ANTIVIRAL ACTIVITY OF RNA PREPARATIONS ISOLATED FROM INTERFERON-TREATED CELLS (MESSENGER RNA OR ANTIVIRAL PROTEIN?)

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A biologically active RNA which, when transferred to chick embryonic cells, produced an antiviral state in them, was isolated from cultures of chick embryonic cells treated with interferon. The level of reproduction of vesicular stomatitis virus in these cells was 2-30% of the level of reproduction observed in cells treated with control RNA. Maximal activity of the experimental RNA was observed after exposure to interferon for 3 h.

KEY WORDS: *interferon; RNA; antiviral state.*

The action of interferon is based on the formation of a specific antiviral protein (AVP) in the cells treated with it; this protein modifies the cell ribosomes so that they do not translate viral messenger RNA [5]. The mechanisms of formation of AVP have not yet been explained. However, there is indirect evidence (for example, the sensitivity of the action of interferon to actinomycin D) which suggests that its appearance is preceded by a phase of derepression of specific messenger RNA. In this investigation an attempt was made to isolate this RNA and to work out a scheme for determining its action.

EXPERIMENTAL METHOD

Chick interferon was prepared by infecting chick embryos with influenza virus A/WSN in a dilution of 10^3 PFU per embryo. After cultivation for 48 h the allantoic fluid was drawn off and subjected to differential centrifugation to sediment the large particles and virions (at 5000 rpm for 30 min and at 70,000 rpm for 2 h). The supernatant was treated with hydrochloric acid (pH 2.0) for 3 days to inactivate the residual virus. The pH of the medium was then adjusted to neutral with 2 N NaOH and the resulting interferon was titrated by the usual method of inhibition of the cytopathic action of vesicular stomatitis virus (VSV) [3]. Interferon (100-200 units/ml) was added to a monolayer culture of chick embryonic cells (CEC) and kept at 37° for between 1 and 18 h depending on the experimental conditions. The liquid was decanted, and RNA isolated from the cells by the phenol-detergent method in the modification of De Maeyer-Guignard et al. [4]. In this way the cytoplasmic (fraction C) and membrane-bound with nuclear (fraction N) RNA could be obtained separately, and this method had been widely used by the writers to study messenger RNA for interferon [1, 2]. After double reprecipitation with alcohol the residue of RNA was dissolved in a solution of DEAE-dextran (75 µg/ml in 0.02 M phosphate buffer, pH 7.17) and added to the CEC, which had first been treated for 2 h with actinomycin D (2 µg/ml), which liberates the cell polysomes from endogenous mRNA and facilitates the translation of added messenger RNA [1, 4]. Contact between RNA and the cells lasted for between 45 min and 1 h, after which the RNA was poured off, the cells were treated with medium No. 199 with 5% bovine serum, and they were cultured for 4 h, on the assumption that by that time the AVP should have gone through translation. Next, VSV was added to the cells with a multiplicity of

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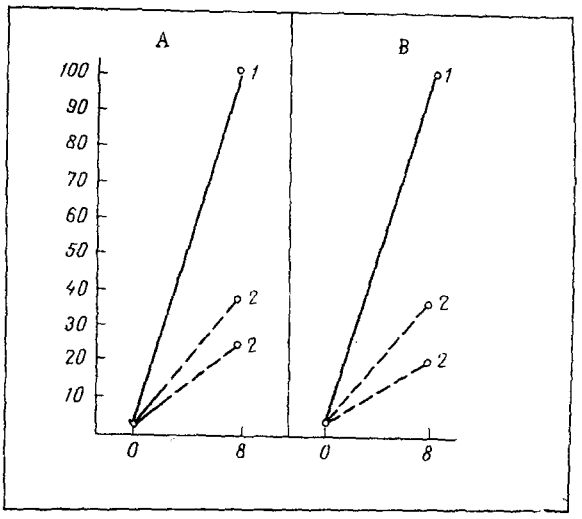

Fig. 1. Reproduction of vesicular stomatitis virus in cells treated with RNA: 1) control RNA, 2) experimental RNA; A) fraction C, B) fraction N. Ordinate, reproduction of virus (in %); abscissa, time (in h).

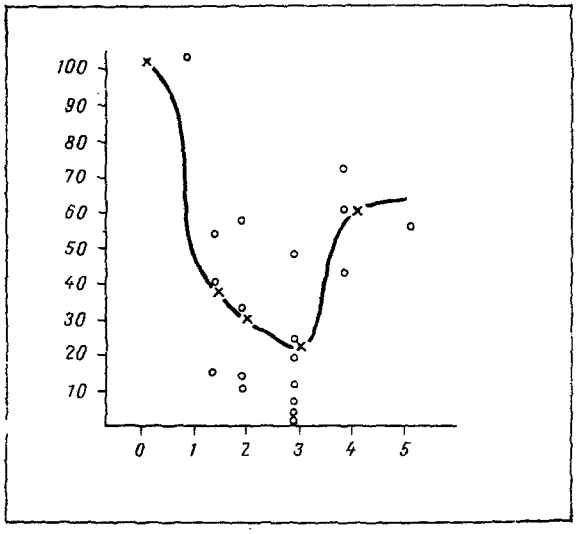

Fig. 2. Dynamics of appearance of biologically active RNA in cells treated with interferon. Ordinate, reproduction of virus (in %); abscissa, time of action of interferon (in h). Circles indicate result of each experiment; curve drawn through arithmetic mean values.

0.01-0.001 PFU per cell and cultured for 8-12 h, after which the yield of virus was determined. The presence of antiviral activity of RNA was judged from the difference in reproduction of VSV in the control and experimental CEC. The control for the experiment consisted of CEC treated with RNA isolated from normal cells, not in contact with interferon and CEC not treated with RNA at all.

EXPERIMENTAL RESULTS

The antiviral state of the cells is known to begin to develop 1 h after the addition of interferon and to reach its maximum after 6-8 h; the intensity thereafter remains at the same level so long as interferon remains in the medium. In the first series of experiments RNA was therefore isolated from CEC which had been in contact with interferon for 18 h. It will be clear from Fig. 1 (plotted from the results of two experiments) that the intensity of reproduction of the virus in cells treated with experimental RNA (Fig. 1, curves 2) was considerably below the level of reproduction in cells treated with the control RNA, namely 25-37%. Both the C fraction and the N fraction of the experimental RNA possessed this property.

Next,the dynamics of formation of this particular RNA was studied, i.e., the RNA was isolated from cells exposed to the action of interferon for different times. Altogether nine experiments were carried out, of which two gave negative results, i.e., inhibition of virus reproduction was not found. The results of seven experiments are illustrated in Fig. 2. The appearance of active RNA was observed 1.5 h after the addition of interferon (the arithmetic mean level of virus reproduction in cells treated with this RNA was 35%). Activity then increased and the level of reproduction of the virus after treatment of the cells with RNA isolated after exposure with interferon for 2 h was 28%, whereas after exposure for 3 h it was 21%. The last RNA proved to be the most active; in some experiments reproduction was lowered to 2-6%.

The results are in good agreement with the dynamics of formation of the antiviral state of the cells under the influence of interferon. As was pointed out above, it was after 4-6 h that maximal activity of AVP was observed. On the basis of the results of these experiments the maximal activity of RNA from cells treated with interferon preceded the time of development of the antiviral state. After exposure to interferon for 4 h the activity of the isolated RNA was reduced, evidently in connection with the utilization of this RNA in the translation of AVP.

It must also be added that the experiments to determine antiviral activity of RNA of cells treated with interferon were associated with certain difficulties, for sometimes the RNA isolated from the control cells had antiviral action. This could evidently by due to the presence of certain persistent viruses in the cells. Only those experiments in which the control RNA had no action on reproduction of VSV were therefore considered in the calculation.

It can be concluded by saying that a biologically active RNA was isolated from cells treated with interferon and, under the chosen conditions, it produced an antiviral state in the cells. The maximum of activity of this RNA was observed 3 h after the addition of interferon. The results can be interpreted as the first attempt to isolate and describe a messenger RNA for AVP.

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