STUDIES ON NUCLEAR PROTEIN METABOLISM AFTER INFECTION OF EHRLICH ASCITES CELLS WITH MAUS-ELBERFELD (ME) VIRUS*

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Received January 31, 1964

The striking early suppression of DNA-dependent RNA-synthesis following infection of the host-cell by a number of different picornaviruses has been well documented (Martin et al., 1961; Franklin and Rosner, 1962; Hausen, 1962; Zimmerman et al., 1962; Holland, 1962; Fenwick, 1963). The mechanism of this suppression is not presently understood. However, the fact that p-fluoro-phenylalanine (Verwoerd and Hausen, 1963) and puromycin (Franklin and Baltimore, 1962) prevent the viral-induced suppression of cellular RNA synthesis suggests a mechanism requiring protein synthesis.

The hypothesis (Stedman and Stedman, 1951) that histones may represent functional regulators of gene expression through combination with sites on DNA is gaining experimental support (Allfrey et al., 1963; Huang and Bonner, 1962; Barr and Butler, 1963). The experiments described here represent an exploration of the possibility that the inhibition of cellular RNA synthesis following viral infection is the result of a general blockade of DNA-expression by histones by following the synthesis of nuclear histones during the process of viral development. It will be shown in this report that a marked increase in the labelling rate of the nuclear histone fraction of Ehrlich ascites cells occurs shortly after infection with ME-virus.

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*This investigation was supported in part by Public Health Service Research Grant, AI 01267 from the National Institute of Allergy and Infectious Diseases, Public Health Service, and by the Cancer Research Fund, University of California.

MATERIALS AND METHODS

ME-virus, a picornavirus belonging to the Columbia SK group (Hausen and Schäfer, 1962) exhibits in Ehrlich ascites suspensions an eclipse period of 3 to 5 hours and affords maximal yields of virus 8 to 10 hours after infection.

Virus was prepared by homogenizing infected mouse brains in 10 volumes (v/w) Dulbecco's buffer (DB) and 5 volumes (v/w) fluorocarbon (Genetron 113) 2 minutes in a Waring blendor in the cold. The emulsion was then centrifuged 10 minutes at 2,000 xg and the supernatant subjected to two further cycles of fluorocarbon treatment as described above. The resulting clear supernatant fluid containing 1-2 x 10^8 plaque-forming-units (pfu) per ml was used in all experiments.

Tumors were harvested 7 days after intraperitoneal inoculation of about 4 x 10^6 cells, washed free of erythrocytes with DB, suspended in Earle's saline to 5 x 10^6 cells/ml and at a time hereafter referred to as "zero-time" infected at 37° C in swirling cultures by addition of virus to about 6-10 pfu per cell. Washed cells, assayed as infectious centers by a modification of the ascites-cell plaque test (Martin et al., 1961), typically registered 40 to 70% of the cells plated. For reasons as yet undetermined clusters of "negative runs" characterized by failure to produce hemagglutinin or infectious centers occurred at sporadic intervals and such experiments were discarded.

Suspensions of infected and uninfected control cells were pulse-labelled at various intervals by simultaneous addition 30 minutes prior to the time of harvest of 2.5 μC of L-arginine-C^{14} (specific activity 150 mC/mmole from Schwarz BioResearch Inc.) and either thymidine-methyl-H^3 (specific activity 6700 mC/mmole from New England Nuclear Corporation) or uridine-H^3 (specific activity 600 mC/mmole from New England Nuclear Corporation). At the times indicated in Figure 1 the cells were chilled in an ice bath and washed with chilled DB and unlabelled carrier.

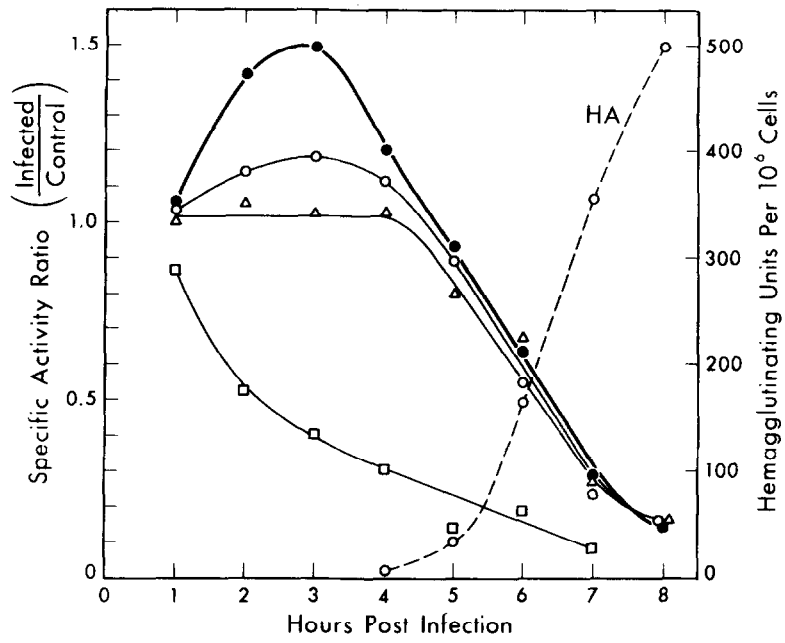

Fig. 1. Effect of ME-virus infection on nuclear macromolecular syntheses in Ehrlich ascites cells. Cells were pulse-labelled and harvested at the indicated time intervals. Measurements were made on isolated nuclei as described in the text. Hemagglutinin (HA) was found mainly in the cytoplasmic fraction.

●——● "Histone-Fraction"; ○——○ "Acid-insoluble nuclear proteins"; △——△ DNA; □——□ RNA: ○-------○ Hemagglutinin.

Nuclei were isolated from washed cells by repeated osmotic shock with distilled water and freed of cytoplasmic contamination by the procedure of Chaveau et al. (1957).

Acid soluble nuclear proteins, consisting mainly of histones with small amounts of other proteins (Holoubek, unpublished data), were extracted in 5 volumes (v/w) 0.25 N HCl, the extract clarified by ultracentrifugation (60 minutes at 100,000 xg) and dialyzed 48 hours against several changes of 500 volumes distilled water in the cold room. This acid soluble, non-dialyzable nuclear fraction is referred to as the "histone-fraction". The insoluble material reconverted from the HCl extracted nuclei was freed of RNA, DNA and lipids by the procedure of Ogur and Rosen (1950), and the residual material referred to as "acid insoluble nuclear protein".

RNA and DNA were determined spectrophotometrically. Protein was determined by the biuret procedure (Kabat and Mayer, 1962). C^{14} and H^3 were counted simultaneously in the Packard automatic two-channel Tri-carb liquid scintillation spectrometer with a split channel setting.

Hemagglutinating activity was assayed by the procedure of Jungeblut (1958).

RESULTS AND DISCUSSION

The effect of virus infection on the incorporation by intact cells of isotopic precursors into DNA, RNA, histone-fraction and acid-insoluble nuclear proteins of the cell nucleus is shown in Figure 1. Also presented for reference purposes is the hemagglutinating activity (HA) of the cytoplasmic fraction. HA first appeared about 4 hours post-infection (p.i.) and was generally complete 8 to 10 hours p.i.

An increase in specific activity of the pulse-labelled nuclear histone fraction was detected within an hour p.i. and rose 50% above controls within 2 to 3 hours, then fell sharply to less than 20% of controls by 8 hours. The early increase in histone labelling considerably exceeded a parallel but much smaller increase in the specific activity of the acid-insoluble nuclear protein, a result arguing against interpretation of the effect in terms of changing amino acid pool size.

Concommitant with the rise in specific activity of the histone-fraction was a sharp decrease in the rate of labelling of RNA by uridine while no detectable change in the rate of labelling of DNA by thymidine was observed until about 5 hours p.i.

It is clear from these experiments that the gross effects of infection of Ehrlich ascites cells with ME-virus are in most respects similar to those observed with a number of other picornavirus-infected cell systems. Of particular interest, however, for the present discussion, is the observed increase in histone-labelling following viral infection which occurs almost simultaneously with the virus-induced suppression of nuclear RNA synthesis.

It has been shown previously that an enzymatically active DNA-protein complex (called"aggregate enzyme" and believed to represent the major cellular RNA synthesizing machinery) isolated from Mengo-infected L-cells (Baltimore and Franklin, 1962) or polio-infected HeLa cells (Holland, 1962) exhibited decreased RNA-synthesizing capacity in a cell-free environment. In both cases DNA isolated from the inhibited nucleoprotein aggregate possessed unimpaired "priming" activity and was physically indistinguishable from normal DNA. Attempts to demonstrate suppression of cellular RNA synthesis with viral coat protein (uv-irradiated virus) or to detect activation of cellular nucleases also proved fruitless (Franklin and Baltimore, 1962). Furthermore, studies on the effects of FPA and puromycin cited earlier strongly suggest that synthesis of a protein-like material is a necessary requirement for expression of the picornavirus-induced suppression of nuclear RNA synthesis.

Our observation of a temporal correlation between stimulation of nuclear histone labelling and suppression of nuclear RNA synthesis in conjunction with previous reports that DNA-dependent RNA synthesis is inhibited by shielding of nuclear DNA with histones (Allfrey et al., 1963; Huang and Bonner, 1962; Barr and Butler, 1963), plausibly implicate the histones in the suppression of nuclear RNA synthesis induced by ME-virus.

To summarize, evidence is presented that the synthesis of nuclear histones is markedly accelerated following infection of Ehrlich ascites cells with ME-virus. It is postulated that the characteristic picornavirus-induced suppression of nuclear RNA synthesis is the result of blockade of host DNA by histones, the synthesis of which are initiated or accelerated shortly after virus infection.

The authors are indebted to Professor Werner Schäfer for making ME-virus available to them and to Professors Wendell M. Stanley and Timothy Crocker for their encouragement and support. We also wish to acknowledge the competent technical assistance of Mrs. Carroll Hatie and Miss Malinda Keeser.

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