of messenger and ribosomal RNAs.) (B) $\blacksquare$, total cell RNA from Me_2SO -induced 745A cells; $\square$, total cell RNA from Me_2SO +IF-treated 745A cells; $\blacktriangle$, total cell RNA from untreated 745A cells. All samples were collected at day 5 from cell seeding (10^5 cells per ml) and Me_2SO addition (1.5%).

a prerequisite for other inducers (37). Because heme synthesis was not the factor restricting Hb production, we went on to investigate whether globin mRNA was being transcribed.

Production and Characterization of Globin mRNA in Me_2SO +IF-Treated 745A Cells. The saturation curve of reticulocyte globin mRNA hybridized to ^3H]cDNA (Fig. 2A) was used to calculate the amount of globin mRNA present in RNAs from treated cultures. Fig. 2B shows the saturation curve of ^3H]cDNA with whole-cell RNA extracted from Me_2SO - and Me_2SO +IF-treated cells on day 5. The 50% saturation points show that the amount of globin mRNA from Me_2SO +IF-treated cells is about 30% lower than that from Me_2SO -induced cells. Kinetics of globin mRNA accumulation in total cell RNA (Fig. 3A) show quantitative differences between the two experimental conditions; in fact, globin mRNA content of IF-treated cells, on day 2, is 2.5-fold lower than that of Me_2SO -stimulated cells, while it is only 1.5-fold lower on day 5.

To elucidate whether these differences were due to a decreased production or to an impaired flow of mRNA from the nucleus to the cytoplasm, nuclear and cytoplasmic amounts of globin mRNA were determined. Data, shown in Fig. 3B, indicate that in Me_2SO +IF-treated cells there is no evidence of nuclear storage of globin transcripts. Comparison of *insets* from Figs. 3 and 4 suggests that the IF-mediated inhibition of globin mRNA accumulation in total cell RNA can be accounted for solely by inhibition of its accumulation in the cytoplasm.

On day 5, the great decrease in Hb synthesis was unexpected in view of the fact that there was only 30% less globin mRNA in Me_2SO +IF-treated cells than in Me_2SO -stimulated FLC. Therefore, experiments were carried out to determine whether this was due to an alteration in the properties of the globin mRNA. The analysis of the thermal stability of hybrids formed between globin ^3H]cDNA and cytoplasmic RNA from Me_2SO -induced and from Me_2SO +IF-treated FLC showed no difference in the melting point value. Similarly, when glycerol density gradients of the same RNA preparations were analyzed for their capacity to hybridize to globin ^3H]cDNA, the two resulting patterns were superimposable. Within the limitations of these techniques, no major IF-induced alterations in size or base sequence of globin mRNA were detected.

Effect of Interferon on Globin Synthesis. The marked inhibition of Hb production and a somewhat less severe decrease of globin mRNA transcription could not be attributed to an effect on protein synthesis. The arginine pool sizes in Me_2SO -

FIG. 3. (A) Comparison of globin mRNA accumulation in Me_2SO - and $\text{Me}_2\text{SO}+\text{IF}$ -treated 745A cells. Total cell RNAs extracted at the indicated intervals from (●) Me_2SO -induced 745A cells and from (○) $\text{Me}_2\text{SO}+\text{IF}$ -treated 745A cells were hybridized to 1000 cpm of $[^3\text{H}]\text{cDNA}$. Data on the abscissa indicate days after cell seeding and addition of Me_2SO (IF was added on day 1). Data on the ordinates were calculated according to the following formula: $(0.05 \text{ ng of cDNA})/(\mu\text{g of total cell RNA})$ at 50% hybridization value. (Inset) Data were calculated according to the following formula: $100 \times [(\text{ng of globin mRNA in } \text{Me}_2\text{SO}+\text{IF-treated cells})/(\text{ng of globin mRNA in } \text{Me}_2\text{SO-induced cells})]$. (B) Nuclear and cytoplasmic globin mRNA accumulation in Me_2SO - and $\text{Me}_2\text{SO}+\text{IF}$ -treated 745A cells. Experimental conditions and calculations are the same as reported in A. The RNAs employed in hybridization reactions were extracted from nuclear and cytoplasmic fractions obtained as described in *Materials and Methods*. RNAs extracted from Me_2SO -induced 745A cells: ●—●, cytoplasmic fraction; ●+++●, nuclear fraction. RNAs extracted from $\text{Me}_2\text{SO}+\text{IF}$ -treated 745A cells: ○—○, cytoplasmic fraction; ○+++○, nuclear fraction. (Inset) Data were calculated according to the following formula: $100 \times [(\text{ng of globin mRNA in the cytoplasm of } \text{Me}_2\text{SO}+\text{IF-treated cells})/(\text{ng of globin mRNA in the cytoplasm of } \text{Me}_2\text{SO-induced cells})]$.

and $\text{Me}_2\text{SO}+\text{IF}$ -treated cells at various days after cell seeding were found to be similar (data not shown) and there was little difference in the incorporation of $[^3\text{H}]\text{arginine}$ into acid-precipitable material from each at the intervals tested, after short pulses, as well as after continuous labeling (Table 1).

Table 1. Incorporation of $[^3\text{H}]\text{arginine}$ into trichloroacetic acid-precipitable material from Me_2SO - and $\text{Me}_2\text{SO}+\text{IF}$ -treated 745A cells

Day from cell seeding	Time of labeling, hr	Arginine incorporation, cpm/ 10^6 cells	
		Me_2SO	$\text{Me}_2\text{SO}+\text{IF}$
3	1/4	1,540	1,266
	1/2	4,350	4,707
3	24	7,860	8,592
4	24	11,980	13,748

In short-pulse experiments, $[^3\text{H}]\text{arginine}$ ($5 \mu\text{Ci/ml}$, specific activity 270 mCi/mmol) was added to arginine-starved (10 min) cells, which were suspended in otherwise arginine-free medium for the indicated times; in 24 hr continuous labeling experiments, $2 \mu\text{Ci/ml}$ of $[^3\text{H}]\text{arginine}$ were added at the indicated time intervals to cells growing in regular medium. After centrifugation, cell pellets were dissolved in $\text{NaOH}/\text{sodium dodecyl sulfate}$ buffer ($0.01 \text{ M Tris}/0.01 \text{ M NaCl}/0.001 \text{ M MgCl}_2/0.01 \text{ M EDTA}/0.5 \text{ M NaOH}/1\% \text{ sodium dodecyl sulfate}$), and incubated for 30 min at 60° . Aliquots were precipitated in 10% trichloroacetic acid, collected on Millipore filters, and the radioactivity was determined in a liquid scintillation spectrometer.

FIG. 4. Carboxymethyl-cellulose (CM-52) chromatography of acid acetone extracts of radiolabeled lysates of untreated, Me_2SO -, and $\text{Me}_2\text{SO}+\text{IF}$ -treated 745A cells. 745A cells ($10^6/\text{ml}$) were seeded in Dulbecco's medium supplemented with 15% dialyzed fetal calf serum and with $10 \mu\text{Ci/ml}$ of $[^3\text{H}]\text{valine}$ (specific activity 294 mCi/mmol) and $10 \mu\text{Ci/ml}$ of $[^3\text{H}]\text{histidine}$ (specific activity 30 Ci/mmol). IF was added, as usually, on day 1. Cultures collected on day 5 were thoroughly washed, cell pellets were lysed with CCl_4 , and globin was extracted with acid acetone according to Clegg *et al.* (33). Aliquots of each sample ($250,000 \text{ dpm}$) were mixed with 30 mg of globin similarly extracted from DBA/2 mouse hemolysates. The mixtures were chromatographed according to Clegg *et al.* (33) on CM-52 columns developed at pH 6.9 with a 5–30 mM linear salt gradient containing 8 M urea and $0.05 \text{ M 2-mercaptoethanol}$. Thin lines indicate the absorbance at 254 nm of DBA/2 mouse globin added as a marker. From left to right, the peaks are pre- β , β , and α . (A) untreated cultures; (B) Me_2SO -induced cultures; (C) $\text{Me}_2\text{SO}+\text{IF}$ -treated cultures.

To determine whether globin synthesis was selectively impaired, elution profiles of globin chains, separated by CM-52 chromatography, from untreated, Me_2SO -, and $\text{Me}_2\text{SO}+\text{IF}$ -treated ^3H -labeled cells were analyzed (Fig. 4). To each sample, equal amounts of globin extracted from DBA/2 mouse erythrocytes were added to monitor elution of β and α chains. Profile A, obtained from untreated control FLC, shows that no radioactivity is present in β and α chain regions, whereas amounts of labeled material are present in the "pre- β " region (6.1% of total radioactivity recovered). In profile B, obtained from Me_2SO -induced cultures, there are considerable amounts of radiolabeled material, which cochromatograph with authentic carrier adult mouse globin β and α chains in addition to 7.8% of radioactivity present in the "pre- β " region. Profile C, obtained from Me_2SO -induced IF-treated cells, is strikingly different and contains little or no radioactivity in the β and α chain region. However, considerable amounts of labeled proteins are eluted in the "pre- β " region, which represent 19.9% of recovered radioactivity. It is interesting that the increase in percent radioactivity detected in the "pre- β " region of profile C over

Table 2. Immunoprecipitation of eluted pooled fractions of CM-52 columns with rabbit serum against DBA/2 mouse globin

Pooled fractions	Precipitated radioactivity, %	
	Me ₂ SO	Me ₂ SO+IF
44–57 (pre- β region)	63.0	78.7

Fractions from CM-52 chromatographies shown in Fig. 4 were pooled as indicated, exhaustively dialyzed against distilled water, lyophilized, dissolved in 0.9 ml of undiluted rabbit immune serum against DBA/2 mouse globin prepared in our laboratory, and kept at room temperature. After 48 hr, the samples were centrifuged for 20 min at $5000 \times g$. The pellets and 100 μ l aliquots of supernatants, dried at 75°, were dissolved in 0.3 ml of 1 M Hyamine 10X hydroxide and diluted in 10 ml of acidified toluene-based scintillation fluid. The radioactivity was determined in a Packard Tri-Carb. Data are given as percentages of radioactivity recovered in precipitates.

that of profile *B* may account for the amount of radioactivity eluted in the “ β and α chain” regions of profile *B*.

Because similar “pre- β ” peaks have been observed by others (2, 38) and were thought to be related to hemoglobin (2), rabbit serum against DBA/2 mouse globin was used to immunoprecipitate pooled fractions eluted in “pre- β ” regions from the Me₂SO- and Me₂SO+IF cell lysates. Table 2 shows that 63% of the proteins eluted in the “pre- β ” region of profile *B* (Me₂SO-globin) were precipitated by globin antiserum as compared to 78.7% of globin profile *C* (Me₂SO+IF).

DISCUSSION

The studies presented here demonstrate that treatment with high doses of IF causes a distinct reduction in the amount of globin mRNA accumulated and the almost complete inhibition of the synthesis of globin in Me₂SO-stimulated FLC, which had not been observed with lower amounts of IF (39, 40).

The IF-induced reduction of globin mRNA is maximal on day 2 and tends to diminish thereafter (*insets* of Fig. 3). It is possible that the concentration of IF in the culture medium decreases from day 2 to day 5, perhaps because of thermal denaturation and culture metabolic changes (unpublished data), and allows a fraction of the previously uninduced cell population to start synthesizing globin mRNA in that time interval. In addition, the ratio of IF molecules to the number of FLC also diminishes because, even in the presence of IF, the cell population expands, although to a limited extent (Fig. 1C).

Evaluation of nuclear and cytoplasmic amounts of globin mRNA (Fig. 3B) shows that there is no accumulation of globin mRNA-related sequences in the nuclei of IF-treated FLC. This makes it unlikely that IF may prevent globin mRNA processing from its nuclear precursor. Interferon does not affect the size or base sequences of the globin mRNA synthesized in its presence. On the other hand, the possibility that increased degradation of globin mRNA occurs in Me₂SO+IF-treated FLC cannot yet be ruled out. The fact that Hb synthesis is promptly resumed after IF removal argues against an extensive mRNA degradation, as do our preliminary data showing that resumption of Hb synthesis following IF removal also occurs in the presence of α -amanitin concentrations that prevent any *de novo* synthesis of mRNA (unpublished data). The determination of the half-life of labeled globin mRNA in the presence and absence of IF should resolve this issue.

That the observed effects are attributable to IF itself rather than to unknown contaminants of IF preparations is indicated

by the following evidence: (i) effects induced by IF preparations of various degrees of purification (10^6 or more units/mg of protein for Gresser preparations, 10^5 or 10^4 units/mg of protein for Dianzani preparations) are indistinguishable; (ii) mouse IF proved equally effective regardless of the cell type used as source of IF (brain, fibroblasts, ascites cells) and of the various inducers (West Nile virus or Newcastle Disease virus); (iii) rabbit heterologous IF or mouse mock IF (medium from uninduced cultures) was ineffective (data not shown).

In most virus-cell systems investigated, inhibition of translation appears to be the primary mechanism of IF action (41). Nevertheless, there have been reports attributing the effect of IF to inhibition of primary transcription of vesicular stomatitis virus (42, 43) and influenza virus (44) genomes, but these findings are open to dispute (45). Recently, however, the IF-induced inhibitions of transcription of simian virus 40 *early* virus-specific RNA (46) and of early species of single-stranded RNA from reovirus (47) have been described. Further evidence on the simian virus 40 system suggests that the IF primary effect is “either to inhibit the transcription of early virus RNA or to enhance its turnover in the nucleus” (48).

In the Me₂SO-induced Friend cells system, a similar quantitative inhibition of globin mRNA production occurs upon treatment with 5-bromo-2'-deoxyuridine (30, 49). This effect, however, appears to be basically different because bromodeoxyuridine-induced inhibition of globin mRNA synthesis is accompanied by a marked decrease of heme synthesis (49) as well, which could by itself account for the decreased induction of globin gene transcription (15). Thus, the inhibitory action on globin mRNA accumulation is a novel effect of IF since it deals with the transcription of a homologous cellular mRNA.

The almost complete inhibition of globin synthesis observed in Me₂SO+IF-treated FLC is not accounted for by the reduction in globin mRNA production. It is also not dependent upon an overall inhibition of protein synthesis, which was comparable in Me₂SO- and Me₂SO+IF-treated FLC (Table 1).

Of particular interest are the marked differences in the elution profiles of the IF-treated and untreated cultures. Little radioactivity was found in the β and α chain regions of the Me₂SO+IF-treated material, yet the portion of the pre- β material precipitated by a globin antiserum was larger than that of the corresponding region of Me₂SO material. The nature of this antiserum-precipitable material is not yet known. Similar IF inhibitory effects were reported with reference to the production of steroid-induced tyrosine aminotransferase in IF-treated rat HTC cells (50). Translation of mRNAs of conventional cytopathic viruses which do not integrate into host cell genomes is completely blocked so that infectious virus and intracellular viral antigens are eliminated from IF-treated cultures (51).

The observed inhibition of globin mRNA translation in IF-treated FLC is apparently reminiscent of the IF effect on translation of mRNAs of RNA (27, 51–54) and DNA (55) tumor viruses, the common trait being the fact that the corresponding genes either belong to homologous cells or are integrated into the cell genome, respectively. We do not have information about the biochemical steps involved in the IF effects described. Apparently tRNAs restore the translational capacity of cell-free lysates from IF-treated FLC (56).

The mechanisms by which IF interacts with cells remain obscure. Our results suggest that IF has a dual mechanism of action because it appears to have an effect on both transcription and translation of Me₂SO-induced globin mRNA.

It remains to be established whether the membrane is a possible target site for Me₂SO and IF interactions with mammalian cells. The fact that mitotic activity and expression of

genes involved in cell differentiation can be triggered by specific membrane receptors (57) suggests that those compounds that affect differentiation may have this property in common, i.e., the ability to modulate the expression of a specific gene, through an interaction with the cell membrane.

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