Low Temperature Treatment of Namalwa Cells Causes Superproduction of Interferon

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SUMMARY

Interferon production in Namalwa cells, a human lymphoblastoid line, was enhanced by lowering the incubation temperature after induction. The optimum conditions for this effect were established. At the lower temperature interferon synthesis proceeded at a lower rate but continued for longer. Interferon mRNA was shown to be associated with the polysomes for longer after induction. Addition of drugs that inhibit transcription did not prevent the increased production of interferon. Thus, the increased production of interferon is due to the prolonged translation of interferon mRNA.

INTRODUCTION

Human interferons are proteins that have antiviral and other activities (Stewart, 1979). They can be induced after treatment of human cells with viruses or double-stranded RNA to yield a product which can be of two types, α or β, previously called leukocyte or fibroblast interferon (Interferon Nomenclature, 1980). Induction involves the de novo synthesis of interferon mRNA and the corresponding protein. Interferon production ceases after a certain time, and it has been suggested that this is due to the action of a second (repressor) gene product. Thus, the production of interferon provides a model for the control of the synthesis of some eukaryotic proteins.

The control of the formation of β interferon by human fibroblasts has been extensively studied. The judicious use of inhibitors of RNA and protein synthesis enabled a phenomenon termed superinduction to be demonstrated. In superinduction the yields of interferon are substantially increased rather than decreased, by appropriate use of metabolic inhibitors. This effect is probably due to an increase in the half-life of the interferon mRNA, which is lengthened from 18 min to 68 min (Cavalieri et al., 1977).

Superinduction has not been reported for the production of α interferon from leukocytes or lymphoblastoid cells induced with virus (Zoon et al., 1978; Morser et al., 1979). However, yields of α interferon can be enhanced by treating these cells with butyric acid (Adolf & Swetly, 1979; Baker et al., 1980; Johnston, 1980), or with 5-bromodeoxyuridine (BrdUrd; Tovey et al., 1977; Baker et al., 1979), before they are induced. Both of these treatments lead to the presence of more interferon mRNA in induced cells (Morser et al., 1980), suggesting that the increased interferon yield is due to either an increase in the rate of transcription or of processing of the interferon mRNA. However, neither the period of interferon synthesis nor the half-life of the interferon mRNA is altered (J. Shuttleworth, J. Morser & D. C. Burke, unpublished results).

In this paper we describe a treatment that enhances the production of interferon in Namalwa cells, a human lymphoblastoid line, after induction with Sendai virus. This treatment consists of lowering the temperature of incubation after induction. We show that interferon mRNA is found for longer within the cells and that the period of interferon
production is lengthened, and we conclude that the effect is caused by prolonged translation of
the interferon mRNA. The enhanced yields are therefore due to superproduction rather
than superinduction (Stewart, 1979).

METHODS

Cells and virus. Namalwa cells, obtained from the Wellcome Research Laboratories, were
grown in RPMI 1640 medium buffered with 20 mM-Hepes containing 10% newborn calf
serum (NCS, Flow Laboratories), 60 µg/ml penicillin and 100 µg/ml streptomycin. When a
cell count of 1.0 × 10^6 to 1.5 × 10^6/ml was reached, the cells were diluted in the same
medium to a concentration of 0.5 × 10^5/ml. EBTr cells, a gift from Dr J. Vilcek (New York
University Medical School, New York, N.Y., U.S.A.) were grown in Glasgow modified Eagle's
medium (GMEM) containing 10% NCS. Human foreskin fibroblasts (HFF) cells, a gift from
Dr T. Merigan (Stanford University, Ca., U.S.A.) were grown in GMEM supplemented with
10% foetal bovine serum. Sendai virus was grown in the allantoic cavity of 10-day-old eggs
and was harvested after 72 h. Each batch of virus was tested to determine the optimum dose
required for interferon induction.

Interferon induction. Namalwa cells were centrifuged (2000 g for 5 min), resuspended in
RPMI 1640 with 2% NCS at 1 × 10^6 cells/ml, and incubated at 37 °C for 48 h with 1 mM-
butyric acid (Baker et al., 1980). In some experiments Namalwa cells, at a concentration
of 5 × 10^5 cells/ml, were treated with 25 µg/ml BrdUrd (Baker et al., 1979) or were used
without any treatment. After centrifugation the cells were resuspended at 2 × 10^6/ml, Sendai
virus added (Baker et al., 1980) and incubated for a further period in temperature-controlled
water baths. Subsequently the cells were centrifuged and the supernatant fluid was adjusted to
pH 2 with concentrated HCl and left for at least 24 h at 4 °C. Before the samples were
assayed for interferon they were neutralized by the addition of 10 M-NaOH. In order to
measure the intracellular titre of interferon, cells were washed three times in 1 ml cold
phosphate-buffered saline (PBS), lysed by addition of 30 µl concentrated HCl, diluted to 1 ml
with RPMI 1640 containing 2% NCS, and the cell debris was removed by centrifugation. The
samples were then left overnight at 4 °C and neutralized before being assayed.

When the rate of interferon production was to be measured the induced cells were pelleted.
The incubation medium was kept and assayed to determine the cumulative yield up to that
time. The cells were washed with warm medium and incubated for a further hour in medium at
the appropriate temperature before the cells were pelleted and the amount of interferon
produced and secreted into the medium during that hour was measured.

Interferon assay. Interferon was assayed by the RNA-reduction method (Atherton &
Burke, 1975) using EBTr cells. The results are expressed in international units based on the
human leukocyte interferon research standard (69/19). In this assay 1 unit is equivalent to 0.1
international units. In some experiments interferon was assayed on HFF cells, in which case 1
unit is equivalent to 2 international units.

RNA extraction and assay. Namalwa cells, from 30 ml cultures, were pelleted, washed
once with cold PBS before lysis with 4M-guanidinium isothiocyanate (Fluka, A. G., Buchs,
S. G., Switzerland), 1 M-2-mercaptoethanol in 20 mM-sodium acetate pH 4.9. The lysate
was layered over 1.5 ml 5.7 M-caesium chloride and centrifuged overnight at 180000 g at
20 °C in an MSE 6 × 5-5 ml rotor. The RNA was redissolved in 300 µl of a buffer containing
10 mM-tris-HCl pH 7.6, 100 mM-NaCl and 1 mM-EDTA, extracted twice with a 4:1 mixture
of chloroform and n-butanol and precipitated with 2.5 vol. ethanol. The precipitate was
collected by centrifugation, reprecipitated twice more, washed twice with 80% ethanol, dried
and redissolved in water at a concentration of 5 mg/ml. Use of this method ensured that
recoveries of RNA were the same, and that different preparations of RNA caused similar
stimulations of incorporation in an in vitro protein-synthesizing system.
Oocytes from *Xenopus laevis* were microinjected with 30 nl of the RNA solution/oocyte and incubated for 24 h as described previously (Colman & Morser, 1979). The incubation medium was then assayed for interferon activity. In this assay the amount of interferon produced by the oocytes is directly proportional to the amount of interferon mRNA injected. In some experiments the interferon present inside the oocytes was measured by homogenizing the oocytes in 400 μl GMEM + 2% NCS and pelleting the debris before assaying the homogenate.

**Polysome preparation.** Polysomes were prepared from induced cells by a modification of the method used previously (Morser et al., 1979). Cycloheximide was added to the cells to a final concentration of 100 μg/ml. The cells were pelleted, washed in cold PBS containing 100 μg/ml cycloheximide, resuspended and allowed to swell in cold buffer containing 10 mM-KCl, 5 mM-magnesium acetate, 20 mM-tris–HCl pH 7.5, and 100 μg/ml cycloheximide. A detergent, Nonidet P40 (NP40), was added to a concentration of 0.5% and the cells were then lysed by ten strokes of a Dounce homogenizer. All subsequent operations were carried out at 4 °C. Further lysis was prevented by addition of 0.25 vol. 1.25 M sucrose in 460 mM-KCl, 5 mM-magnesium acetate and 20 mM-tris–HCl pH 7.5. The lysate was then centrifuged at 7000 g for 10 min. The supernatant was treated with 0.1 vol. 10% (w/v) sodium deoxycholate, and was then layered over a discontinuous gradient consisting of 2 ml 1 M-sucrose in 100 mM-KCl, 5 mM-magnesium acetate and 20 mM-tris–HCl pH 7.5 above 2 ml 1.8 M-sucrose in the same buffer. The gradients were then centrifuged at 140000 g for 3 h in a 10 x 10 ml angle rotor (MSE). Under these conditions the polysomes were pelleted. RNA was then extracted from them by the method described above. Recoveries of RNA from the polysomes were the same.

**Incorporation of [³H]uridine and [³S]methionine into acid-insoluble material.** Incorporation of precursors into both RNA and protein were followed by the methods described by Baker et al. (1980). Briefly, duplicate 0.5 ml cultures were incubated for 1 h in the presence of 10 μCi/ml [³H]uridine or 20 μCi/ml [³S]methionine (both from The Radiochemical Centre, Amersham). Incorporation was stopped by addition of 5 ml cold 10% trichloroacetic acid (TCA). The precipitate was collected by filtration, washed twice with 5 ml cold TCA, once with cold ethanol, dried and counted.

Protein samples for gel analysis were prepared by washing the 0.5 ml cultures (1 x 10⁶ cells) with warm methionine-free GMEM and resuspending the cells in 150 μl of the same medium containing 60 μCi [³S]methionine. The cells were then incubated for 30 min, pelleted and prepared for electrophoresis as described earlier (Colman & Morser, 1979). Proteins were analysed on 7.5 to 22.5% exponential gradient gels using the discontinuous buffer system of Laemmli (1970). Gels were fixed, stained, dried and autoradiographed as previously described (Colman & Morser, 1979).

**RESULTS**

**Effect of incubation temperature on interferon production**

Cell growth and interferon induction are routinely carried out at a temperature of 37 °C. During experiments on the secretion of interferon (Morser & Colman, 1980), the temperature was lowered after induction to test whether the amount of interferon secreted had changed. Although the proportion of the interferon made that was secreted remained constant, we were surprised to find that the overall interferon yield was increased by lowering the temperature of incubation to 28 °C at 3.5 h after induction. We carried out further experiments to characterize this phenomenon.

The optimum temperature of incubation was determined by inducing the cells at 37 °C and subsequently shifting cells from 37 °C to another temperature at 3.5 h or 7 h after induction
Fig. 1. Effect of altering the incubation temperature on interferon production. Cells were induced at 37 °C and the temperature was changed 7 h later. The cells were pelleted at 24 h and the interferon in the supernatant assayed.

Fig. 2. Effect of altering the incubation temperature at different times after induction. Cells were induced at 37 °C and their incubation temperature was changed at various times. The interferon produced by 24 h was measured. The control sample had been incubated at 37 °C throughout.

and assaying the interferon produced at 24 h. Fig. 1 shows the results of one such experiment, in which the temperature was changed 7 h after induction, where it can be seen that the yield was enhanced by lowering the temperature. The largest enhancement was observed when the cells were shifted to 28 °C, but only when they were shifted to 19 °C was the yield lower than when the cells were incubated at 37 °C throughout. On the other hand, raising the temperature of incubation to 41 °C inhibited interferon production. Similar results were obtained when the temperature was altered 3.5 h after induction rather than 7 h, but the cells that had been incubated for 7 h before changing to another temperature gave higher yields than those that had been at 37 °C for only 3.5 h. Therefore, the effect of the length of incubation at 37 °C on the enhancement of interferon yield was investigated by inducing the cells and shifting the temperature to 28 °C at various times after induction. Fig. 2 shows that the optimum time to shift the temperature was after 7 h, but that an enhancement could be seen at all times up to 10 h. The higher yield brought about by changing to 28 °C at induction, rather than at 1 h after induction, is reproducible, but its cause is unknown. We have subsequently demonstrated that yields were still enhanced if the temperature was lowered to 28 °C up to 3 h before induction.

In Namalwa cells at 37 °C interferon production has apparently ceased by 10 h after induction (Morser et al., 1979). Thus, it appeared that the change to a lower temperature could enhance yields at any time up to the cessation of interferon synthesis. This observation could be explained if interferon synthesis continued for longer at the lower temperature. Fig. 3 shows an experiment in which this was tested by inducing the cells at 37 °C, shifting the temperature to 28 °C at 3.5 h or 7 h after induction and following the course of interferon production. It can be seen that, at the lower temperature, interferon production is slower but continues for longer.

To test if the rate of interferon secretion was altered, the intracellular interferon titres were also measured in this experiment. When the cells were incubated throughout at 37 °C, the intracellular titre rose to a maximum of 10^2.4 international units/10^6 cells at 9 h after induction and then fell. When the temperature was lowered to 28 °C at either 3.5 h or 7 h after
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induction the maximum intracellular titre was lower, but the ratio of intracellular to secreted interferon was the same. However, intracellular interferon could be detected later after induction at 28 °C than at 37 °C. This is the expected result since the rate of interferon production is slower, but continues for longer at 28 °C and shows that the rate of interferon secretion is not altered at the lower temperature. We concluded that the enhanced yields at the low temperature were not due to an increased rate of secretion, but to a prolonged period of interferon synthesis.

In these experiments the cells had been treated for 48 h with butyric acid before induction. The enhancement of yields could also be demonstrated in both untreated cells and in cells that had been treated with BrdUrd for 48 h before induction (Baker et al., 1979). The enhancement in yield obtained by lowering the temperature at 3.5 h or 7 h after induction was approximately the same, although overall yields were lower (data not shown). Thus, the effect does not depend on the way in which the cells were treated before induction.

The average yield of interferon under the normal induction conditions was $10^{5.2}$ international units/ml (arithmetic average of 16 experiments). When the temperature was reduced to 28 °C at 7 h post-induction the yield of interferon was enhanced on average by fivefold (average of the enhancement observed in the same 16 experiments). The yields under normal conditions varied from one experiment to another (range $10^{4.8}$ to $10^{5.9}$ international units/ml). However, an enhancement was seen in every experiment but one. The highest yield was $10^{7.2}$ international units/ml in an experiment in which a 20-fold enhancement was observed.

Characterization of the interferon produced at 28 °C

The interferon produced by Namalwa cells is a mixture of α and β interferons (Havell et al., 1977), and the α interferon at least is itself a mixture of related polypeptides (Allen & Fantes, 1980). The enhanced yield at 28 °C could be caused by a change in the proportion of α to β interferon, or in the amounts of the constituent polypeptides of α interferon. This was tested by comparing some of the properties of interferon synthesized at 28 °C with those of a
control preparation produced at 37 °C. One of the characteristics of α interferon is that the ratio of its titre on EBTr cells (used in the assays here) to its titre on HFF cells is high. In the case of β interferon this ratio is low. Therefore, we assayed the preparations on both cell types. No change in the ratio of activity on EBTr cells to HFF cells was observed, suggesting that the interferons are similar.

This was further confirmed by measuring the neutralization of the two preparations by either anti-α interferon or anti-β serum. No difference in their neutralization was observed, suggesting again that the preparations contain a similar mixture of interferons present within them.

Interferon produced by incubating the cultures at 28 °C was tested for its ability to bind to an affinity column of a monoclonal antibody to α interferon (Secher & Burke, 1980). The binding and elution properties of this interferon were identical to those of a control preparation (data not shown).

Finally, the titres of interferon produced at 28 °C were compared by a radioimmune assay with those of a control preparation of interferon produced at 37 °C. In this experiment eightfold more interferon had been synthesized at 28 °C. The radioimmune assay is based on the specificity of the monoclonal antibody (Secher, 1981). The results of the radioimmune assay demonstrated that the interferon prepared at 28 °C had sevenfold more interferon in it than the control preparation. Since the radioimmune assay measures the amount of interferon protein present, it follows that at 28 °C more interferon is made, that is the enhanced yield is not due to a change in specific activity. Thus, in summary, the interferon produced by incubation at the lower temperature was indistinguishable from control interferon by the tests used here.

**Continued transcription is not necessary for the enhanced yields**

Interferon synthesis might be prolonged in cells incubated at lower temperatures because transcription of the interferon mRNA continued for longer. This hypothesis was tested by addition of the inhibitor of RNA synthesis actinomycin D (Act. D) which blocks all messenger RNA synthesis within a very short time. The cells were induced at 37 °C and then 3.5 h later the temperature was reduced to 28 °C and sufficient was added to reduce RNA synthesis by more than 99%. The subsequent production of interferon was then followed at both temperatures in the presence or absence of the drug. It had previously been shown that addition of Act. D up to 5 h after induction caused partial but not complete inhibition of subsequent interferon production (Zoon et al., 1978; Morser et al., 1979). A similar effect was found when the inhibitor was added at 3.5 h after induction (Table 1), but if it was added earlier there was complete inhibition. When the temperature was lowered to 28 °C at the same time as the inhibitor was added, the expected enhancement in yields was still obtained (Table 1). This result was confirmed by varying the time at which the temperature was lowered to 28 °C and also the time at which the inhibitor was added. In all cases the enhancement in yields at 28 °C was observed even in the presence of the inhibitor. Since the addition of the inhibitor prevented transcription but did not prevent the stimulation in yield,
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Fig. 4. Production of interferon and interferon mRNA at different times after induction. Cells were induced at 37 °C and shifted to 28 °C 7 h later. (a) The cumulative yield of interferon was then measured at 28 °C (○) or at 37 °C (●). (b) RNA was extracted from these cultures and the amount of interferon mRNA determined by microinjection into oocytes as described in Methods. The interferon produced by injecting into oocytes RNA from cells at 28 °C (○) or 37 °C (●) was then assayed.

Table 2. Interferon mRNA in polysomes

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<tr>
<th>Treatment</th>
<th>Interferon yield from polysomal RNA (units/ml)*</th>
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<tr>
<td>14 h p.i. at 37 °C</td>
<td>40</td>
</tr>
<tr>
<td>14 h p.i. at 37/28 °C</td>
<td>125</td>
</tr>
<tr>
<td>16 h p.i. at 37 °C</td>
<td>16</td>
</tr>
<tr>
<td>16 h p.i. at 37/28 °C</td>
<td>25</td>
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* RNA was extracted from polysomes isolated from induced cells, some of which had been incubated at 28 °C from 7 h after induction. The RNA solution (5 mg/ml) was microinjected into oocytes and the resultant interferon was assayed.

we concluded that continued transcription is not the cause of the increased production of interferon.

Measurement of interferon mRNA levels

The amount of interferon mRNA present in induced cells can be measured by extracting the total RNA, microinjecting it into oocytes and subsequently assaying the amount of interferon produced. When cells were induced at 37 °C and the temperature reduced 7 h later the interferon was produced more slowly by the cells incubated at 28 °C but the cumulative yield was greater (Fig. 4a). The levels of interferon mRNA were similar at the two temperatures soon after the temperature was reduced, but at later times more interferon mRNA was present in cells cultured at the lower temperature (Fig. 4b). Thus, the prolonged synthesis of interferon at 28 °C could be due to a lengthening of the half-life of interferon mRNA.

In these experiments interferon synthesis at 37 °C has apparently ceased between 9 h and 12 h after induction. However, at 28 °C interferon synthesis continued for longer, ceasing between 15 h and 22 h after induction. The loss of interferon mRNA activity at 37 °C was
Fig. 5. Rates of interferon production at 28 °C and 37 °C. (a) The rate of interferon synthesis was determined in cultures at 37 °C (●) or at 28 °C (○) from 7 h after induction. (b) The cumulative yield of interferon was measured in the same experiment at 37 °C (●) or at 28 °C (○).

not due to cell death because even at 24 h after induction all the cells at either temperature still excluded trypan blue.

However, it has recently been shown that under some circumstances interferon mRNA is still present in induced cells after interferon synthesis has ceased (Berger et al., 1980). In the experiment shown in Fig. 4 this appears to be so, since mRNA was still present at 14, 16 and 18 h after induction at 37 °C but very little interferon was being made. However, it was possible that this mRNA was not associated with the polysomes. To test this, cells were induced and the temperature was reduced to 28 °C at 7 h and the interferon yields were followed. The results were similar to those shown in Fig. 3 and 4(a). Polysomes were also prepared from these cells, and the RNA was subsequently extracted from them and assayed by microinjection into oocytes. The results showed that there was more interferon mRNA present on the polysomes at later times at the lower temperature (Table 2). Therefore, the enhanced yields of interferon at 28 °C were due to the prolonged presence of more interferon mRNA on the polysomes. However, at 37 °C interferon mRNA was still present on the polysomes at times after induction when interferon synthesis had apparently halted.

Rates of interferon synthesis

We therefore decided to measure the rates of interferon synthesis and compare those with the rates of RNA and protein synthesis. In experiments such as those reported in Fig. 3 and 4(a) the cumulative yield of interferon was measured, making it difficult to detect the continuing synthesis of a small amount of interferon. However, when the rate of interferon synthesis was determined by pelleting and resuspending the cells in fresh medium at intervals, and assaying the interferon released over a 1 h period, it was found that interferon synthesis was still taking place at both temperatures (Fig. 5a), even though no increase in the cumulative interferon yield could be detected (Fig. 5b). It was concluded that the experiments in which the cumulative yield was being measured were misleading because of the error in determining a small difference between two large numbers. Interferon production was still continuing and interferon mRNA was still present on the polysomes 17 h after induction.
The rate of interferon synthesis at 37 °C was higher than at 28 °C at early times after induction, but subsequently the rate at 37 °C dropped below that at 28 °C. Thus, these results confirm those obtained by determining the levels of interferon mRNA, namely that the enhanced yield at 28 °C is due to prolonged translation of the interferon mRNA.

**Incorporation of precursors into acid-insoluble material**

Macromolecular synthesis was measured by the incorporation of radioactive precursors (uridine and methionine) into TCA-insoluble material (Baker *et al.*, 1980) in experiments in which induced cells were shifted to 28 °C 7 h after induction. Incorporation of both fell rapidly over the next few hours, uridine incorporation declining to 30% of the control at
37 °C and methionine incorporation to 40% (Fig. 6). The reduction in rate of interferon synthesis when the temperature is changed to 28 °C exactly followed the reduction in methionine incorporation, and it seems probable that this is the cause of the lowered rate of interferon production at 28 °C.

It was possible that reducing the temperature changed the spectrum of proteins being synthesized. This was tested by labelling the proteins with [35S]methionine and analysing them by electrophoresis on polyacrylamide gels. The results of a typical experiment are shown in Fig. 7. In track A are shown the proteins from induced cells that were labelled from 12 h to 12.5 h after induction when the temperature was kept at 37 °C throughout, while track B shows the proteins labelled under the same conditions except that the temperature had been reduced to 28 °C 7 h after induction. The one change detectable in the proteins synthesized is indicated with the arrow. However, this change does not constitute a major change in the spectrum of proteins being synthesized. This change became detectable within 1 h of reducing the temperature (data not shown). No further changes in the proteins being synthesized were detectable, even at 24 h post-induction (data not shown). This protein had a mol. wt. of 75 000. It is neither interferon nor a virus protein, because the same change was detectable when uninduced cells were labelled at 28 °C (Fig. 7, tracks C and D).

These results show that reduction in temperature to 28 °C does not lead either to enhanced production of all proteins or to a major change in the spectrum of proteins synthesized. Thus, the effect on interferon synthesis, and possibly on the 75 000 mol. wt. protein, could be either that they are intrinsically translated better at lower temperatures, or that the system that stops interferon production operates more slowly at 28 °C, allowing more interferon to be synthesized.

Does lowering the temperature enhance yields of interferon from oocytes?

When interferon mRNA levels are assayed by microinjection into oocytes they are then incubated usually at 21 °C (Colman & Morser, 1979). Oocytes continue to synthesize some proteins even if the temperature is lowered to 4 °C, but their rate of secretion is substantially reduced below 10 °C (Colman et al., 1981). Their effect on the translation of interferon mRNA was investigated by incubating the oocytes at different temperatures. Lowering the temperature at which the oocytes were incubated did not lead to an enhanced yield of interferon (data not shown), but rather a fall. This result suggests that the enhanced yield of interferon obtained when the incubation temperature of Namalwa cells is lowered is not due to an intrinsic property of interferon mRNA, enabling it to be translated better at lower temperatures. Instead it seems more probable that the reduction in temperature is affecting the system that controls interferon production.

DISCUSSION

The experiments described here show that if the temperature of incubation is lowered after induction the yield of interferon is increased. The interferon produced under these conditions is similar to, if not the same, as that produced normally. It therefore provides a useful way of increasing the interferon yield by existing processes. We have recently shown that the yield from leukocytes can also be increased by lowering the incubation temperature (J. Morser, unpublished results), showing that the phenomenon occurs in other interferon-producing systems.

Indeed, it had earlier been reported that fibroblasts, induced with double-stranded RNA, also show a similar effect (Havell & Vilecek, 1973). These authors suggested that when the temperature is lowered the mechanism that controls the translation of interferon mRNA is altered, allowing its prolonged translation. In this paper we show directly that not only are interferon mRNA levels higher at 28 °C than at 37 °C but also that there is more present on
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the polysomes. Thus, more interferon is synthesized at 28 °C than at 37 °C because interferon mRNA is more stable at the lower temperature and is translated on the polysomes for a longer time.

Lowering the temperature does not lead to a rise in the synthesis of many other proteins. Indeed, because the overall rate of methionine incorporation falls, the rate of interferon synthesis falls in line with it. This makes it unlikely that lowering the temperature increases the translatability of the interferon mRNA. This was confirmed in experiments in which oocytes that had been microinjected with interferon mRNA were incubated at different temperatures. The results show that lowering the temperature decreased the translation of interferon mRNA. Hence, the increased yields observed when induced Namalwa cells were incubated at 28 °C were not due to any inherent properties of the interferon mRNA. Rather the lower temperature is probably altering processes controlling translation of the interferon mRNA.

It is unlikely that the enhanced yields of interferon at 28 °C are caused by continued transcription of the interferon genes because Act. D did not prevent the yield enhancement. This is underlined by the fact that at 28 °C RNA synthesis falls to under 50% of its value at 37 °C. It is of interest to note that although a range of conditions was tried for the drug treatments, a superinduction of interferon was never observed.

Earlier, Morser et al. (1979) had suggested that the cessation of interferon synthesis was due to the disappearance of interferon mRNA. This view was recently challenged by Berger et al. (1980) who showed that interferon mRNA could be detected after interferon production had halted. They proposed that interferon synthesis stopped because, for some reason, the interferon mRNA was prevented from being translated. In this paper we show that the interferon mRNA is present late after induction at 37 °C when interferon synthesis has apparently halted. However, when we reinvestigated the rate of interferon synthesis rather than the cumulative yield of interferon, it was possible to show that interferon production was continuing. Therefore, the presence of interferon mRNA in the cells and on the polysomes correlates with the continuing synthesis of interferon, and the reason that interferon synthesis stops is because its mRNA has decayed.

In the system described in this paper for increasing yield, interferon mRNA translation is prolonged but increased transcription is not necessary. It is therefore not superinduction but superproduction (as defined by Stewart, 1979) that leads to the enhanced production of interferon.

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