Control of Interferon mRNA Levels and Interferon Yields in Butyrate and 5'-Bromodeoxyuridine-treated Namalwa Cells

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SUMMARY

Treatment of Namalwa cells with butyrate or 5'-bromodeoxyuridine (BrdUrd) before induction with Sendai virus caused an increase in the production of both interferon (IFN) and interferon mRNA (IFN mRNA). However, the increase in IFN mRNA did not completely account for the increase in IFN yield. The treatments did not affect the time course of IFN mRNA transcription and translation, or the association of IFN mRNA with polysomes. Likewise, the treatments did not alter the post-translational fate of the IFN produced. We conclude that butyrate and BrdUrd affect IFN production at the level of transcription or processing of IFN mRNA and suggest that increased efficiency of translation provides an additional level of control.

INTRODUCTION

The interferons (IFNs) are a group of biologically active proteins which have attracted a great deal of interest as potential therapeutic agents because of their ability to inhibit both virus replication and the proliferation of transformed cells in culture (Stewart, 1979). The human lymphoblastoid cell line, Namalwa, produces high yields of IFN when induced with Sendai virus, and for this reason it has become an important source of human IFN for clinical use (Finter & Fantes, 1980). The IFN produced by Namalwa cells is a mixture of IFN-α (leukocyte) and IFN-β (fibroblast) in which IFN-α predominates (Interferon Nomenclature, 1980; Havell et al., 1977). Several reports have recently shown that significant enhancement of IFN production can be achieved by treatment of Namalwa cells with butyrate or bromodeoxyuridine (BrdUrd) before induction (Adolf & Swetly, 1979; Johnston, 1980; Baker et al., 1980; Tovey et al., 1977; Baker et al., 1979). These agents are not only useful commercially for increasing IFN yields but also provide a tool for studying the control of IFN production.

Gene expression in a variety of cells is affected by treatment with butyrate or BrdUrd. Butyrate induces differentiation in Friend cells (Leder & Leder, 1975), affects the expression of a range of differentiated cell functions (Prasad & Sinha, 1976), and induces the transcription and translation of new proteins (Reeves & Cserjesi, 1979). Incorporation of BrdUrd into cellular DNA enhances the production of various proteins and enzymes (Bick & Soffer, 1976; O'Brien & Stellwagen, 1977), initiates new mRNA and protein synthesis in murine melanoma cells (Price, 1976), and induces C-type particle production in many types of mouse cells (Besmer et al., 1975).

The levels of IFN mRNA have been measured in Namalwa cells during the course of IFN production by quantitative assay of the IFN mRNA in *Xenopus* oocytes (Morser et al., 1979; Berger et al., 1980). In a preliminary report, the enhancement of IFN yield by butyrate was shown to be accompanied by an increase in mRNA levels (Morser et al., 1980). We decided to examine more closely the relationship between IFN and IFN mRNA levels in both butyrate- and BrdUrd-treated cells, and report our findings here.
Fig. 1. Calibration of the oocyte translation assay. Various concentrations of IFN mRNA were prepared by mixing increasing proportions of RNA from induced untreated cells with RNA from non-induced untreated cells such that the total RNA concentration remained constant (5 mg/ml). These were microinjected into oocytes and the amount of IFN secreted was measured.

**METHODS**

**Cells and virus.** Namalwa cells, obtained from the Wellcome Research Laboratories, Beckenham, Kent, U.K., were grown in suspension culture in medium RPMI 1640 containing 10% newborn calf serum (NCS, Flow Laboratories), 60 μg/ml penicillin, 100 μg/ml streptomycin and buffered with 20 mM-HEPES (Baker et al., 1979). Sendai virus was grown in the allantoic cavity of 11-day-old embryonated eggs using seed stock provided by the Wellcome Research Laboratories. HFF (human fibroblast cells), a gift from Dr T. C. Merigan, Stanford University, Ca., U.S.A., and MDBK (bovine kidney cells) were grown in the Glasgow modification of minimal essential medium (GMEM) containing 10% foetal calf serum (Flow Laboratories). EBTr (embryonic bovine tracheal cells) a gift from Dr J. Vilcek, New York University Medical Center, N.Y., U.S.A., were grown in GMEM containing 10% NCS.

**Treatment of Namalwa cells with butyrate and BrdUrd.** Cells were pelleted and resuspended either at 1 × 10⁶ cells/ml in maintenance medium (RPMI 1640 plus 2% NCS) containing 0.8 mM-butyrate (Baker et al., 1980) or at 5 × 10⁵ cells/ml in maintenance medium containing the appropriate concentration of BrdUrd (Baker et al., 1979), then incubated at 37 °C for the times indicated. Untreated control cells were incubated at the same cell density for the same period of time in maintenance medium.

**IFN induction.** Cells were pelleted and resuspended at 2 × 10⁶ cells/ml in maintenance medium and induced using 100 haemagglutinating units of Sendai virus per 10⁶ cells (Baker et al., 1979). At the appropriate time the cells were pelleted and retained for RNA extraction whilst the supernatant was adjusted to pH 2 by addition of concentrated HCl and stored at 4 °C for 24 h before assaying for IFN activity. Intracellular IFN was determined as described previously (Morser et al., 1980).

**Preparation and extraction of RNA.** Total cellular RNA was extracted using the guanidinium isothiocyanate (Fluka, A. G., Buchs, S. G., Switzerland) technique described previously (Morser et al., 1979). Polysomes were prepared and their RNA extracted as described elsewhere (Morser & Shuttleworth, 1981). Polyadenylated RNA was prepared using oligo(dT)—cellulose.

**IFN assay.** IFN was assayed using the inhibition of nucleic acid synthesis method (Atherton & Burke, 1975) in either MDBK, EBTr or HFF cells. The results are expressed in international units. In these assays one international unit of human IFN-α was found to be equivalent to 10 biological units when assayed in EBTr, 4 biological units in MDBK and 0.25 biological units in HFF cells, using Semliki Forest virus as the challenge virus.

**Oocyte microinjection.** IFN mRNA was assayed by translation in Xenopus oocytes (Colman & Morser, 1979). Batches of 10 oocytes were injected with a total of 500 nl RNA
dissolved in distilled water at a concentration of 5 mg/ml, then incubated at 21 °C for 24 h in 400 μl modified Barth-X medium. The IFN secreted by the oocytes during this period was taken as a measure of the IFN mRNA content of the RNA. The oocyte translation assay was calibrated by injecting into oocytes increasing proportions of induced RNA diluted in a solution containing non-induced RNA so that the RNA concentration was constant. The amount of IFN secreted was directly proportional to the amount of IFN mRNA injected when plotted arithmetically (Fig. 1).

**Incorporation of [35S]methionine.** The rates of incorporation of [35S]methionine into TCA-insoluble material were determined as described elsewhere (Baker et al., 1980).

**Immunoradiometric assay of IFN.** IFN was measured by an immunoradiometric assay using NK2 monoclonal antibody to IFN-α (Secher, 1981; Secher & Burke, 1980). 125I-labelled NK2 and antiserum-coated beads used in the assay were a kind gift from Dr D. Secher, Laboratory of Molecular Biology, Cambridge, U.K.

**RESULTS**

**IFN yields and IFN mRNA levels in treated cells**

Treatment of Namalwa cells with 0.8 mM butyrate or 25 μg/ml BrdUrd for 48 h before induction always resulted in an enhancement of IFN yield at 24 h after induction. We have compared IFN yields and IFN mRNA levels in treated and untreated cells in order to investigate the cause of this enhancement. Fig. 2(a) shows the results of 16 experiments in which it can be seen that both of these agents cause an increase in IFN yield at 8 h after induction. Both the overall yield and the level of enhancement varied. The mean enhancement, calculated from that observed in each experiment, was 77-fold in butyrate-treated cells and 11-fold in BrdUrd-treated cells.

IFN mRNA levels in RNA extracted from treated and untreated cells at 8 h after induction were measured by translation in oocytes. Fig. 2(b) shows the IFN mRNA levels from the same 16 experiments. These indicate that both treatments cause an increase in IFN mRNA levels. Again, the enhancement was variable. The mean enhancement, calculated from that measured in each experiment, was 26-fold in butyrate-treated cells and threefold in BrdUrd-treated cells. The enhancement of IFN yield in treated cells is therefore three- to fourfold greater than the enhancement in IFN mRNA levels. Although the ranges of values obtained from treated and untreated cells overlap, in every experiment butyrate-treated cells produced more IFN and IFN mRNA than did BrdUrd-treated cells, both producing more than untreated cells. We concluded that increased production of IFN was always accompanied by, but not due solely to, an increase in the amount of IFN mRNA.

There are several sources of error to consider when evaluating data such as these. Apart from variations in culture conditions due to media and handling, the IFN assay used is subject to an error of up to twofold (i.e. ± 0.3 log₁₀, J. Shuttleworth & J. Morser, unpublished observation). The efficiency of translation of standard RNA preparations injected into oocytes varied by up to fourfold (i.e. ± 0.6 log₁₀). However, inspection of the scatter diagrams (Fig. 2) shows that even so the data are consistent within each treatment. In order to test the statistical significance of the increase in IFN and IFN mRNA caused by treatment, we applied a one-way analysis of variance test, the null hypothesis being that this distribution of values from treated cells could be accounted for by the variance of data from untreated cells. The probability of obtaining these results by chance was less than 0.1% (i.e. P < 0.001) and, therefore, treatment is significantly affecting both IFN yields and IFN mRNA.

**Time course of IFN and IFN mRNA production in treated cells**

The disproportionate increase in IFN yield could be caused by prolonged transcription and/or prolonged translation of the IFN mRNA. We tested this by comparing the cumulative
IFN yield and IFN mRNA levels at various times after induction of cells that had been treated with butyrate or BrdUrd. As we have reported earlier (Baker et al., 1979, 1980), these treatments did not alter the time course of IFN production. Fig. 3 (a) shows that the increased yields of IFN caused by both treatments were seen at all times. The amount of IFN mRNA in total RNA extracted at these times was measured by translation in oocytes. Fig. 3 (b) shows that in both treated and untreated cells IFN mRNA levels rose to a maximum at 9 h after induction. It was concluded that the time course of IFN mRNA accumulation and decay and its resultant IFN production are unaltered by butyrate or BrdUrd treatment and, therefore, prolonged transcription or translation was not the cause of the enhancement. However, at no time did the increase in IFN mRNA levels completely account for the increase in IFN yield.

Effect of alterations in treatment on the production of IFN and its mRNA

We next investigated the relationship between length of treatment, IFN mRNA levels and IFN yields to determine if an increase in the amount of IFN mRNA always accompanied increased IFN production. We suspended cells in maintenance medium and added 0.8 mM-butyrate or 25 μg/ml BrdUrd at various times from 4 to 48 h before induction. IFN yields and mRNA levels were measured at 8 h after induction and compared with cells suspended in maintenance medium alone for 48 h. We detected increases in both IFN yields and IFN mRNA levels after only 4 h of treatment and with both agents the effects rose in parallel reaching a maximum after 48 h of treatment (Fig. 4). Thus, the two effects closely followed each other but at no time did the increase in mRNA levels completely account for the increase in IFN yield.

Increased IFN mRNA levels also accompanied the increase in IFN yields resulting from treatment of cells with various concentrations of BrdUrd. Both increased rapidly when the concentration was raised from 0 to 10 μg/ml, then gradually declined as the concentration
Butyrate and BrdUrd enhance IFN mRNA levels

Fig. 3. Time course of IFN accumulation and amount of IFN mRNA. The cumulative IFN yields (a) from butyrate-treated (○), BrdUrd-treated (▲) and untreated (■) cells were compared at various times after induction. RNA was extracted from the cells at these times and the amount of IFN mRNA (b) determined by measuring the IFN produced by oocytes microinjected with RNA from butyrate-treated (○), BrdUrd-treated (▲) and untreated cells (■).

Fig. 4. Effect of varying times of treatment on subsequent IFN yields and amount of IFN mRNA. The cumulative IFN yield at 8 h after induction (a) was determined for cells treated with butyrate (○) or BrdUrd (▲) for various times before induction. RNA was extracted from these cells at 8 h after induction and translated in oocytes. The amount of IFN produced (b) by oocytes injected with RNA from butyrate-treated (○) or BrdUrd-treated (▲) cells was then assayed.

Table 1. Effect of combined treatment on IFN yield and IFN mRNA levels*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0–48 h</th>
<th>48–96 h</th>
<th>IFN (log₁₀ units/10⁶ cells)</th>
<th>IFN mRNA (log₁₀ units/10 oocytes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BrdUrd</td>
<td>Butyrate</td>
<td>4·20 (12)</td>
<td>2·20 (11)</td>
<td></td>
</tr>
<tr>
<td>BrdUrd</td>
<td>None</td>
<td>3·70 (4)</td>
<td>1·60 (3)</td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
<td>None</td>
<td>3·50 (3)</td>
<td>1·65 (3)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Butyrate</td>
<td>4·40 (20)</td>
<td>2·40 (18)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>BrdUrd</td>
<td>3·80 (5)</td>
<td>1·65 (3)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>3·10</td>
<td>1·15</td>
<td></td>
</tr>
</tbody>
</table>

* Cells were treated with 0·8 mM butyrate, 25 μg/ml BrdUrd or maintenance medium for the times indicated. At 8 h after induction the accumulated IFN was measured, and total RNA was extracted and microinjected into oocytes. The IFN secreted was used to determine the relative amounts of IFN mRNA. Numbers in parentheses indicate the relative enhancements in treated cells.

was increased further (data not shown). Again the enhancement of IFN mRNA levels did not completely account for the enhancement of IFN yield.

In another set of experiments we investigated the effects of sequential treatment of cells with both butyrate and BrdUrd. Simultaneous treatment would be unlikely to have any additive effect because the inhibition of DNA synthesis by butyrate (Adolf & Swetly, 1979)
would prevent any effects due to the incorporation of BrdUrd (Baker et al., 1979). We tested various regimens of sequential treatment and as Table 1 shows we could find no additive effects on enhancement of IFN yield or IFN mRNA. Therefore, the mechanisms by which these agents affect IFN production are either mutually exclusive, or mediated by common pathways or, alternatively, there is another rate-limiting process in IFN formation so that cells are already producing their maximum yield. Surprisingly though, the conditions of treatment result in increases in IFN mRNA that are equal to the enhancement in IFN yield. It would appear that cells that have been held in maintenance medium for the extended period of these experiments are altered in such a way that IFN mRNA and IFN yields are increased proportionally by treatment with butyrate or BrdUrd.

Effect of treatment on translation of IFN mRNA

Since we have shown that, except for the conditions just described, the increase in IFN mRNA does not completely account for the enhanced yields of IFN from treated cells, we next considered other factors which might give rise to this disproportionality. We considered the possibility that treatment was affecting the relative proportions of RNA classes within the cells. For example, if the proportion of mRNA to ribosomal RNA was decreased by treatment then the specific activity of IFN mRNA in total RNA extracted from these cells would be lower. It is known that butyrate reduces [3H]uridine incorporation by 96% after 36 h treatment (Baker et al., 1980). We observed no significant reduction in the yield of either total or polyadenylated RNA/cell after induction in butyrate- or BrdUrd-treated cells (J. Shuttleworth & J. Morser, unpublished data). Furthermore, the enhancement of IFN mRNA levels was found to be similar when either total or polyadenylated RNA was tested. Therefore, the proportion of mRNA to other RNA is not significantly affected by the treatments.

mRNA species are known to differ in their efficiency of translation particularly between homologous and heterologous systems. It is conceivable that treatment could alter the mRNA population of the cells such that increased competition for ribosomes in the oocyte would result in reduced translation of IFN mRNA. In mixing experiments we co-injected RNA from induced treated and untreated cells together with RNA from non-induced treated and untreated cells. The IFN mRNA was translated with equal efficiency regardless of which non-induced RNA background was used (data not shown). Moreover, when we injected various concentrations of induced RNA from treated and untreated cells we found no difference in efficiency of translation. Therefore, treatment is not selectively affecting the assay of IFN mRNA from treated cells.

Alternatively, increased production of IFN by treated cells could occur if the ratio of polysome bound to free IFN mRNA was increased. We tested this by extracting total RNA and RNA associated with polysomes at 8 h after induction in treated and untreated cells. As
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Table 3. Intracellular IFN, its rate of secretion and extracellular accumulation in treated and untreated cells*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cumulative extracellular at 8 h</th>
<th>Secreted from 8–9 h</th>
<th>Intracellular at 8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrate</td>
<td>4.40 (38)</td>
<td>4.10 (16)</td>
<td>2.40 (18)</td>
</tr>
<tr>
<td>BrdUrd</td>
<td>3.60 (6)</td>
<td>3.60 (5)</td>
<td>1.85 (5)</td>
</tr>
<tr>
<td>None</td>
<td>2.80</td>
<td>2.90</td>
<td>1.15</td>
</tr>
</tbody>
</table>

* Cells were treated for 48 h with either 0.8 mM butyrate, 25 μg/ml BrdUrd or maintenance medium. At 8 h after induction the cumulative extracellular yield and intracellular levels of IFN were measured, together with the amount of IFN secreted from 8 to 9 h after induction. Numbers in parentheses indicate the relative enhancement of yield in treated cells.

Table 2 shows, the levels of IFN mRNA were increased to the same extent in both total RNA and polysomal RNA preparations from treated cells. Therefore, the proportion of available IFN mRNA being translated is the same in both treated and untreated cells.

Rates of incorporation of [35S]methionine

If butyrate or BrdUrd treatment caused an overall increase in the rate of protein synthesis after induction then the enhancement of IFN mRNA levels need not match the enhancement of IFN yields as translation would occur more efficiently. For this reason we measured the rates of incorporation of [35S]methionine into acid-insoluble material in treated and untreated cells. Cells were treated for 48 h with either 0.8 mM butyrate or 25 μg/ml BrdUrd, then induced. During the 12 h period following induction [35S]methionine incorporation in treated cells never rose sufficiently above the incorporation in untreated cells (data not shown). The general rate of protein synthesis in treated cells is therefore no greater than in untreated cells and in most experiments treatment resulted in a lower rate of [35S]methionine incorporation. Therefore, an overall increase in the rate of translation is not contributing to increased IFN production.

Intracellular IFN and its rate of secretion

Extracellular yields of IFN need not necessarily reflect the actual amount of IFN synthesized by cells if treatment was affecting the intracellular accumulation or secretion of IFN. We therefore measured intracellular levels of IFN, the rate of its secretion and extracellular accumulation in treated and untreated cells. Cells were treated for 48 h with butyrate or BrdUrd, induced, and after 8 h they were pelleted and the supernatant was used to determine the cumulative extracellular IFN yield. The cells were washed and resuspended in fresh medium for 1 h and the amount of IFN produced was used to determine the rate of IFN secretion. Intracellular IFN was measured in washed cells at the same time. Table 3 shows the effect of butyrate and BrdUrd on each of these parameters. The results clearly show that intracellular levels, rate of secretion and extracellular yields of IFN are equally enhanced. We concluded that we are not simply observing a difference in the post-translational fate of IFN produced by treated cells.

Characterization of IFN produced by treated and untreated cells

Namalwa cells produce several different IFN-α species (Allen & Fantes, 1980), which are known to vary in their relative antiviral activities particularly in heterologous cells (Lin et al., 1978; Stewart et al., 1980; Streuli et al., 1980; Yelverton et al., 1981). For example, IFN-α1 has a markedly higher activity in heterologous cells compared to homologous cells. Thus, an apparent increase in IFN yields could be due to the formation of IFN-α species with a higher
Table 4. Characterization of IFN produced by treated and untreated cells*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Assayed in EBTr (log10 units/10^6 cells)</th>
<th>Assayed in HFF (log10 units/10^6 cells)</th>
<th>RIA (log10 units/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrate</td>
<td>4.40 (38)</td>
<td>4.60 (19)</td>
<td>3.10 (22)</td>
</tr>
<tr>
<td>BrdUrd</td>
<td>3.60 (6)</td>
<td>3.75 (3)</td>
<td>2.60 (7)</td>
</tr>
<tr>
<td>None</td>
<td>2.80</td>
<td>3.30</td>
<td>1.75</td>
</tr>
</tbody>
</table>

* Cells were treated for 48 h with either 0.8 mM butyrate or 25 μg/ml BrdUrd or maintenance medium. At 8 h after induction the cumulative IFN yield was assayed in EBTr cells, HFF cells and by immunoradiometric assay (RIA). Titres are all expressed in international reference units. Numbers in parentheses indicate the relative enhancement of yield in treated cells.

Specific activity rather than increased amounts of IFN. IFN-β, on the other hand, has no detectable activity in EBTr cells, and we were interested to see if treatment was having any effect on its production. We therefore assayed the IFN produced by treated and untreated Namalwa cells in homologous cells (HFF), heterologous cells, bovine cells (EBTr) and by an immunoradiometric technique which utilizes the NK2 monoclonal antibody to IFN-α (Secher & Burke, 1980; Secher, 1981). Table 4 shows that a similar enhancement of activity can be measured in both bioassays and in the immunoradiometric assay. The ratio of heterologous to homologous cell activity in these samples and also the samples obtained to measure intracellular IFN and its rate of secretion showed that treatment did not alter the specificity of the IFN produced. Furthermore, the increase in antigenic mass measured by the immunoradiometric assay must be due to IFN-α because of the specificity of the antibody. Therefore, there is a genuine increase in the amount of IFN-α synthesized by butyrate- and BrdUrd-treated cells. The absence of any additional enhancement of activity in homologous cells, above that seen in heterologous cells, indicates that the proportion of IFN-β is not affected by treatment.

The IFN produced by the translation in oocytes of polyadenylated RNA from treated and untreated cells was characterized in the same way. The relative increase in IFN activities of the translation products were the same when assayed in either HFF or EBTr. Similarly, the immunoradiometric assay showed a corresponding increase in antigenic mass showing that the RNA from treated cells is directing the translation of more IFN protein than RNA from untreated cells (data not shown). Thus, the enhancement of IFN mRNA yield by butyrate and BrdUrd represents a genuine increase in transcription and translation and not simply a change in specific activity of the IFN.

**DISCUSSION**

Increased yields of IFN are obtained from Namalwa cells that have been treated with butyrate or BrdUrd before induction. This increase has previously been measured only by enhanced activity in an IFN bioassay. Recent developments have indicated that leukocytes possess at least eight IFN-α genes (Nagata et al., 1980; Goeddel et al., 1981). A similar number of genes are likely to be expressed in lymphoblastoid cells, as at least five distinct polypeptide sequences have been identified in purified Namalwa IFN-α (Allen & Fantes, 1980). This heterogeneity is accompanied by differences in biological properties, and IFN-α species have been shown to differ significantly in their specificities (Lin et al., 1978; Streuli et al., 1980; Stewart et al., 1980; Yelverton et al., 1981). We have shown that the IFN produced by treated cells has the same species specificity as that produced by untreated cells, and that the enhancement of IFN yield can be measured in both homologous and heterologous cells. Moreover, we have demonstrated that this enhancement of activity is accompanied by a corresponding increase in the amount of IFN-α protein measured in the immunoradiometric assay.
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assay using a monoclonal antibody to IFN-α. Therefore, butyrate and BrdUrd are not causing the production of IFN species with higher specific activity or different heterologous cell activity.

We have investigated the mechanism of the effect of butyrate and BrdUrd on IFN production by measuring IFN mRNA levels in treated cells by microinjection of RNA into oocytes and have shown that enhanced IFN yields are always accompanied by an increase in the amount of translatable IFN mRNA. When treatment conditions were varied the increases in IFN mRNA were found to parallel the increases in IFN yield. Oocytes have been shown to translate faithfully and process the products of a wide range of mRNAs, including IFN mRNA (Cavaliere et al., 1977; Lane et al., 1980). We have confirmed that the antiviral activity secreted by oocytes is due to IFN-α by using the immunoradiometric assay, and also that this activity is directly proportional to the concentration of induced RNA injected.

Treatment does not affect the efficiency with which extracted IFN mRNA is translated in oocytes and, therefore, this method accurately reflects the relative amounts of IFN mRNA in treated and untreated cells. However, under most conditions the increase in IFN mRNA is not sufficient to account entirely for the increase in IFN yield. Since the time course of IFN production and the accumulation and decay of its mRNA are not altered by treatment, we conclude that other factors must contribute to the enhancement of IFN yield in addition to elevated mRNA levels. We have shown that the association of IFN mRNA with polysomes is not altered by treatment, which is not surprising since >80% of the IFN mRNA is already associated with polysomes in both treated and untreated cells (J. Shuttleworth & J. Morser unpublished observations). Similarly, treatment does not alter the post-translational fate of IFN. There are two possible explanations to account for the disproportionality. Firstly, the relationship between the amount of IFN mRNA and its product may differ in oocytes and Namalwa cells, such that the increase in concentration of IFN mRNA leads to a disproportionate increase in translation by Namalwa cells but a proportionate increase in oocytes. Secondly, the treatments may be affecting the efficiency with which IFN mRNA is translated in Namalwa cells. The data shown in Table 1 suggest that the second possibility is more likely to be correct since under these conditions of treatment there is no disproportionality between increase in IFN mRNA assayed by oocyte translation and IFN made by Namalwa cells. In other words, treatment can alter the efficiency of IFN mRNA translation in Namalwa cells. Furthermore, the data shown in Fig. 2 (a, b) can be used to compare the relationship between amounts of IFN mRNA and IFN yields in treated and untreated cells. If treatment affects the efficiency of translation then the correlation between IFN mRNA and IFN yield will show differences. When the data are plotted, the lines of best fit show markedly different slopes for the dose-response curves in treated and untreated cells. When the values for treated and untreated cells are plotted together the deviance of points is considerably more than when the data are taken separately, thus favouring the interpretation that butyrate and BrdUrd are altering the slopes of the dose-response curve and are therefore affecting the efficiency with which IFN mRNA is translated. It is unlikely that this effect is due to alterations in the population of competing mRNAs as the results obtained from co-injection of treated and untreated RNA into oocytes and also the measurements of polysome-associated mRNA (Table 2) would have made this apparent. By exclusion the increased efficiency must be the result of an increased rate of elongation and/or termination or to a larger number of ribosomes on each IFN mRNA molecule.

We conclude that production of IFN by Namalwa cells can be modulated by butyrate and BrdUrd at two levels. Firstly, these agents cause an increase in the amount of IFN mRNA produced after induction. It is not possible to determine whether this is due to increased transcription (or processing) of mRNA from those genes expressed in untreated cells, or to activation of additional IFN-α genes coding for IFN with similar biological and antigenic
characteristics. Secondly, treatment increases the efficiency with which IFN mRNA is translated. A similar pleiotropic effect has been reported for the modulation of tyrosine aminotransferase in hepatoma cells (Snoek et al., 1981). In that case dibutyryl cyclic AMP appeared to be affecting tyrosine aminotransferase synthesis at both the transcriptional and post-transcriptional level.

We have recently reported that superproduction of IFN by Namalwa cells can be achieved by lowering the temperature of incubation of cells after induction (Morser & Shuttleworth, 1981). This phenomenon differs from enhancement by butyrate and BrdUrd in that increased IFN mRNA stability and prolonged translation of IFN are causing the increased IFN yield. Taken together, these findings provide useful information for investigating the control of IFN production after virus induction of Namalwa cells. Firstly, the effects of butyrate and BrdUrd show that IFN production is controlled at the level of transcription. The temperature step-down experiments show that regulation of IFN mRNA stability provides a second point of control, and lastly, the efficiency with which IFN mRNA is translated is also capable of being controlled.

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