Interferon Priming
EFFECTS ON INTERFERON MESSENGER RNA*

Sergio L. Abreu, F. Carter Bancroft, and William E. Stewart II†
From the Interferon Laboratories, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

The effects of priming mouse cells with interferon on the production of interferon and its mRNA were investigated. Interferon-treated (primed) mouse L<sub>262</sub> cells produce 3 to 10 times more interferon than do nonprimed cells following induction with Newcastle disease virus. Interferon appears 2 to 4 h sooner in the primed cultures than in nonprimed cultures and interferon production by primed cells becomes resistant to inhibition by actinomycin D about 4 h sooner than interferon production in nonprimed cells. Interferon mRNA is detected in primed-induced cells about 2 h earlier than in nonprimed-induced cells. It reaches peak levels about 2 to 4 h earlier in primed cells, but it also disappears sooner in primed cells. The total amounts of interferon mRNA isolated from primed-induced cells and nonprimed-induced cells were indistinguishable, by the methods utilized. Therefore, although primed cells can produce significantly more interferon and make interferon mRNA sooner than nonprimed cells, the total amount of interferon mRNA produced is apparently not increased, nor is its half-life prolonged in primed cells. Thus, enhanced interferon production in primed cells may result from enhanced efficiency of translation of interferon mRNA in the primed cells.

In recent years, it has become clear that, in addition to their well known inhibitory effects on virus multiplication, interferons alter cells in other ways. Thus, interferons can prime cells (1), increase their sensitivities to double-stranded RNA toxicity (2), inhibit cell multiplication (3-5), and induce a variety of immune modulations (6). The priming effect of interferon, itself, appears to consist of several cellular alterations. Thus, primed cells can produce interferon in response to several viruses that are unable to induce interferon in nonprimed cells (1) and interferon primed mouse cells can produce interferon in response to poly(rI)-poly(rC) alone, whereas normal mouse L cells cannot produce interferon in response to polyribonucleotides unless also treated with DEAE-dextran (2, 7). This suggests that interferon priming can alter the initial interaction of cells with inducers. In addition, it has been repeatedly demonstrated that cells treated with interferon can produce more interferon than untreated cells, when exposed to viral or nonviral interferon inducers (1, 8-12). This priming effect of interferon pretreatment on interferon production can also shorten the lag period between addition of inducer and the appearance of interferon in induced cultures (2, 13-15). Further, the interferon response of primed cells becomes resistant to inhibition by actinomycin D sooner than the response of nonprimed cells (1, 10, 14, 16), suggesting that primed cells can somehow produce interferon mRNA more quickly than can nonprimed cells when both are exposed to the same inducer, either a replicating virus or a synthetic double-stranded RNA. It has not, however, been directly demonstrated that interferon mRNA appears more quickly following induction in primed cells than it does in nonprimed cells.

Interferon mRNA has been extracted from a number of cells systems induced to produce interferons, and these mRNAs have been translated in a number of heterologous cells (17, 18, 36), in cell-free protein-synthesizing systems (19-21), and in Xenopus laevis oocytes (21-24). Apparently, little attention has been paid to the time of extraction of mRNA after cells have been induced. DeMaeyer-Guignard et al. (17) extracted RNA from mouse embryo cells 12 h after their induction with either Newcastle disease virus or poly(rI)-poly(rC). Other workers have extracted interferon mRNA from mouse cells at 8 to 9 h after induction (20, 25) or at 16 to 17 h after induction (26). Recently, Lebleu and collaborators (27), who extracted interferon mRNA from primed mouse cells at 11 to 12 h after induction, have even commented on the apparent paradox that the amount of interferon mRNA activity derived from nonprimed cells, at that time, was often 10 times higher than that obtained from primed cells. In view of the extensive literature showing earlier appearance of interferon in primed cells and the earlier resistance of this interferon response to actinomycin D, it would be anticipated that in primed and nonprimed cells the times after induction at which interferon mRNA levels occur might be different.

In these studies, we have compared the kinetics of appearance and disappearance of interferon mRNA in primed and nonprimed mouse cells induced to produce interferon and have asked the following questions. 1) Do interferon-primed cells synthesize interferon mRNA sooner than nonprimed cells? 2) Does the increased interferon production in primed cells arise from increased production of interferon mRNA, an increased interferon mRNA half-life, or from an increased translational efficiency of this mRNA?

MATERIALS AND METHODS

Cells and Viruses—Interferon induction was performed in cloned cultures of mouse L<sub>262</sub> cells designated Lpa (1). Interferon assays were performed on L cells designated L<sub>262</sub> obtained from G. Bokesi, Mt. Sinai Hospital, New York. All cells were grown in Eagle's minimal medium containing 10% heat-inactivated fetal calf serum. Newcastle disease virus stocks were prepared in 11-day-old embryonated chicken eggs and titrated about 10<sup>5</sup> plaque-forming units/ml in primary chicken embryo monolayer cultures. Vesicular stomatitis virus stocks were prepared as previously described (1).

4114
Priming and Induction of Interferon in Cells—Mouse Lpa cells were grown in roller bottles to confluency (about 2 × 10^6 cells). To primed cells, mouse interferon was added to the growth medium to a final concentration of 100 units/ml 2 h before cells were induced with Newcastle disease virus at about 1 plaque-forming unit/cell. After virus adsorption for 1 h, cultures were incubated at 37°C with 50 ml of medium containing 5% fetal calf serum. The nonprimed cultures were treated in a similar manner except no interferon was added to the medium.

Cell Fractionation—At selected intervals postinduction (2, 4, 6, 8, 11, and 16 h), medium was decanted from three or five roller bottles of both primed and unprimed cells. An aliquot of the medium was stored at 4°C for interferon assay and the cells were scraped into cold phosphate-buffered saline, pH 7.0, and washed three times with 100 volumes of phosphate-buffered saline. Pelleted cells were then resuspended in a hypotonic buffer (10 mM Tris·HCl, pH 7.6, 10 mM NaCl, 1.5 mM MgCl_2) containing the nonionic detergent Nonidet P-40, 0.5%. Lysis was completed by vortex stirring for repeated 15- to 20-s periods. A postnuclear supernatant was obtained by centrifugation at 10,000 × g for 10 min. All steps were performed at 0-4°C.

RNA Extraction—Total RNA was extracted from the postnuclear supernatant with phenol/chloroform as described by Palmiter (28). Poly(A)-rich RNA was prepared by oligo(dT)-cellulose chromatography (29) of the preparation which was first heated for 2 min at 80°C, made 0.4 M in NaCl, and quickly cooled. The solution was then warmed to 25°C before application to the oligo(dT) column. Two cycles of binding and elution were performed.

Translation of mRNA in Xenopus laevis Oocytes—Small segments of the ovaries of adult Xenopus laevis frogs were removed under ether anesthesia and incubation medium (31) for 24 h at 22°C. At the end of the incubation periods, they were prepared for assay as described previously (24). Briefly, oocytes were homogenized in the incubation medium and a supernatant was obtained by centrifugation at 10,000 × g for 5 min.

Interferon Assay—All interferon assays were performed by microtiter assay of inhibition of cytopathology of vesicular stomatitis in Lpa cells (32). The titers expressed in this paper correspond to NIH titer assay of inhibition of cytopathology of vesicular stomatitis in reference mouse interferon units (GOO2-902-026). The titers expressed in this paper correspond to NIH titer assay of inhibition of cytopathology of vesicular stomatitis in reference mouse interferon units (GOO2-902-026).

Kinetics of Interferon Production in Nonprimed and Primed L Cells Induced with Newcastle Disease Virus—The cumulative production of interferon is shown in Fig. 1A. It is seen that, in agreement with earlier reports (1, 10, 11, 14), the primed cells began producing interferon about 2 to 4 h earlier than the nonprimed cells. Furthermore, the total interferon yield from the primed cultures was significantly greater than from the nonprimed cultures. In the experiment illustrated in Fig. 1, the total amount of interferon produced in primed cultures is about 3 times that made in nonprimed cultures; however, this difference is often as much as 10-fold (Fig. 2).

The results in Fig. 1A also suggest that interferon production ceases earlier in the primed cells. To investigate this point further, the medium was changed on cultures every 2 h, and medium harvested during each interval was assayed for inter-
feron activity. It is seen (Fig. 1B) that interferon production both peaked and terminated 2 to 4 h earlier in the primed cultures than in the nonprimed cultures. Thus, while priming shortens the lag preceding interferon production and increases total interferon production, these indirect data suggest that it does not prolong the half-life of interferon mRNA activity.

Sensitivity of Interferon Production in Nonprimed or Primed L Cells to Actinomycin D—Both RNA and protein synthesis are required for the production of interferon in either nonprimed or primed mouse cells (1). The results described in the previous section suggested that interferon mRNA might be synthesized earlier in primed cells than in nonprimed cells. If this were so, one would expect interferon production to remain sensitive to actinomycin D longer in the nonprimed cells than in primed cells. In agreement with this prediction, it was observed that interferon production in primed cells became resistant to inhibition by 5 μg/ml of actinomycin D (a dose which inhibits cellular RNA synthesis by more than 95%) 2 to 4 h earlier than in nonprimed cells (Fig. 2). Thus, interferon production by nonprimed cells was completely inhibited when actinomycin D was added as late as 6 h after induction, and was depressed by about 90% when the inhibitor was added at 8 h after induction. By contrast, interferon production in primed cultures was completely inhibited when actinomycin D was added 2 h postinduction and about 90% inhibited when the inhibitor was added at 4 to 6 h, but was completely resistant by 8 h.

Translation of Interferon mRNA in Oocytes—To determine more directly the effect of priming on interferon messenger RNA synthesis, we decided to utilize the Xenopus oocyte system for translation of isolated mRNA. Preliminary studies showed that translation of interferon mRNA in the oocytes was optimal at a temperature of 22–25°C (data not shown); therefore, all incubations in these studies were performed in this range.

The time course of translation of interferon mRNA in the oocytes is seen in Fig. 3; as most of the interferon synthesis had occurred by 20 h, a 24-h incubation period was used throughout these studies.

The concentration dependence of interferon mRNA translation in Xenopus oocytes was then established using the previously determined time and temperature conditions. Fig. 4 shows that a linear response of the oocytes to RNA injection was obtained with RNA doses ranging from 10 to 75 ng/oocyte; therefore, during these studies, all oocytes were injected with 50 to 75 ng of RNA. In contrast to a previous report (25), we have not found evidence for a form of interferon mRNA which lacks the poly(A) tract (Fig. 5).

Measurements of Interferon mRNA Production in Primed and Nonprimed Cells by Translation in Xenopus Oocytes—As illustrated in Fig. 5, the production of interferon mRNA in both primed and nonprimed cells paralleled, but in each case, significantly preceded, the appearance of interferon (Fig. 1). In nonprimed cells, interferon mRNA activity is first detectable about 4 h after induction and remains sensitive to actinomycin D addition until 8 h after induction. In contrast, the interferon first appears in these cultures about 8 h after induction and peaks at about 12 to 14 h. Primed cells
produce interferon mRNA much sooner than do the nonprimed cells, mRNA being detectable by 2 h after induction and peaking at about 6 h. Again, there is a significant lag in the primed cells between the appearance of interferon mRNA and the appearance of interferon. The peak levels of translatable interferon mRNA in both nonprimed and primed cells were virtually identical. It is, therefore, not likely that the significant increase in interferon production could be attributed to induction of greater amounts of interferon mRNA in primed cells. It is also noteworthy that the extractable interferon mRNA activity disappeared from primed cells earlier than it did from nonprimed cells. Therefore, in agreement with the interferon production kinetics data (Fig. 1 B), enhanced interferon yields from primed cells do not appear to result from stabilization of the interferon mRNA.

Effect of Priming and Induction of Interferon on Total Protein Synthesis—The results described above suggest that the increase in interferon production observed in interferon-treated cells could result from increased translation efficiency of total cellular mRNA. To determine if priming has any gross effect on protein synthesis, we pulsed cells with [3H]leucine at 6 h of induction is similarly not grossly affected by interferon priming. The differences in protein synthesis between the control and Newcastle disease virus-induced cells (about 70% inhibition in induced cells) is likely due to the effect of the virus on L cell macromolecular synthesis. However, again there is no significant difference in protein synthesis between simply induced and primed and induced cells.

**TABLE I**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incorporation of [3H]leucine into acid-insoluble material (cpm x 10^3</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Nonprimed</td>
<td>382 ± 30</td>
<td>110</td>
</tr>
<tr>
<td>Primed</td>
<td>455 ± 50</td>
<td></td>
</tr>
<tr>
<td>B. Nonprimed and induced with Newcastle disease virus</td>
<td>433 ± 18</td>
<td>30</td>
</tr>
<tr>
<td>Primed and induced with Newcastle disease virus</td>
<td>130 ± 10</td>
<td>31</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The interferon-primed cell clearly differs from a nonprimed cell in a number of ways. In this report, we have investigated the origin of some of these differences between nonprimed and primed cells. Thus, in the present studies and others (3, 13-16), interferon-primed cells have been observed to produce interferon sooner than nonprimed cells (Fig. 1), and the interferon production in primed cells has been observed to become resistant to inhibition by actinomycin D more quickly (Refs. 1, 10, 14, 16; Fig. 2). The latter results provided indirect evidence that interferon mRNA appears sooner following induction in primed cells than in nonprimed cells. We have utilized the Xenopus oocyte translation system to show directly that this occurs (Fig. 5).

In the present studies (Fig. 1) and others (1, 8-12), it has been shown that interferon-primed cells are able to produce more interferon than nonprimed cells when exposed to an inducer able to induce interferon in nonprimed cells. We have, therefore, asked whether the increased amount of interferon produced in primed cells results from increased interferon mRNA production, from increased interferon mRNA half-life, or from enhanced translational efficiency of this mRNA. Our data (Fig. 5) suggest that primed cells and nonprimed cells produce the same amounts of interferon mRNA and that there has not been an increase in the half-life of the interferon mRNA cells in the primed cells. Thus, it appears that the translational machinery of the primed cells is altered so that translation of interferon mRNA is more efficient. Since no gross differences in protein synthesis between primed and nonprimed cells were observed, it may be that the putative alteration is specific for interferon mRNA.

It is also important to note that active interferon messenger RNA is detectable in nonprimed cells long after it has disappeared in primed cells. Therefore, the greater production of interferon in primed cultures does not result from stabilization of interferon mRNA, thus increasing its active half-life, as seems to account for the greater interferon yields from "superinduced" cell (22, 34). These results clarify the apparent paradox observed by Lebleu et al. (27) who found 10-fold more interferon mRNA in nonprimed cells than in primed cells, when they extracted the RNA at 11 to 12 h postinduction in both cases.

It could be imagined that the 2- to 4-h lag observed between appearance of interferon mRNA and production of interferon might result from a lag in the processing of this mRNA from the nucleus to the cytoplasm. However, since RNA was extracted only from postnuclear supernatants, this cannot be the explanation. It has been reported that the lag between appearance of active intracellular interferon and the appearance of extracellular interferon is only about 20 min (35), therefore, glycosylation and transport mechanisms do not seem to account for this delay between appearance of active interferon mRNA and appearance of active interferon. Rather, these data suggest that some prolonged post-translational modification of the interferon protein must occur in the cells before active interferon is produced.

These data also suggest another alteration of primed cells. It has been repeatedly suggested that interferon mRNA translation is terminated by a post-transcriptional regulatory mechanism and that "superinduction" works by inhibiting this repression system (34). As interferon-primed cells (which produce interferon sooner than nonprimed cells) also terminate interferon production sooner than nonprimed cells, it appears that interferon treatment may also prime this post-transcriptional, mRNA-inactivating mechanism.

In conclusion, priming changes the time course of translatable interferon messenger RNA appearance in the cytoplasm. It does not seem to affect the total amount of interferon mRNA produced or its half-life. The increased production of assayable interferon protein observed in primed cells must then be explained in terms of a translational mechanism.

**Acknowledgment**—We wish to express our appreciation to Marzena Wiranowska-Stewart for excellent assistance with certain aspects of these studies.

**REFERENCES**

Priming and Interferon mRNA

5. De Maeyer-Guignard, J., Tovey, M. G., Gressier, I., and De Maeyer, E. (1978) *Nature* 271, 622-625
Interferon priming. Effects on interferon messenger RNA.
S L Abreu, F C Bancroft and W E Stewart, 2nd


Access the most updated version of this article at http://www.jbc.org/content/254/10/4114

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/254/10/4114.full.html#ref-list-1