NONSPECIFICITY OF ACTION OF ANTIVIRAL PROTEIN

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UDC 576.858.042.2:577.112

The antiviral activity of antiviral protein messenger RNAs (AVP mRNA) isolated from cells after the superinduction of interferon in them was studied in order to ascertain the specificity of action of their translation product – AVP. After injection of AVP mRNA a marked decrease (from 1 to 5 log PFU/ml) in the infectious titers was observed in both homologous and heterologous cells. RNA preparations from control (uninduced) cells possessed weak (0.4-0.1 log PFU/ml) antiviral activity.

KEY WORDS: interferon; antiviral protein; messenger RNA; translation.

Previous investigations have shown that mRNAs isolated from superinduced cells possess two types of biological activity: interferon and antiviral activity [1, 2, 8]. The first is connected with the presence of interferon mRNAs in the preparation, which are effectively translated in both homologous and heterologous cells with the formation of species-specific interferons. The second is due to the presence of antiviral protein (AVP) mRNAs in the preparation, during the translation of which the rapid development of antiviral resistance is observed in homologous cells.

In the present investigation the antiviral activity of AVP mRNA was investigated in homologous and heterologous cells in order to study the specificity of action of its translation product – AVP. In the modern view, AVP is the final link in the chain of action of interferon and it determines the development of antiviral resistance in the cell. The study of its specificity is therefore of fundamental importance.

EXPERIMENTAL METHOD

Interferon superinduction was set up in accordance with the scheme of Vilcek et al. [10]. Cells were treated with polyI-polyC (10-50 μg/ml) in the presence of DEAE-dextran (100-200 μg/ml) for 30 min or 1 h, after which cycloheximide was added (10-20 μg/ml) to the culture medium. Actinomycin D (1-2 μg/ml) was added to the medium 5 h later for 1 h, after which the culture medium was poured off, the cells were washed three times with Hanks' salt solution, and they were covered with medium 199 containing 2% bovine serum. After incubation for 18 h at 37°C the interferon titers were determined in the culture medium, whereas the cells were washed with isotonic sodium chloride solution and used for the isolation of RNA. Messenger RNAs were isolated by the methods described by Perry et al. [8]. The cells were disintegrated in a homogenizer and centrifuged at 1000g to remove nuclei at 4°C. RNAs were isolated at room temperature with a mixture of phenol and chloroform (1:1), saturated with STE buffer (0.01M Tris-HCl, 0.1M NaCl, 0.001M EDTA) at pH 7.0 or 9.0 in the presence of 1% SDS. These conditions ensured quantitative isolation of the polyA content of the messenger RNAs. After extraction of the RNAs twice or three times they were precipitated with ethyl alcohol with the addition of sodium chloride to a concentration of 0.1M. To prevent the toxic action of phenol on the cells the RNAs were reprecipitated with alcohol. Primarily trypsinized chick embryonic (FEK) and human embryonic (FECH and PECH) cells and transplantable mouse (L-1210) and monkey (BFC-1) cells were used as donors and recipients of AVP mRNA. mRNA preparations isolated from superinduced cells were added in concentrations of 50-100 μg/ml were applied in medium 199 containing 200-500 μg/ml DEAE-dextran to the cell monolayer. To prevent induction of homologous interferon, before the addition of the RNA the cells were treated for 2-4 h with actinomycin D (2 μg/ml). RNA was adsorbed for 1 h at 37°C or at room temperature, after which the cells were washed to remove RNA with medium or Hanks' salt solution and infected with Venezuelan equine encephalomyelitis (VEE) virus with a multiplicity of 10-50 PFU/cells. After incubation for 18 h the infectious titers of virus were determined in the culture fluid in PFU/ml.

D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 6, pp. 720-721, June, 1978. Original article submitted October 4, 1977.

 0007-4888/78/8506-0788$07.50

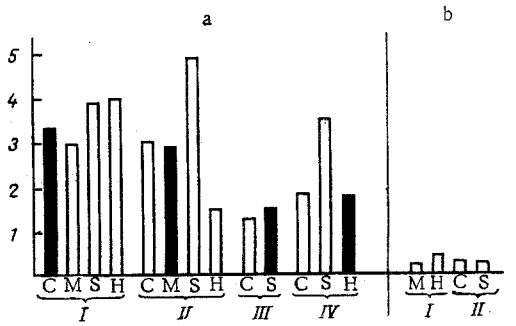

Fig. 1. Antiviral activity of AVP mRNA in cells of different origin. Abscissa, species of AVP mRNA: I) Chick, II) mouse, III) monkey, IV) human; ordinate, depression of yield of VEE virus compared with control (in log PFU/ml). Unshaded columns show antiviral activity after introduction of different AVP mRNAs and control RNAs from noninduced cells into chick (C), mouse (M), monkey (S), and human (H) cells; black columns show antiviral activity in homologous systems: a) AVP mRNAs; b) control RNAs.

EXPERIMENTAL RESULTS

The results of the various experiments to determine the antiviral activity of four preparations of AVP mRNA in chick, mouse, monkey, and human cells are summarized in Fig. 1. The degree of activity of mRNA was judged from the reduction in the yield of VEE virus, expressed in log PFU/ml. As Fig. 1 shows, after administration of the mRNA a marked decrease (from 1 to 5 log) in the titers of VEE virus was observed in both homologous and heterologous cells, evidence of the nonspecificity of action of their translation products – AVP. After treatment of the AVP mRNA preparations with pancreatic RNase, the antiviral effect was completely abolished. RNA preparations isolated from normal (noninduced) cells possessed weak inhibitory action (0.1-0.4 log).

The same conclusion regarding the nonspecificity of AVP by contrast with the marked species specificity of interferon [1] was drawn by Samuel and Farris [9]. AVPs extracted by them from ribosomes of human and monkey cells previously treated with interferon effectively suppressed the translation of rheovirus RNA in cell-free extracts of Krebs-2 mouse cells. The other approach to the study of the specificity of action of AVP used in the present investigation, namely the translation of AVP mRNA in homologous and heterologous cells, is simpler and more reliable, for in this case complete biological cell systems are used with all their inherent regulatory processes. The phenomenon discovered opens up fresh prospects for the study of discrimination of translation of viral and cell templates, which is a matter of great practical importance for the chemotherapy of virus infection.

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