Biochemical Events During Interferon Synthesis in L Cells Infected with Newcastle Disease Virus[1] (35431)

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(Introduced by G. P. Youmans)
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Despite the inefficient nature of Newcastle disease virus (NDV) replication in mouse L cells, several studies have indicated that the cell–virus interaction results in the development of cytopathogenic effects leading to cell death (1, 2). These observations suggest that despite the abortive nature of viral replication, the infection of L cells with NDV resulted in a substantial alteration in cellular metabolism. Although this system has been extensively employed in biochemical studies relating to the induction and synthesis of interferon (3–5), very little information is presently available regarding the effects of this abortive infection on cellular macromolecular biosynthesis. The present study involves an investigation of the temporal relationship between interferon production in L cells infected with NDV and subsequent changes in cellular protein and nucleic acid synthesis.

Materials and Methods, The cultivation of mouse L-929 cells and the propagation of NDV (Roakin strain) have been reported (3, 6). Interferon was produced by infecting L cell monolayers with NDV at a multiplicity of 30 plaque-forming units/cell (pfu/cell). Following an adsorption period of 60 min at 37°, the monolayers were washed two to three times to remove the unadsorbed virus (6). After incubation at 37° for the time periods stated in the specific experiments, the culture medium was collected and the NDV was inactivated overnight at pH 2.0 (4°). Interferon was titered by a plaque reduction assay with mengo virus and L cell monolayers by pretreating the cells for 24 hr with serial dilutions of the inactivated medium as previously described (6). A unit of interferon was designated as the amount of interferon that produced a 50% reduction in viral plaques.

The effect of viral infection on cellular protein synthesis was measured by infecting L cell monolayers with NDV (30 pfu/cell). After a 60-min adsorption period, infected and noninfected (control) monolayers were carefully washed twice with normal saline and fed 4 ml of double strength Eagle's medium (6). At various times after viral adsorption (p.i.), the medium from infected and control monolayers was replaced with 1 ml of medium containing the labeled amino acids of interest. The cells were incubated at 37° for 30 min to allow incorporation of the radioactive precursors into protein and then the monolayers were washed several times with cold saline. Cellular protein was extracted and precipitated with hot 5% trichloroacetic acid as previously described (6). The resulting protein precipitate was solubilized in 0.05 *N* sodium hydroxide and an aliquot was used to determine radioactivity in a Beckman LS-100 scintillation counter. The results are expressed as counts per minute per milligram of cellular protein.

Nuclear RNA synthesis was measured by the incubation of cell monolayers with 1.0 ml of medium containing ^{3}H-uridine for 60 min. Following incubation, the cell monolay-

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ers were washed twice with cold saline, the cells were removed by trypsinization and washed to remove the trypsin. The cells were then resuspended in 2 ml of cold hypotonic buffer (10 m*M* Tris·HCl, pH 7.4; 10 m*M* KCl; 1.5 m*M* $MgCl_2$) and disrupted by 8 to 12 strokes of a motorized Teflon–glass homogenizer. The nuclei were pelleted by centrifugation, the nuclear RNA was deproteinized with hot phenol–sodium dodecyl sulfate and purified by several precipitations with cold ethanol (7). Since RNA synthesis in nuclei obtained from NDV-infected L cells was decreased by 80 to 90% within 30 min after addition of actinomycin D (Lancz and Johnson, unpublished observations), the nuclear preparations were not further purified by detergent treatment to remove residual cytoplasmic constitutents. The radioactivity in the final RNA preparation was determined by counting a suitable aliquot as described above.

Protein concentrations were determined by the colorimetric procedure of Oyama and Eagle (8). Crystalline bovine serum albumin was used as the protein standard.

^{14}C-Valine (200 mCi/mmole), ^{3}H-valine (2.97 Ci/mmole) and a ^{3}H-amino acid mixture were purchased from New England Nuclear Corp., Boston, Mass. ^{3}H-Uridine (9.53 Ci/mmole) was purchased from Schwarz BioResearch Inc., Orangeburg, New York. Actinomycin D was a gift from Merck, Sharp and Dohme Research Laboratories, Rahway, New Jersey.

Results. The relationship between RNA synthesis and interferon production was examined in L cells during the first 12 hr after their infection with NDV. Actinomycin D was added at various time periods after infection of the cells and the interferon concentration was measured at the time of addition and at 24 hr postinfection. The results indicated that the synthesis of interferon was extremely sensitive to actinomycin D during the first 8 hr of infection (Table I). At 8 hr, less than 5% of the final interferon yield had been synthesized and the inhibition of RNA synthesis completely suppressed further interferon production. However, when actinomycin D was added 12 hr after infection, when approximately 50% of the interferon was already synthesized, continued production of the interferon was resistant to the interruption of transcriptional events by the antibiotic.

TABLE I. Time Course for the Induction of Interferon.[a]

Actinomycin D addition (hr p.i.)	Actinomycin D	Interferon titer: At time of actinomycin addition (units/ml)	Interferon titer: 24 hr post-infection (units/ml)
1	0	0	2,400
1	+	0	0
8	0	100	2,400
8	+	70	50
12	0	1,200	2,400
12	+	1,400	2,400

[a] L cell monolayers were infected with NDV at a multiplicity of 30 pfu/cell. At the times indicated, a portion of the medium was removed to determine the interferon titer and actinomycin D was added to the appropriate cultures to a final concentration of 1 μg/ml. At 24 hr after infection, the medium was removed from all cultures, acidified to inactivate the virus, dialyzed overnight at 4°, and titered for interferon.

Although we have previously shown that most of L cell interferon synthesis occurs relatively late in the viral replication cycle (3) (after 10 to 12 hr), the above results suggest that transcriptional events leading to interferon production are primarily initiated between 8 to 12 hr after infection of the cells with NDV. Therefore, studies were performed to examine the effects of NDV infection on cellular protein synthesis prior to interferon production. Infected and noninfected L cell monolayers were incubated with ^{14}C-valine medium at 1, 3, and 7 hr after viral adsorption. The results showed that the infection of the cells had little effect within the first 3 hr although by 7 hr, there was a 60% decrease in protein synthesis in the infected cells (Table II). These data would suggest that interferon, a host-cell directed protein, might be synthesized when overall protein synthesis was being inhibited by the viral infection. The relationship between macromolecular synthesis and interferon synthe-

TABLE II. The Effect of NDV Infection on the Incorporation of ^{14}C-Valine by L Cells.[a]

Time after infection (hr)	Sp act (cpm/mg of protein)	Percentage of control
1 control	59,500	—
1 infected	65,200	110
3 control	58,000	—
3 infected	58,000	100
7 control	56,100	—
7 infected	30,500	54

[a] L cell monolayers were infected with NDV (30 pfu/cell) or sham infected as described in the methods. At various times after viral adsorption the monolayers were incubated with 1.0 ml of medium containing ^{14}C-valine (0.25 μCi/ml) for 30 min.

sis was further examined by comparing the rate of both amino acid and uridine incorporation at various time periods following infection. The results (Fig. 1) clearly show that both protein and nucleic acid synthesis are drastically altered during the viral infection. Approximately 3 hr after infection, protein synthesis rapidly decreased and within 10 hr, when interferon was being synthesized, only 20% of the cellular protein synthesis remained. During this same time period a decrease in nuclear RNA synthesis was also observed and 10 hr after viral adsorption approximately 50% of RNA synthesis remained.

Discussion. Several studies have shown that the production of interferon in virus-infected cells requires protein and nucleic acid synthesis (9–11). Investigators have been able to estimate the time course of the induction and synthesis of interferon messenger RNA by measuring the effect of actinomycin D on interferon production (12–14). The present data confirm and extend these observations as well as present an overall view of the relationship between cellular macromolecular synthesis during the production of interferon. Previous studies on the NDV–L cell interaction indicated that interferon was synthesized relatively late in the virus replication cycle and was initially detectable intracellularly at 7 hr after infection (3). The present study substantiates this finding and shows that interferon is actively synthesized and production is effectively blocked by actinomycin D during this time period (Table I). However, actinomycin D had little, if any, effect on interferon production when it was introduced 12 hr after viral adsorption. The time of this actinomycin sensitive period appears to be somewhat different to that measured in chick embryo fibroblasts exposed to ultraviolet light inactivated NDV (12).

Although interferon synthesis presumably involves the induction and synthesis of host-directed protein, the infection of L cells with NDV actually resulted in a pronounced decrement in macromolecular synthesis. Protein synthesis rapidly decreased during the virus infection and the bulk of the interferon was apparently produced by the infected cells when 75 to 80% of the original synthetic activity had been depressed. Although alter-

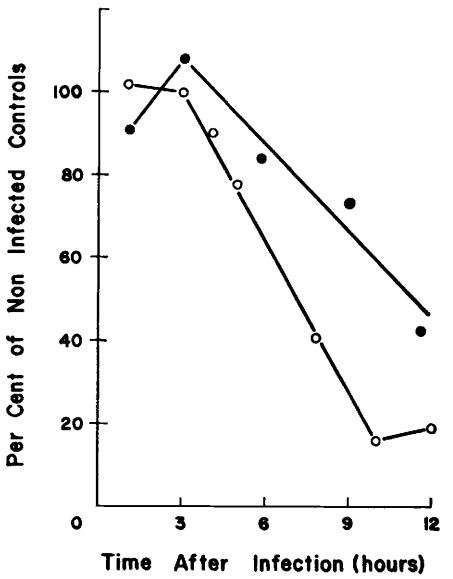

FIG. 1. The effect of NDV infection on L cell nuclear RNA and protein synthesis: (●) nuclear RNA; (○) protein. Protein synthesis was measured in infected and noninfected cells by incubating the cell monolayers for 30 min with medium containing either ^{3}H-valine (0.5 μCi/ml) or a ^{3}H-amino acid mixture (1.0 μCi/ml). Nuclear RNA synthesis was measured as described in Methods with medium containing ^{3}H-uridine (2.0 μCi/ml). The data plotted represent the mean determinations from six experiments and are expressed as the percentage of the control (noninfected cells) specific activity.

ations in host cell macromolecular synthesis have been observed in both virulent (15, 16) and other inefficient (17, 18) cell–virus interactions, the metabolic events of L cells infected with NDV are somewhat unique in that interferon synthesis occurs during a period when host cell metabolism is generally depressed by the viral infection. The actual mechanism which renders interferon production relatively resistant to the inhibitory effects of the viral infection is presently unknown. This phenomenon may be the result of a special property of interferon messenger RNA or the intracellular compartmentalization of its translation.

It is interesting to note that, although NDV replication is primarily associated with cytoplasmic events, the infection of L cells with this virus resulted in a marked inhibition of cellular nuclear RNA synthesis. However, the loss in nuclear activity was somewhat less striking than that measured with translational events and the time course was somewhat delayed.

Summary. The time of interferon induction in L cells has been shown to occur from 8 to 12 hr after infection with NDV. Although interferon synthesis represents the production of a new cellular protein, the rates of protein and nuclear RNA synthesis were found to actually undergo a rapid decrease following NDV infection. A comparison of the temperal relationship between alterations in overall macromolecular synthesis and interferon production indicated that the synthesis of L cell interferon occurred when macromolecular biosynthesis was considerably depressed.

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