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Reovirus Inhibition of Cellular DNA Synthesis: Role of the S1 Gene

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Type 3 reovirus inhibits L cell DNA synthesis, whereas type 1 reovirus exerts little or no effect on L cell DNA synthesis. By using recombinant viruses containing both type 1 and type 3 double-standard RNA segments, we determined that one double-stranded RNA segment, the reovirus type 3 S1 double-stranded RNA segment which encodes the viral hemagglutinin, segregates with and is responsible for the capacity of reovirus type 3 to inhibit L cell DNA synthesis.

Cytocidal viruses alter cellular metabolism and cytoskeletal organization, ultimately causing cellular degeneration and death. The role of individual viral components in the production of cellular damage is poorly understood. We have chosen to study how reovirus, an animal virus with a segmented double-stranded RNA (dsRNA) genome, alters cellular metabolism and cytoarchitecture.

Reoviruses contain 10 segments of dsRNA surrounded by a double capsid shell of proteins (9). The dsRNA segments are named according to size classes; there are three large segments (L1, L2, and L3), three medium segments (M1, M2, and M3), and four small segments (S1, S2, S3, and S4). The outer capsid consists of three polypeptides, σ_1, σ_3, and μ1C (18), which are encoded by the S1, S4, and M2 dsRNA segments, respectively (10, 12).

There are three serotypes of mammalian reoviruses: type 1, type 2, and type 3. Recombinant viruses containing dsRNA segments from two serotypes have been generated by coinfecting cells with both serotypes (11, 14, 16). Such recombinant viruses have been used to define a number of biological properties of the mammalian reoviruses (15, 19).

In this report, we examine the effects of reoviruses type 1 and type 3 and recombinant viruses on mouse L cell DNA synthesis. Reovirus type 3 previously has been shown to inhibit L cell DNA synthesis (1), although the viral component causing this inhibition has not been identified. By using a genetic approach, we have determined the viral dsRNA genome segment responsible for inhibiting L cell DNA synthesis.

To measure inhibition of host cell DNA synthesis, subconfluent monolayers of mouse L cells were infected or mock infected with 15 to 30 PFU of reovirus type 1 or type 3 per cell. As previously reported (1), reovirus type 3 Dearing markedly inhibited L cell DNA synthesis (Fig. 1). The rate of DNA synthesis in infected monolayer cultures began to decline by 12 h after infection. By 24 h postinfection, DNA synthesis of the infected monolayers was 20 to 35% of DNA synthesis in mock-infected control cells. Type 1 reovirus, on the other hand, did not significantly decrease the rate of host cell DNA synthesis.

The different effects of type 1 and type 3 reoviruses on L cell DNA synthesis could not be explained by differences in the growth characteristics of these viruses in mouse L cells because both serotypes grew at the same rate and to the same extent. By 26 h postinfection, each infected cell produced approximately 1,000 infectious progeny particles. Furthermore, no cytopathic effects were observed in the L cells at the time inhibition of DNA synthesis occurred.

In contrast to the effect in monolayer cultures, the inhibition of L cell DNA synthesis in suspension cultures occurs much earlier and at lower multiplicities (1). To determine whether type 1 and type 3 reoviruses also differed in their capacity to inhibit DNA synthesis in suspension culture, we examined virally infected cells in suspension culture (4). At a multiplicity of infection of 5 to 10 PFU per cell, type 3 reovirus inhibited DNA synthesis by ~60% as compared with control cells beginning at 6 to 8 h postinfection. Under these conditions, type 1 reovirus inhibited L cell DNA synthesis by ~20%. By 12 h postinfection, DNA synthesis of monolayers infected with type 3 reovirus was inhibited by ~88% as compared with mock-infected monolayers, whereas DNA synthesis of monolayers infected with type 1 was reduced by ~25% of that found in control monolayers (data not shown).

To determine which viral genome segment was responsible for inhibiting DNA synthesis, a series of recombinant viruses derived from coinfection of L cells with reoviruses type 1 and type 3 were studied. The details of the isolation and characterization of these recombinants has been reported previously (11, 14, 16). The recombinant viruses behaved like the type 1 or the type 3 parent virus, and there was no intermediate pattern of DNA synthesis inhibition. Recombinants containing a type 3 S1 dsRNA segment inhibited L cell DNA synthesis, whereas recombinants having a type 1 S1 dsRNA segment did not (Table 1). It was especially striking that recombinant clone 1.HA3 exhibited the type 3 pattern of response (Fig. 1). This recombinant contains all type 1 dsRNA segments, except for the S1 dsRNA segment, which is derived from type 3. The reciprocal recombinant 3.HA1, which contains all type 3 dsRNA segments except for the type 1 S1 dsRNA segment, exhibited the reovirus type 1 pattern of response (Fig. 1).

UV irradiation of reovirus type 3 does not abolish its ability to inhibit cellular DNA synthesis (6). Reovirus type 3 inhibited DNA synthesis even when its infectivity was reduced by approximately 10^9-fold by UV irradiation (Fig. 2). Reovirus type 1, inactivated to the same extent, had little or no effect on cellular DNA synthesis. The effect of UV-inactivated recombinant viruses on L cell DNA synthesis correlated solely with the parental origin of the S1 dsRNA segment. After irradiation, the recombinant virus 3.HA1 behaved like the type 1 parent. It had little or no effect on DNA synthesis (Fig. 2). The purified recombinant clone 204, containing all type 1 dsRNA segments except for the S1 and L1 segments of type 3, was used instead of 1.HA3 in the irradiation studies. High yields of purified 1.HA3 were difficult to obtain. This recombinant behaved like the type 3 parent and inhibited DNA synthesis.

In addition to the laboratory strain of type 1 and type 3, five additional isolates of reovirus type 1 and type 3 (8) were examined for their capacity to inhibit L cell DNA synthesis. Two type 1 human isolates did not inhibit L cell DNA synthesis, whereas three type 3 isolates (two bovine isolates and one mouse isolate) inhibited L cell DNA synthesis in a manner similar to that of the type 3 Dearing strain (data not shown).

These results demonstrate that the type 3 S1 dsRNA segment segregates with and is responsible for the capacity of reovirus type 3 to inhibit L cell DNA synthesis. Because inactivated and live reovirus inhibits DNA synthesis, the S1 gene product and not the dsRNA appears to be the viral component responsible for this inhibition. None of the three isolates of reovirus type 1 significantly inhibits DNA synthesis, whereas all four isolates of reovirus type 3 inhibit DNA synthesis. Therefore, the capacity of reovirus type 3 to inhibit DNA synthesis appears to be a serotype-specific property and not just peculiar to the laboratory strain of reovirus (type 3 Dearing). This finding is consistent with the fact that the S1 gene product is the determinant of serotype specificity (20, 21).

This report is the first genetic identification of a single viral gene responsible for inhibiting host cell macromolecular synthesis. Studies with intertypic recombinants of herpes simplex virus have shown that a specific region of the viral DNA contains a gene or genes whose products

TABLE 1. *Effect of reovirus recombinant clones on L cell DNA synthesis*

Clone	Origin of genome segment (location of polypeptide encoded by genome segment)[a]										% Inhibition[b]	Pattern of DNA synthesis effect[c]
	Outer capsid			Core					Nonstructural			
	M2	S1	S4	L1	L2	L3	M1	S2	M3	S3		
54	1	1	3	3	1	1	1	3	3	3	16–20	1
65	1	1	1	3	3	3	3	1	3	1	10–15	1
802	3	1	3	1	3	3	3	3	3	3	15–25	1
3.HA1	3	1	3	3	3	3	3	3	3	3	12–18	1
63	1	3	3	3	1	3	1	3	1	1	79–88	3
112	3	3	1	1	3	1	1	1	3	1	85–88	3
204	1	3	1	3	1	1	1	1	1	1	65–78	3
1.HA3	1	3	1	1	1	1	1	1	1	1	75–85	3

[a] Type of reovirus from which the genome segment of the hybrid clone originated (see Mustoe et al. [15] for nomenclature).

[b] Range of DNA synthesis inhibition in 10 experiments.

[c] Number 1 refers to the minor inhibition of L cell DNA synthesis (10 to 25%) characteristic of type 1, whereas number 3 refers to the marked inhibition characteristic of type 3 (70 to 85%).

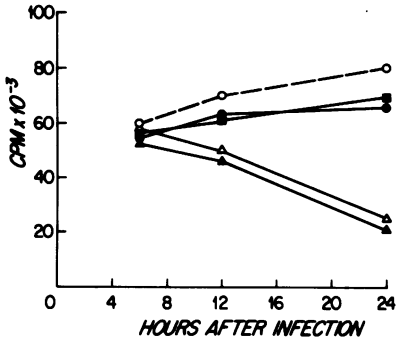

FIG. 1. *Effect of reovirus type 1 and type 3 and recombinant viruses on L cell DNA synthesis. Reovirus type 1, strain Lang, and type 3, strain Dearing, were the same as described by Ramig et al. (13). The origin and RNA genotypes of the recombinant clones used in this study are described by Sharpe et al. (16), Ramig et al. (14), and Mustoe et al. (11). Additional isolates of type 1 and type 3 reovirus used in these studies were collected by Leon Rosen and described by Hrdy et al. (8). Virus was purified by the method of Mustoe et al. (11). Mouse L cells were transferred from suspension culture to 35-mm² plastic petri dishes (5 × 10⁵ cells per dish). After 24 h, the culture fluid was removed from the dishes, and the cells were infected or mock infected with a multiplicity of infection of 15 to 30 PFU of reovirus of the desired genotype per cell. Cells were incubated with virus for 2 h at 37°C in an incubator containing 5% CO_2 in air (NAPCO no. 3221). After adsorption, 2 ml of minimal essential medium containing 5% fetal calf serum was added to each dish, and the dishes were returned to the incubator. At 6, 12, and 24 h postinfection, the growth medium was replaced by 2 ml of minimal essential medium containing 0.5 μCi of [methyl-³H]thymidine per ml (2 Ci/mmol; New England Nuclear Corp.). The cell cultures were then incubated at 37°C for 30 min. Incorporation of radioisotope was stopped by removal of the medium containing [methyl-³H]thymidine and the addition of phosphate-buffered saline containing 0.2% EDTA. The cells were removed from the dishes by agitation with a Pasteur pipette and transferred to disposable glass tubes (13 by 100 mm). The cell suspensions were sonically treated to disrupt the cells and then adjusted to a final concentration of 5% trichloroacetic acid. The cell suspensions were incubated at 4°C for 1 h and then filtered onto Whatman 2.4-mm GF/c filter paper. The filter papers were washed with ethanol, dried, and transferred to plastic scintillation vials. Triton X-100–toluene scintillation fluid was added to each vial and assayed for radioactivity in a Beckman liquid scintillation counter. Previous studies have shown that decreased thymidine incorporation reflects a true decrease in DNA synthesis (2) and that a DNA precursor block is not involved (1). The amount of DNA synthesized in infected cells correlates with the extent of thymidine incorporated (6). Therefore, decreased thymidine incorporation was interpreted as decreased DNA synthesis in these studies. The effect of reovirus type 1 (■) and type 3 (△) and recombinant clones 1.HA3 (▲) and 3.HA1 (●) on L cell DNA synthesis is shown. Mock-infected monolayers of L cells (○) were included as controls.*

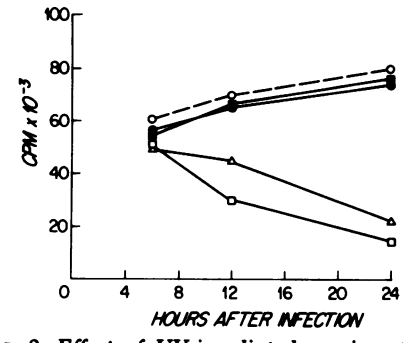

FIG. 2. *Effect of UV-irradiated reovirus types 1 and 3 and recombinant clones on L cell DNA synthesis. Reovirus type 1 and type 3 and recombinant clones were purified by the method of Mustoe et al. (11). Purified reovirus was diluted to 10⁹ PFU/ml, placed in a 35-mm² plastic petri dish, and irradiated for 12 min. A Blak-Ray UV meter (Ultra-violet Products, Inc., San Gabriel, Calif.) indicated that the UV radiation dose was 600 μW/cm². Titration of irradiated virus revealed a decrease in titer of about 10⁹ PFU/ml. DNA synthesis was measured as described in Fig. 1. Subconfluent monolayers were infected with inactivated type 1 (■), type 3 (△), recombinant 204 (□), or 3.HA1 (●). Subconfluent monolayers of uninfected L cells (○) served as controls.*

inhibit host cell DNA and protein synthesis. This region has a molecular weight of about 7 × 10^6 and contains structural genes for at least six polypeptides (3). In a further study, we studied the effect of reovirus on cellular protein synthesis and found that reovirus type 2 Jones and reovirus type 3 Dearing differ in their capacity to inhibit L protein synthesis. Another viral gene, the S4 gene, which encodes the σ3 outer capsid polypeptide, is responsible for this effect (Sharpe and Fields, manuscript in preparation). Thus, there is clearly a difference in the way that the reoviruses cause inhibition of cellular DNA and protein synthesis.

Our findings extend those of Shaw and Cox (17), who found that UV irradiation of reovirus did not abolish its inhibitory effect on DNA synthesis and suggested that either a component of the virus is capable of causing inhibition or that RNA can function after irradiation to give rise to an inhibitory component. Although we have identified the S1 gene as responsible for the inhibition, we cannot determine whether the initial reovirus-cell interaction leads to inhibition of DNA synthesis or whether UV-treated virus transcribes limited amounts of S1 mRNA. In the latter case, newly synthesized S1 gene

products could mediate the inhibition. Similar to Hand et al. (6), we have found that empty capsids did not inhibit DNA synthesis (unpublished data). Possibly, empty capsids do not have the proper configuration to interact with cells. The fact that 80% of intact particles adsorb to L cells during a 2-h period, whereas only 15% of empty capsids adsorb during the same time period would support this possibility (6).

The identification of the S1 gene as the viral component responsible for DNA synthesis inhibition may provide a clue as to the mechanism of inhibition. The S1 gene product, the viral hemagglutinin (21), plays a key role in a number of virus-cell surface interactions. The viral hemagglutinin is the viral polypeptide that interacts with the surface of erythrocytes; it is the viral component that is recognized by primed lymphocytes, probably via a specific lymphocyte receptor (5), and also determines cell tropism in the central nervous system, presumably by interacting with cell surface receptors that determine whether reovirus can infect ependymal cells (type 1 pattern) or neuronal cells (type 3 pattern) (19). It is possible that inhibition of cellular DNA synthesis is similarly mediated through an interaction at the cell surface. However, further study will be needed to determine if a virus-membrane interaction does indeed affect cellular DNA synthesis.

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