Communication

Induction of Unique mRNAs by Human Interferons*
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Treatment of human fibroblast cells with human interferon (INF-α, INF-β, or INF-γ) resulted in the accumulation of at least four newly synthesized mRNAs. The mRNAs code for proteins having molecular weights of 56,000, 57,000, 62,000, and 68,000 when characterized in a wheat germ cell-free translation system. A direct relationship was observed between the amount of IFN used and the degree of both the accumulation of the induced mRNAs and the development of an antiviral state. In the case of IFN-α or INF-β, time course studies indicated that the induced mRNAs appeared as early as 40 min, accumulated for 2 h, then remained ribosome bound for up to 16 h. The ability of fibroblast cells to develop an antiviral state always coincided directly with both the appearance and the level of accumulation of the induced mRNAs. Further mRNA synthesis beyond 2 h had a minimal effect on the development of an antiviral state. Human INF-γ also induced the synthesis of the same four mRNAs but required higher interferon titers and a longer incubation time. In addition, INF-γ induced a disproportionate amount of the mRNA coding for the 68,000 molecular weight protein and three new mRNAs not detected in cells treated with INF-α or INF-β. Mouse interferon induces the original four mRNAs in human cells but to a far lesser extent. This correlated with the inability of these cells to develop much resistance to viral infection.

Interferons are proteins with antiviral, antitumor, and immunomodulator activities which are secreted in response to various inducers (1). Three major human IFN species, namely IFN-α (leukocyte), IFN-β (fibroblast), and IFN-γ (immune) have been identified by differences in their antigenic determinants and pH 2 lability. Despite having different amino acid sequences (2), all three species of IFN exhibit similar biological activities. Notably, they induce human cells to develop an antiviral state always coincided directly with both the appearance and the level of accumulation of the induced mRNAs. Further mRNA synthesis beyond 2 h had a minimal effect on the development of an antiviral state. Human INF-γ also induced the synthesis of the same four mRNAs but required higher interferon titers and a longer incubation time. In addition, INF-γ induced a disproportionate amount of the mRNA coding for the 68,000 molecular weight protein and three new mRNAs not detected in cells treated with INF-α or INF-β. Mouse interferon induces the original four mRNAs in human cells but to a far lesser extent. This correlated with the inability of these cells to develop much resistance to viral infection.

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The abbreviations used are: IFN, interferon; VSV, vesicular stomatitis virus; NaDodSO₄, sodium dodecyl sulfate.

Interferon Titration—INF titration assays were based on the inhibition of cytopathic effect in 96-well plates using either human diploid FS-7 fibroblast cells (INF-α) or FS-2 fibroblast cells (INF-β)
challenged with encephalomyocarditis virus or VSV, respectively (15). IFN-γ was assayed on GM-258 cells challenged with VSV. Mouse interferon was assayed on L cells challenged with VSV.

RESULTS

Previous work indicated that treatment of human diploid fibroblast cells with human IFN-β induces the synthesis of four mRNAs after 2 h of incubation (8). Based on in vitro translation experiments, these four mRNAs were shown to code for proteins with apparent molecular weights of 56,000, 57,000, 62,000, and 68,000 (formerly referred to as 61,000, 62,000, 64,000, and 68,000). To further prove that the appearance of the induced mRNAs was indeed related to interferon treatment, cells were treated with various concentrations of IFN-β for 2 h at 37 °C. Polysomal mRNA was isolated as described and translated in a wheat germ cell-free translation system. The [35S]methionine labeled translation products were then analyzed on a polyacrylamide gel. Results (Fig. 1A) show the accumulation of the four induced mRNAs previously shown to code for proteins having molecular weights of 56,000, 57,000, 62,000, and 68,000. These results demonstrate a direct relationship between the quantitative accumulation of the induced mRNAs and the amount of IFN-β added. Maximal accumulation of the four mRNAs was obtained when the cells were treated with 100–200 units/ml of IFN-β. In order to correlate the quantitative appearance of the induced mRNAs with the ability of the cells to develop an antiviral state, one roller bottle of cells was infected with VSV at the time of each mRNA isolation. Actinomycin D (5 μg/ml) was added to the cell culture along with VSV to inhibit further mRNA synthesis. Results (Fig. 1B) show that the development of an antiviral state was near maximum after treating the cells with 100 units/ml of IFN-β. Subsequent experiments were therefore performed with an IFN titer of 100 units/ml.

To more precisely determine the kinetics of appearance of these four mRNA species, polysomal mRNA was prepared from human fibroblast cells treated with 100 units/ml of purified IFN-β for 40, 80, and 120 min. Translation products of the isolated mRNAs reveal (Fig. 2A) that the four induced mRNAs appeared as early as 40 min after the addition of IFN-β. These data suggest that the four mRNA species were transcribed both simultaneously and quantitatively. Maximal accumulation of the induced mRNAs was observed after 2 h of incubation of cells with IFN-β. Longer treatment of cells with IFN-β beyond 2 h did not cause further accumulation of the four mRNA species (8). There appears to be a direct correlation between the amount of the induced mRNAs detected and the degree to which an antiviral state is established in the absence of further mRNA synthesis (Fig. 2C). Complete establishment of an antiviral state by the cells (over 99% reduction of virus titer) was achieved at the time (2 h) when maximal accumulation of the four mRNA species was observed.

Since human IFN-α is also able to protect human fibroblast cells against viral infection, it was of interest to determine whether IFN-α can also induce the same four induced mRNAs. Polysomal mRNA was isolated from cells treated with 100 units/ml of IFN-α as described previously. Results (Fig. 2B) indicate that the time course for the induction of the same four mRNAs was comparable, if not identical, to that induced by IFN-β. In addition, the ability of cells to resist VSV infection again increased directly with the accumulation of the four mRNAs (Fig. 2C).

The kinetics of turnover for each of the four induced mRNA species were analyzed to determine both the stability and the role of the induced mRNAs in the maintenance of the antiviral state. Cells were incubated with IFN-β at 37 °C for 2 h. The interferon was then removed, the cells washed twice with phosphate-buffered saline, and placed back at 37 °C with fresh media containing serum. Cells were then harvested every 8 h up to 48 h to determine the amount of polysome-bound induced mRNAs. Results (Fig. 3A) demonstrate that there are differential rates of release of the four mRNAs from polysomes. The mRNAs coding for the 57,000 and 68,000 molecular weight proteins remain ribosome-associated for at least 8 h while the mRNA coding for the 62,000 molecular weight protein has a half-life shorter than 8 h. The turnover time for the mRNA coding for the 56,000 molecular weight protein appears to be 16–24 h. A fifth mRNA species coding a protein with a molecular weight of 42,000 appeared after the removal of interferon from the cell culture, and its function in the maintenance of the antiviral state is not known. The decay of the antiviral state was also monitored (Fig. 3B). After the disappearance of the polysome-associated IFN-induced mRNAs, the ability of the cells to resist VSV infection was maintained for at least another 24 h. This suggests that the maintenance of the antiviral state does not require continuous translation of the four IFN-induced mRNA species.

It was of interest to study the ability of IFN-γ to induce the four mRNAs species under study. Preliminary experiments showed that, when compared to IFN-α and IFN-β, higher titers of IFN-γ and longer incubation times were needed to demonstrate near comparable protection of cells against VSV replication. Polysomal mRNA was isolated from cells treated with 1000 units/ml of IFN-γ for 3, 5, or 7 h at 37 °C. Results (Fig. 4A) show that the induced mRNAs coding for the 56,000, 57,000, and 62,000 molecular weight proteins were present over a 7-h period in reduced levels when compared to IFN-β induction. Surprisingly, the mRNA coding for the 68,000 molecular weight protein became the predominant mRNA on polysomes after IFN-γ treatment. Furthermore, three new mRNA species were detected which coded for proteins having molecular weights of 24,000, 31,000, and 41,000. The role these three new mRNAs play in the establishment of an antiviral
antiviral state (since they are not seen after treatment with IFN-α or IFN-β) has yet to be determined. It cannot be ruled out that mRNAs may be induced by other lymphokines present in the partially purified IFN-γ preparations. The IFN-γ result does point out that the mRNA coding for the 68,000 molecular weight protein may not be involved in the development of an antiviral state since treatment of cells with IFN-γ for 7 h did not result in an increased antiviral state (Fig. 4C). Alternatively, the 68,000 molecular weight protein may require the presence of the other three proteins to have antiviral activity since the development of the antiviral state appears to coincide with the appearance of the other three mRNAs.

It has been shown that some mouse IFN subspecies are active in human cells (17, 18). Therefore, it was of interest to investigate whether mouse IFN can induce the same four mRNAs in human fibroblasts. Human fibroblast cells were treated with mouse IFN at 100 units/ml for up to 5 h at 37 °C before isolation of mRNAs. Characterization of the mouse IFN-induced mRNAs showed a low level (Fig. 4B) accumulation of the same four mRNAs induced by the three species of human IFN. As predicted, the low level of the mRNAs induced correlated with the limited ability of these cells to resist VSV replication.

**DISCUSSION**

It has been well established that mRNA synthesis and its subsequent translation into proteins are required prerequisites for the development of an antiviral state in cells treated with IFN (1). In the present report, we have clearly demonstrated that treatment of human diploid fibroblast cells with different human interferons resulted in the accumulation of at least four newly synthesized mRNAs. The mRNAs code for proteins having molecular weights of 56,000, 57,000, 62,000, and 68,000 and have been previously shown to sediment in sucrose gradients at 21S, 16.5S, 19S, and 21S, respectively (8). Surveillance of cells for the ability to develop an antiviral state during mRNA isolation has repeatedly demonstrated a direct correlation between the quantitative appearance of these four mRNAs and the degree to which development of an antiviral state can occur (Figs. 1-4). It would be precarious to assume that these four mRNAs account for all the mRNAs required in the development of an antiviral state since additional mRNAs were found in IFN-γ treated cells (Fig. 4). Furthermore, detection of proteins with molecular weights of greater than 95,000 and minor mRNA species are difficult to see using cell-free translation systems.

Recent experiments conclusively show that the 68,000 molecular weight protein coded for by one of the induced mRNAs is found in IFN-treated extracts of fibroblast cells. The fact that there is a preferential accumulation of this mRNA species...
in cells treated with IFN-γ without the concomitant establishment of full antiviral state suggests that this protein may not be involved directly in the development of an antiviral (VSV) state, but instead be involved in other cellular activities resulting from IFN treatment. Clearly, the level of accumulation of the mRNA species coding for the 56,000, 57,000, and 62,000 molecular weight proteins correlates well in all experiments with the degree to which an antiviral state is developed. This suggests that these three mRNAs are directly involved in the development of an antiviral state. The mRNAs themselves appear vary rapidly (within 40 min) in the case of IFN-α or IFN-β (Fig. 2) and remain ribosome-associated for 8–16 h (Fig. 3). The antiviral proteins coded for by the mRNAs must also be very stable because maintenance of the antiviral state does not require continuous translation of these mRNA species (Fig. 3). It is important to emphasize that throughout this study we have correlated mRNA appearance with the ability of cells to resist infection by VSV. Studies on the mouse Mx gene have demonstrated that specific interferon-induced genes may be required to protect cells against specific virus groups (19). Therefore, whether the IFN responsive genes that we have observed are also active against other types of viruses remains to be determined. It is hoped that future cloning of the induced mRNAs will allow us to further study the structure and expression of the IFN-induced genes.

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