VIROLOGY

Inhibition by Interferon of Production of Double-stranded Semliki Forest Virus Ribonucleic Acid

THE formation of virus-specific RNA¹ and of infectious RNA² is inhibited in cells treated with interferon. This communication presents evidence that interferon also inhibits the formation of the double-stranded form of RNA appearing in cells infected with Semliki forest virus (SFV).

Double-stranded forms of virus RNA have been reported in bacterial plant and animal cells infected with RNA viruses³. Such forms appear to play an important part in virus multiplication. A double-stranded form of RNA has also been reported in primary chick embryo fibroblasts infected with SFV⁴. Since SFV is quite sensitive to the antiviral action of interferon, the effect of interferon on the formation of the double-stranded form of SFV was studied.

Preparation of chick embryo fibroblast cultures, virus strains, and media used in this laboratory has been previously described^{1,5}. Each experimental group contained 10⁸ cells. The cells were treated with 50 units of interferon or with buffer for 4 h at 36° C. (A unit of interferon reduced by 50 per cent plaque formation by vaccinia virus on chick cells.) The cells were then washed and infected with SFV at a multiplicity of 10–20 plaque-forming units per cell. After 1 h at 36° C for virus adsorption 1 µg/ml. actinomycin D was added and the cells left overnight at 4° C. The next morning the cells were replaced at 36° C, and this time was considered to be the

onset of virus growth. RNA synthesis and infectious virus production have been closely studied under these conditions⁶.

After 2 h and 5 h of virus growth, a 1-h pulse of 60 µc. and 20 µc. respectively of tritium-labelled adenosine was added to one set of interferon-treated and one set of control cells. After this 1-h labelling period, the cells were scraped off the glass and homogenized in 0.1 M potassium phosphate buffer, pH 7.2, in a Dounce homogenizer. Nuclei were removed by centrifugation and the cytoplasm treated with 5 per cent sodium dodecyl sulphate for 4 min at 37° C. Preparations were analysed by sedimentation in a 6–30 per cent sucrose gradient with chick ribosomal RNA as an optical density marker; fractions were collected by piercing the bottom of the tube. Tritium was counted in a scintillation counter. Details of these methods will be published later⁷.

Fig. 1 shows the sedimentation pattern of RNA extracted from cells labelled at 2–3 or 5–6 h after infection together with the optical density of the unlabelled chick ribosomal RNA used as a marker. The results resemble those of previously reported investigations⁴ in that 2 viral RNA components are seen in material extracted from cells both early and late in infection. In the cells labelled at 2–3 h after infection, however, the slower-sedimenting component predominates. This component is ribonuclease-resistant and has been identified as the double-stranded form of Semliki forest virus RNA.

In the time-periods examined both the slow- and fast-sedimenting components were markedly depressed or completely absent from the cells pre-treated with interferon.

Since interferon markedly inhibits the formation of the double-stranded form of Semliki forest virus RNA, it is evident that the biochemical event(s) blocked by interferon must occur quite early in infection. Levy's results⁸, however, indicate that at least some virus-specific proteins, the inhibitors of cellular RNA and protein synthesis, which are produced very early in infection, are made in interferon-treated cells. The point of action of interferon would therefore appear to be somewhere between the production of virus specific early proteins and the production of double-stranded virus RNA.

Recent investigations indicate that the antiviral action of interferon appears to depend on the synthesis by cells of an induced antiviral protein⁸. These findings are consistent with the hypothesis that interferon acts by inducing the synthesis of a protein which inhibits net synthesis of virus RNA.

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GENETICS

Monosomic Interchange Heterozygote of Diploid Chrysanthemum

DEFICIENCY of whole chromosomes is normally viable in species with polyploid ancestry where previous duplication of chromosomes seems to compensate for missing

Fig. 1