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Immunology

Synthesis of new proteins associated with the induction of interferon in human fibroblast cells

(mRNA translation/radioimmunoprecipitation)

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ABSTRACT **The relative amounts of translatable cellular mRNAs and newly synthesized cellular proteins were examined in poly(I)·poly(C)-induced human fibroblast cells early during induction. At this time interferon and interferon mRNA synthesis are maximal and cells have not acquired their antiviral state. Translation of the mRNA from poly(I)·poly(C)-induced cells in a wheat germ cell-free system led to the synthesis of a [^{35}S]methionine-labeled 22,000-dalton protein that is precipitated by antiserum to highly purified human fibroblast interferon. The synthesis of this protein was detected only with the mRNA preparations that, when translated in *Xenopus* oocytes, coded for the synthesis of biologically active human interferon. Two-dimensional gel analysis of the [^{35}S]methionine-labeled polypeptides translated from the total mRNA of the induced and uninduced cells revealed the presence of 23 new proteins that were translated from mRNAs of the induced cells but not from the mRNAs of the controls. These polypeptides ranged from 15,000 to 70,000 daltons. Thirteen of these proteins were detected in induced cells labeled with [^{35}S]methionine. It is concluded that, in human fibroblasts, poly(I)·poly(C) induces, in addition to interferon, the synthesis of a variety of "interferon-associated" proteins.**

Interferons are cellular glycoproteins induced in eukaryotic cells by viral infection or by treatment with double-stranded nucleic acid—e.g., poly(I)·poly(C) (1–3). When secreted, interferon interacts with the adjacent cells, inducing in them a state in which the multiplication of viruses is impaired. It has been shown in human cells that the induction of interferon and the establishment of an antiviral state are two independent functions coded for by different chromosomes (4). Thus, the genes for human fibroblast interferon seem to reside on chromosomes 2, 5, and 9, whereas the sensitivity to interferon is specified by the presence of a surface receptor governed by chromosome 21.

Interferon treatment of human, mouse, and chicken cells and the subsequent production of the antiviral state lead to a *de novo* synthesis of several other proteins of unknown function not detected in the controls (5–7). Induction of an antiviral state seems to occur simultaneously with the synthesis or increase in activity of several enzymes—e.g., the oligoadenylate-associated nuclease and the eukaryotic protein synthesis initiation factor (eIF-2) phosphorylase, as demonstrated in *in vitro* systems (8–12). Thus the antiviral effect of interferon, which (with a few exceptions) occurs at the translational level (13), may be associated with the synthesis or activation of a number of translational inhibitors.

The induction of interferon by viruses or double-stranded RNA necessitates the synthesis of interferon mRNA (14, 15). Because of interferon's high specific activity (10^9 units/mg of protein) (16, 17), a sensitive biological assay measuring inhibition of viral replication allows detection of picogram quantities of the protein. When mRNA of induced cells is translated *in vitro*, the high sensitivity of this assay enables detection of *in vitro*-synthesized interferon (14, 15, 18–21), in spite of the fact that the interferon mRNA represents less than 1% of the total mRNA population.

It is possible, however, that induction results in a more general derepression of the cellular genome, leading to the transcription of several mRNAs and the synthesis of "interferon-associated" proteins. We compared the relative amounts of translatable cellular mRNAs and the newly synthesized proteins in the poly(I)·poly(C)-induced and uninduced human fibroblast cells at the time of maximal synthesis of interferon and its mRNA. The results show that, in addition to interferon, poly(I)·poly(C) induces in human fibroblast cells the synthesis of several new mRNAs and proteins not detected in the uninduced cells.

MATERIALS AND METHODS

Induction and mRNA Preparation. Human fibroblast cells were grown, interferon production was induced by poly(I)·poly(C) as described (21, 22) and translatable mRNA was prepared by the guanidine hydrochloride method (22). Fifteen 150-mm tissue culture dishes of 100% confluent cells typically yielded 4.0 μg of poly(A)$^+$ RNA; the yields from induced and uninduced cells were comparable.

Translation of mRNA in *Xenopus* Oocytes and the Wheat Germ Cell-Free System. Microinjection of mRNA into oocytes was done as described (20, 21); wheat germ extracts were prepared and treated with micrococcal nuclease as described (23, 24). The reaction mixture was as described (20), but [^{3}H]leucine was replaced by [^{35}S]methionine (400 μCi/ml; 1 Ci = 3.7×10^{10} becquerels).

Interferon Assay. Interferon titers were measured by the colorimetric assay of Finter (1) on human fibroblast cells trisomic for chromosome 21. The titers are given in international reference standard (69/17) units; 1 unit of standard was titrated as 1 unit in our assay.

Radioimmunoprecipitation. Antiserum to human fibroblast interferon was obtained from rabbits repeatedly injected with purified human fibroblast interferon (10^8 units/mg) (unpublished data). The titer, measured by neutralization of 10 units of human fibroblast interferon, was 10^5. The precipitation of the [^{35}S]methionine-labeled peptides synthesized in the wheat germ cell-free system by the antiserum was done as described for viral proteins (25).

Polyacrylamide Gel Electrophoresis of Proteins. Slab gel electrophoresis was carried out as described by Laemmli (26) on 11–13% (wt/vol) polyacrylamide gels. After electrophoresis, gels were fixed and processed for fluorography according to the method of Bonner and Laskey (27). The basic two-dimensional technique and the processing of the gel for fluorography have been described (28), except that *N,N'*-diallyltartardiamide was used as a crosslinking reagent in both gels.

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RESULTS

Quantitation of Interferon mRNA in Poly(I)·Poly(C)-Induced Cells. Interferon production induced in human fibroblasts by poly(I)·poly(C) reaches its maximum within 3–6 hr after induction and falls to undetectable levels by 12–15 hr. The induction of interferon parallels the accumulation of functionally active interferon mRNA. Under conditions previously described (15), the relative amount of translatable interferon mRNA in *Xenopus* oocytes reached a maximum 4 hr after induction (Table 1). No translatable interferon mRNA was detected in the uninduced cells. These results are in agreement with the published data from several laboratories (12, 18–21, 29).

mRNA isolated from the induced and control cells was translated in the nuclease-treated wheat germ system (23), and [^{35}S]methionine-labeled translation products were analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (26). Translation of mRNA from the poly(I)·poly(C)-induced cells (Fig. 1, lane b) yielded an increased amount of at least three proteins compared with the translation product of mRNA from the control cells (Fig. 1, lane a). These new proteins migrated in the 56,000-, 26,000-, and 22,000-dalton regions of the sodium dodecyl sulfate/polyacrylamide gel. These results suggested that the induction of human fibroblasts with poly(I)·poly(C) resulted in increased accumulation of several mRNAs.

When fibroblasts are induced with poly(I)·poly(C) in the presence of cycloheximide (50 μg/ml) for the first 4 hr and treated with actinomycin D later, interferon synthesis is enhanced (superinduced) (30). The relative amounts of translatable interferon mRNA isolated in the early stages of induction from poly(I)·poly(C)-induced and -superinduced cells are comparable, as measured in the oocyte assay (20), whereas the synthesis of interferon and other cellular proteins is substantially inhibited. This system thus affords an evaluation of the effect of poly(I)·poly(C) treatment alone and allows a separation of the interferon induction and the antiviral state.

One-dimensional sodium dodecyl sulfate/polyacrylamide gel electrophoresis was used to compare the *in vitro* translation products of mRNA from induced and superinduced cells (Fig. 1, lanes b and c). ^{35}S-Labeled proteins synthesized in the wheat germ system from mRNA of superinduced cells (isolated 4 hr after induction) showed a pattern identical to that of the translation products of mRNA from poly(I)·poly(C)-induced cells. These data indicated that the new mRNAs identified in the poly(I)·poly(C)-treated cells were induced by poly(I)·poly(C) and not by interferon. Translation products of mRNA isolated from poly(I)·poly(C)-induced cells in the presence of actinomycin D showed a sodium dodecyl sulfate/polyacrylamide gel pattern identical to the products of the translation of the uninduced mRNA (data not shown).

Table 1. Comparison of interferon production and the relative amounts of translatable interferon mRNA in human fibroblasts induced by poly(I)·poly(C)

Time after induction, hr	Interferon mRNA, units/ml	Interferon yield, units/ml/hr
1.5	<10	5
4	640	320
15	20	<10

The poly(A)$^+$ mRNA was isolated from the induced cells at indicated times and dissolved in water at 2 μg/μl; 70 nl was injected into each oocyte. Oocytes were incubated in Barth's medium (20) for 24 hr at room temperature and homogenized. The supernatant material was assayed for the antiviral activity. Interferon mRNA (units/ml) represents the interferon activity detected in 10 oocytes. The medium from the 7×10^5 induced cells was collected every hour and, at the times indicated, the levels of interferon were measured.

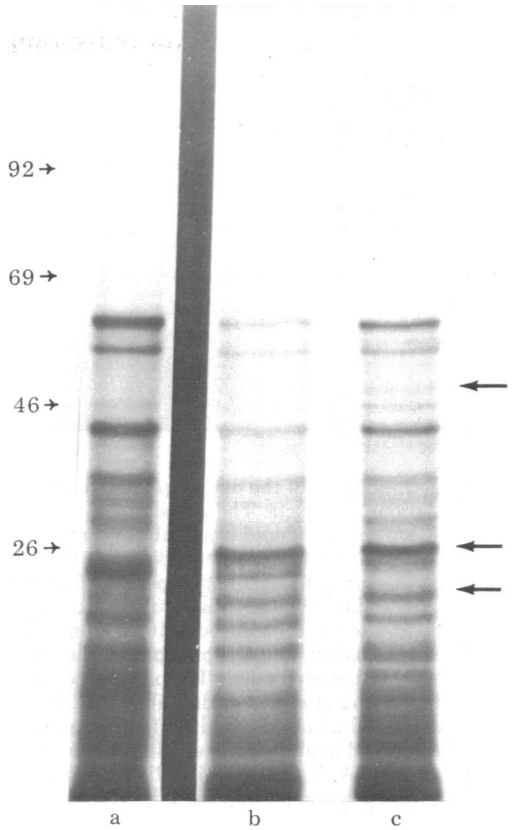

FIG. 1. The effect of poly(I)·poly(C) induction on the translatability of total mRNA in wheat germ cell-free extract. Human fibroblasts were induced with poly(I)·poly(C) (100 μg/ml; 0–1.5 hr) in the absence or presence of cycloheximide (50 μg/ml; 0–4 hr). Poly(A)$^+$ RNA was isolated from the cells 4 hr after induction and translated in the wheat germ cell-free system; the ^{35}S-labeled translational products were analyzed on sodium dodecyl sulfate/polyacrylamide gel. Lanes: a, mRNA from control cells; b, mRNA from induced cells; c, mRNA from cells induced in the presence of cycloheximide. The proteins synthesized from newly induced mRNAs are labeled with arrows; 40,000 cpm were loaded in each lane.

To determine which of the *in vitro*-synthesized proteins are antigenically related to human fibroblast interferon, the [^{35}S]methionine-labeled polypeptides were precipitated by the antiserum to highly purified human fibroblast interferon, and the immunoprecipitate was analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (Fig. 2). Anti-interferon serum precipitated a 22,000-dalton protein from the *in vitro* translational products of mRNA from poly(I)·poly(C)-induced cells. This protein was not precipitated by the nonimmune serum; it also was absent from the products of translation coded by the uninduced mRNA or by the mRNA isolated from the poly(I)·poly(C)-induced cells at the times interferon was not synthesized. Others have translated mRNA from induced cells in the rabbit reticulocyte system and have found a 23,000-dalton protein by the immunoprecipitation of the products with antiserum against human fibroblast interferon (32). Thus both Weissenbach's data (32) and our data indicate that the 22,000-dalton protein may represent an *in vitro*-synthesized interferon polypeptide.

Analysis of the supernatants from the immunoprecipitation revealed another inducible protein of 14,000 daltons. This protein was present in the *in vitro* translational product of pools of mRNA isolated from both the interferon-producing and -nonproducing induced cells and was not recognized by the anti-interferon serum. Therefore, it is not likely that it repre-

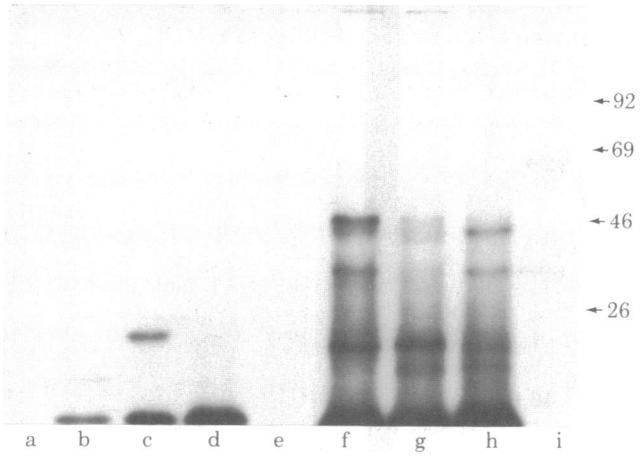

FIG. 2. Electrophoresis of the unprecipitated and immunoprecipitated translational products. The translational mixture (25 μl) of the induced (2 μg) and uninduced (2 μg) mRNA containing 10^6 cpm was incubated with 5 μl of the anti-interferon serum for 1 hr at room temperature. At the end of the incubation period, 25 μl of 1% Sepharose A was added and the mixture was incubated for an additional 30 min. The precipitate was centrifuged at 10,000 rpm, the supernatant material was collected, and the precipitate was washed three times with phosphate-buffered saline. The precipitate was heated in the standard buffer and analyzed as described (31). To 5 μl of supernatant material, 20 μl of standard buffer was added, and the sample was heated and analyzed. Lanes with immunoprecipitates: a, no exogenous mRNA; b, uninduced mRNA; c, induced mRNA isolated 4 hr after induction; d, induced mRNA isolated 8 hr after induction; e, induced mRNA (4 hr after induction) precipitated with the normal rabbit serum. Lanes with supernatant material: f, uninduced mRNA; g, induced mRNA 4 hr after induction; h, induced mRNA 8 hr after induction; i, no exogenous mRNA.

sents a partially cleaved 22,000-dalton protein or an incomplete translational product of the same mRNA.

Two-Dimensional Analysis of Proteins Synthesized in Wheat Germ Cell-Free System. By separating peptides on the basis of both molecular weight and pI, two-dimensional gel electrophoresis revealed more accurately than its one-dimensional counterpart the differences among the *in vitro* translational products of mRNA from induced and uninduced cells (Fig. 3). The autoradiograms revealed 23 new proteins translated from the mRNA from induced cells. The synthesis of the new proteins was not detected in the translational product of mRNA isolated from cells treated with actinomycin D during the induction period. In addition to these qualitative changes, quantitative differences between the translation products of mRNAs of induced and uninduced cells were reflected by differences in the intensity of production of various peptides coded for by these mRNAs. Thus, the new proteins synthesized *in vitro* from mRNA of induced cells differed from their control counterparts both in quantity and nature. Since there is not evidence of glycosylation in the wheat germ system, these proteins should differ in their amino acid sequences, and different mRNAs should code for them.

A number of the polypeptides detected as products of translation of mRNAs from induced cells were larger than human interferon. These proteins were mostly neutral or acidic and were estimated to be 25,000–70,000 daltons.

A number of new polypeptides did appear that shared interferon's molecular weight and pI; however, we cannot designate which of these proteins is interferon. The human interferon produced in the fibroblast cultures is approximately 20,000 daltons (34, 35), whereas the human fibroblast interferon enzymatically deglycosylated is several thousand daltons smaller (36). The pI of human fibroblast interferon seems to be

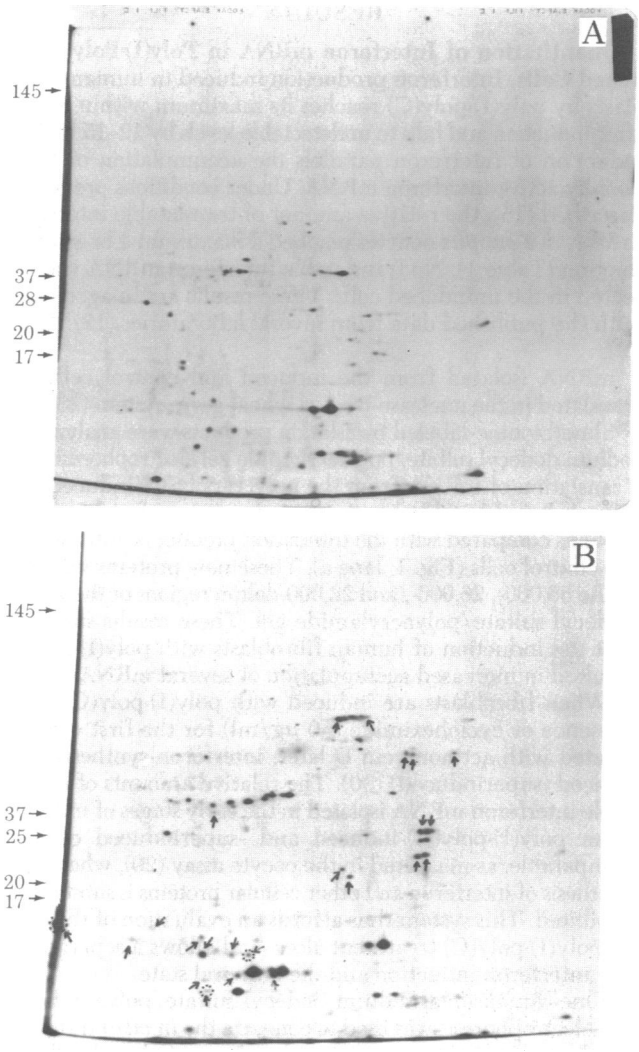

FIG. 3. *In vitro*-synthesized polypeptides analyzed by two-dimensional separations. Poly(A)$^+$ RNA from the control cells (*A*) and poly(I)·poly(C)-induced cells (*B*) were translated at 120 μg/ml for 120 min at room temperature in a wheat germ system with [^{35}S]methionine (400 μCi/ml) as a radioactive label. After incubation, aliquots were frozen and kept at −70°C. The resulting translation products were subjected to two-dimensional separations and subsequent processing of the gel for fluorography; 50,000 cpm was loaded on the first-dimension gel. The crosslinking of the second-dimension gel was 14%. The sample origin was at the left-hand side of this figure and electrofocusing was toward the positive electrode (right-hand side); pH 5.0 is on the right side and pH 7.0 on the left side of this figure. Extrapolated molecular weight estimates ($\times 10^{-3}$) are shown in the left-hand margin, and the arrows indicate new proteins coded for by mRNA from the induced cells. Dotted circles represent proteins that were not synthesized when the mRNA isolated from the cells 15 hr after induction was translated.

in the range of 5.7, and this value changes to a higher value after neuraminidase treatment (37). Therefore, one could expect that the interferon synthesized in the wheat germ system should have a pI higher than 5.7. Our data show that the translation of mRNA from poly(I)·poly(C)-induced cells led to the synthesis of four new proteins with mobility similar to the 22,000-dalton protein that precipitated with the antiserum to human fibroblast interferon. However, two of them are more acidic than the nonglycosylated interferon molecules would be expected to be. We could not determine which of the four proteins (if any) was precipitable with our interferon antiserum, since the specific activity of the 21,000-dalton band was too low to be detected on a two-dimensional gel autoradiogram.

Analysis of Cellular Proteins Synthesized in Poly(I)·Poly(C)-Induced Cells. To determine whether the synthesis of new cellular proteins can be detected in the poly(I)·poly(C)-induced cells, the induced cells were labeled with [^{35}S]methionine (50 μCi/ml) 1.5–4 hr after induction. The labeled medium was removed, and the cells were washed and lysed. The extent of labeling of the induced (2×10^6 cpm/10^6 cells) and control cells (3.5×10^6 cpm/10^6 cells) was comparable. The two-dimensional analysis of the synthesized proteins is shown in Fig. 4. In the induced cells, the synthesis of seven new minor polypeptides (10,000–30,000 daltons) was detected (see arrows); these polypeptides were not synthesized in the control cells during the 2.5-hr exposure to [^{35}S]methionine. Because mRNA isolated from induced cells and translated *in vitro* coded for 23 new proteins, this indicated that not all the polypeptides (detected in the cell-free translation product of mRNA from induced human fibroblasts) were synthesized at detectable levels in the induced cells during the 2.5-hr exposure to [^{35}S]methionine.

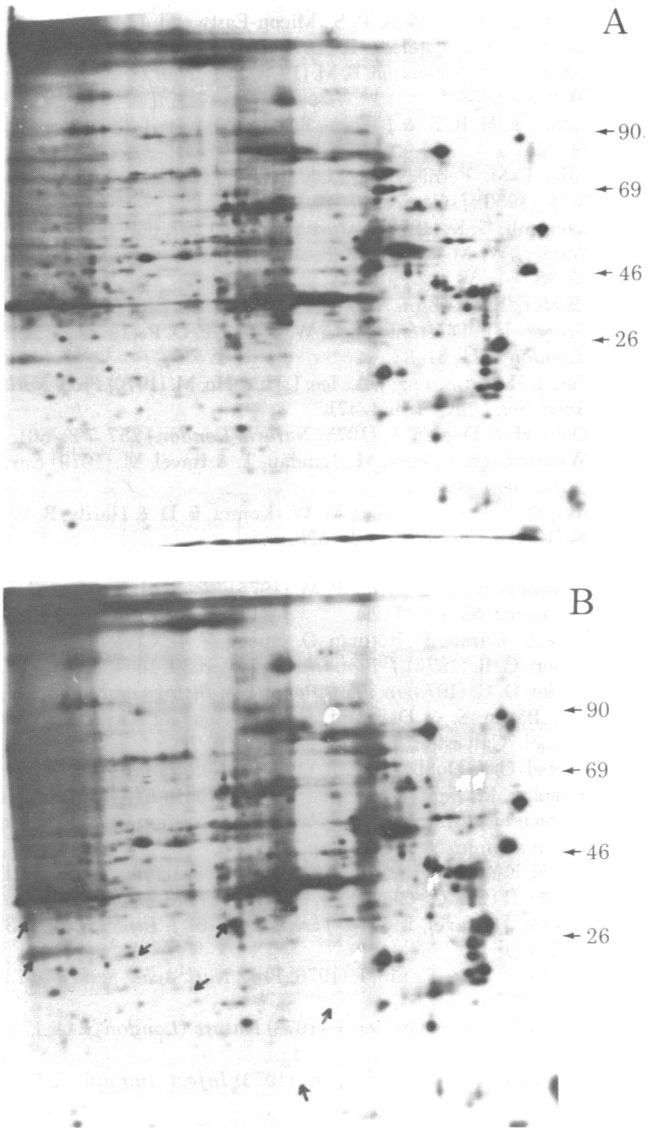

FIG. 4. Polypeptides from cells labeled for 2.5 hr with [^{35}S]methionine in the poly(I)·poly(C)-induced cells and controls. Cells were induced with poly(I)·poly(C) (100 μg/ml) for 1.5 hr; inducer was removed and the cells were labeled for 2.5 hr with [^{35}S]methionine (50 μCi/ml). The uninduced cells were labeled for 2.5 hr with [^{35}S]methionine, the cells were lysed, and the proteins were analyzed. (10^5 cpm were loaded on the first dimensional gel.) The crosslinking of the second dimensional gel was 11.5%; the samples were run as described in Fig. 3. Extrapolated molecular size estimates (daltons) are shown in the left-hand margin; pH 5 is on the right side and pH 7.0 is on the left side of the figure. The arrows indicate new polypeptides present only in the induced cells. (*A*) uninduced cells; (*B*) poly(I)·poly(C)-induced cells.

To determine whether the proteins synthesized in the induced cells during the first 4 hr of induction are antigenically related to human fibroblast interferon, the ^{35}S-labeled cellular proteins were precipitated with the antiserum to human fibroblast interferon and separated on sodium dodecyl sulfate/polyacrylamide gel. The immunoprecipitate of the cellular proteins from the induced cells contained a 15,000- to 16,000-dalton polypeptide that was not detected in the uninduced cells and that was not precipitated by the normal rabbit serum (data not shown). However, in the cells the presence of the 22,000-dalton protein that would correspond to the polypeptide synthesized in the wheat germ translational system was not detected.

DISCUSSION

In this paper we compare the *in vitro* translatable mRNA and [^{35}S]methionine-labeled proteins synthesized in the poly(I)·poly(C)-induced cells during the first 4 hr of induction to those synthesized in the uninduced cells. At this time the rate of interferon synthesis in the induced cells and the relative amount of interferon mRNA are maximal.

The amount of interferon mRNA present in the poly(I)·poly(C)-induced cells was estimated both by translation in *Xenopus* oocytes (22) and in the wheat germ cell-free system. We have shown that human interferon mRNA can be translated with fidelity in the wheat germ cell-free extract (20); however, the amount of biologically active interferon synthesized was 100- to 200-fold lower in the cell-free system (18, 20, 21) than in *Xenopus* oocytes. Immunoprecipitation of the translation products of the mRNA preparations, in which the presence of interferon mRNA was demonstrated by oocyte assay, revealed a synthesis of a 22,000-dalton polypeptide. This polypeptide was not translated from the mRNA preparations negative for interferon synthesis in the oocytes. Therefore, it is tempting to conclude that the 22,000-dalton polypeptide is related to the interferon protein.

[^{35}S]Methionine-labeled interferon molecules synthesized *in vitro* should be detected by fluorography, even though the interferon mRNA represents only a fraction of the total mRNA translated. The amount of biologically active interferon synthesized under the conditions used is 10–100 units, which represents 10–100 pg of synthesized interferon (10^9 units/mg of protein). Since human fibroblast interferon contains three methionine residues (33), 1,500–15,000 cpm are expected in the interferon polypeptide band. The [^{35}S]methionine-labeled 22,000-dalton protein precipitated by the antiserum contains approximately 1,000 cpm, suggesting that it does represent *in vitro*-synthesized interferon. The molecular size of interferon polypeptide synthesized both in wheat germ and rabbit reticulocytes (34) is higher than would be expected for the unglycosylated interferon (35–37). However, it is possible that the 22,000-dalton protein represents a precursor protein (32), which is cleaved into interferon during the extracellular export (38). In the induced cells, the interferon precursor was not detected; however, the immunoprecipitation revealed the presence of a 15,000- to 16,000-dalton protein antigenically related to human interferon that may represent its intracellular form.

The results further indicate that in addition to interferon, poly(I)·poly(C) induces in human cells the synthesis of mRNAs and proteins not detected in the uninduced cells. It is unlikely

that these mRNAs and proteins have been synthesized as a response to interferon for several reasons: (*i*) 4 hr after poly(I)·poly(C) induction, the cells have not yet reached full antiviral state, and subsequent induction of the antiviral state can be prevented by the addition of actinomycin D at this time; (*ii*) the new mRNAs were found also in cells induced in the presence of cycloheximide—cells in which synthesis of interferon is markedly inhibited; and (*iii*) none of the new proteins synthesized in the induced cells seems to be identical to those identified in interferon-treated human fibroblast cells (5). The majority of the proteins induced together with interferon are either not exported from the cells to the medium or are separated during purification from the interferon used for immunization, because they are not precipitated by our anti-interferon serum.

The possibility that induction of interferon leads to the synthesis of additional protein together with interferon was suggested several years ago (39); their biological role is unknown. One of these proteins could represent the hypothetical "repressor" of interferon, which may regulate the cessation of interferon gene expression or the stability of interferon mRNA (40–42); others may have biological functions attributed at present to the interferon molecule (43–44).

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1. Finter, N., ed. (1973) *Interferons and Interferon Inducers* (North Holland, Amsterdam).
2. Taylor, J. (1964) *Biophys. Res. Commun.* **14,** 447–451.
3. Lockhart, R. Z., Jr. (1934) *Biochem. Biophys. Res. Commun.* **15,** 513–518.
4. Creagan, R. P., Tan, Y. H., Chen, S. & Ruddle, F. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34,** 2222–2226.
5. Knight, E. J. & Korant, B. D. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1824–1827.
6. Farrell, P. J., Broeze, R. J. & Lengyel, P. (1979) *Nature (London)* **279,** 523–525.
7. Ball, L. A. (1979) *Virology* **94,** 282–296.
8. Zilberstein, A., Federman, P., Shulman, L. & Revel, M. (1976) *FEBS Lett.* **88,** 119–124.
9. Lebleu, B., Sen, G. C., Sharta, S., Carrier, B. & Lengyel, P. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3107–3111.
10. Brown, G. E., Lebley, B., Kawakiba, M., Sharta, S., Sen, G. C. & Lengyel, P. (1976) *Biochem. Biophys. Res. Commun.* **69,** 114–122.
11. Kerr, I. M. & Brown, R. E. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 256–260.
12. Roberts, W. K., Hovanessian, A., Brown, R. E., Clements, M. D. & Kerr, I. M. (1976) *Nature (London)* **264,** 477–480.
13. Friedman, R. (1977) *Bacteriol. Rev.* **41,** 543–567.
14. De Maeyer-Guignard, J., De Maeyer, E. & Montagnier, L. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1203–1207.
15. Reynolds, T. H., Jr. & Pitha, P. M. (1974) *Biochem. Biophys. Res. Commun.* **59,** 1023–1029.
16. De Maeyer-Guignard, J., Tovey, M. G., Gresser, I. & De Maeyer, E. (1978) *Nature (London)* **271,** 622–625.
17. Rubinstein, M., Rubenstein, S., Familletti, P. C., Miller, R. S., Waldman, A. A. & Pestka, S. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 640–644.
18. Cavalieri, R. L., Havell, E. A., Vilcek, J. & Pestka, S. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4415–4419.
19. Sehgal, P. B., Dobberstein, B. & Tamm, I. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3409–3413.
20. Raj, N. B. K & Pitha, P. M. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1483–1487.
21. Reynolds, F. J., Premkumar, E. & Pitha, P. M. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4881–4885.
22. Strohman, R. C., Moss, P. S., Micou-Eastwood, J., Spector, D., Przybyla, A. & Paterson, B. (1977) *Cell* **10,** 265–273.
23. Roberts, B. E. & Paterson, B. M. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2330–2334.
24. Pelhman, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67,** 247–257.
25. Pitha, P. M., Fernie, B. F., Maldarelli, F., Hattman, T. & Wivel, N. A. (1980) *J. Gen. Virol.* **46,** 97–110.
26. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
27. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46,** 83–88.
28. Hunter, T. & Gibson, W. (1978) *J. Virol.* **28,** 240–253.
29. Greene, J. J., Dieffenbach, C. W. & T'so, P. O. P. (1978) *Nature (London)* **271,** 81–83.
30. Tan, J. H., Armstrong, J. A., Ice, J. H. & Ho, M. (1970) *Proc. Natl. Acad. Sci. USA* **67,** 464–471.
31. Dahl, H. & Degien, P. (1975) *Nature (London)* **257,** 799–801.
32. Weissenbach, J., Beer, M., Landau, T. & Revel, M. (1979) *Eur. J. Biochem.* **98,** 1–8.
33. Knight, E. J., Hunkapiller, M. W., Korant, B. D. & Hardy, R. W. F. (1980) *Science* **207,** 525–526.
34. Knight, E., Jr. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 520–523.
35. Reynolds, R., Jr. & Pitha, P. M. (1975) *Biochem. Biophys. Res. Commun.* **65,** 107–112.
36. Bose, S., Gurari, T., Rotman, D., Ruegg, V. T., Corley, L. & Anfinsen, C. B. (1976) *J. Biol. Chem.* **251,** 1659–1664.
37. Burke, D. C. (1978) in *Texas Reports on Biology and Medicine,* eds. Baron, S. & Dianzani, F. (University of Texas Medical Branch, Galveston, TX), pp. 11–16.
38. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67,** 835–851.
39. Paucker, K., Berg, K. & Ogburn, C. (1975) in *Effects of Interferon on Cells, Viruses and the Immune System,* ed. Geraldes, A. (Academic, New York), pp. 638–658.
40. Havel, E. A. (1977) in *Interferons and Their Actions,* ed. Stewart, W. E., II (C.R.C., Cleveland, OH), pp. 37–48.
41. Vilcek, J., Havel, E. A. & Tase, M. (1976) *J. Infect. Dis.* **133,** 422–429.
42. Seghal, P. B. & Tamm, I. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1621–1625.
43. McNeil, T. A. & Gresser, I. (1973) *Nature (London)* **244,** 173–174.
44. Donahoe, R. M. & Huang, K. (1973) *Infect. Immun.* **7,** 501–503.