Repair Replication of HeLa Cell Deoxyribonucleic Acid in Cells Infected with Newcastle Disease Virus or Mengovirus or Treated with Puromycin

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We examined repair replication of HeLa cell deoxyribonucleic acid (DNA) in cells infected with mengovirus or Newcastle disease virus or treated with puromycin. Cellular DNA was damaged by ultraviolet light and then pulse-labeled with ^3H -thymidine. Autoradiographic analysis of non-S-phase DNA synthesis (repair replication) showed that there was no inhibition of this process at a time when overall cellular DNA synthesis was severely inhibited by either virus infection or puromycin treatment.

Mengovirus and Newcastle disease virus (NDV), two cytocidal ribonucleic acid viruses belonging to different major virus groups, inhibit cellular deoxyribonucleic acid (DNA) synthesis in mammalian cells in tissue culture (11, 16). The step in semiconservative replication of cellular DNA that seems most sensitive to inhibition by infection with these viruses is initiation of replication of new DNA chains (8, 12). Chain elongation, the continued polymerization of nucleotides on the template and the joining of short polynucleotide chains by ligase, is inhibited slightly, if at all. Repair replication, the nonsemiconservative replication of short strands of DNA occurring after damage to DNA by such agents as ultraviolet (UV) light (4, 6, 14, 15), also involves chain elongation, but it is not known whether the same enzymes function in the repair process as are used in semiconservative DNA replication. We have examined repair replication in HeLa cells infected with mengovirus or NDV by using autoradiography of whole cells to analyze non-S-phase or unscheduled DNA synthesis occurring after irradiation of cells with UV light (15). In mammalian cells, the occurrence of repair replication as measured by this technique has been well correlated with the occurrence of repair replication as measured by analysis of density-labeled DNA by isopycnic centrifugation (5, 13).

The characteristics of the small-plaque variant of mengovirus (1) and the Hickman strain of NDV (16) have been described. Monolayers of HeLa cells were grown to mid-log phase on

cover slips in 60-mm petri dishes in reinforced Eagle minimal essential medium (2) with 5% fetal calf serum. They were then infected with mengovirus at a multiplicity of 300 or with NDV at a multiplicity of 500. At times after infection when cellular DNA synthesis was markedly inhibited, but before the onset of virus-induced morphological cell damage, infected and uninfected cells were irradiated with UV light at doses of either 225 or 1,800 ergs/mm². The UV light source was a 15-w General Electric Sterilamp delivering 14.8 ergs per mm² per sec at 253.7 nm, as quantitated by an Eppley thermopile. Controls of infected and uninfected cells were identically handled but not exposed to UV light (mock irradiation). The irradiated and mock-irradiated cells were then immediately pulsed for 1 hr with ^3H -thymidine (^3H -dThd, 1.0 $\mu\text{Ci}/\text{ml}$, 22 to 26 Ci/mmol, Amersham-Searle Corp., Arlington Heights, Ill.). The cells on cover slips were washed, fixed, extracted four times with trichloroacetic acid, and processed for autoradiography by standard techniques (3). We quantitated repair replication in these cells by counting grains over nuclei (5, 7, 9, 10). When cells were exposed to 225 ergs/mm², S-phase cells could still be recognized, and only non-S-phase nuclei were scored. A dose of 1,800 ergs/mm² caused practically complete inhibition of S-phase synthesis (15), and all labeled nuclei were scored. The results at the two dose levels were comparable. Grains over 30 nuclei were counted to obtain mean values.

In all experiments we estimated overall DNA synthesis as follows. Replicate cultures of infected and uninfected cells were pulse-labeled with ^3H -dThd (1.0 $\mu\text{Ci}/\text{ml}$) for 15 min at the same time that experimental cultures were irradiated or mock-irradiated. We precipitated the cells with 5% trichloroacetic acid and measured incorporation of ^3H -dThd into acid-insoluble material by liquid scintillation counting. The results are expressed as percentage of incorporation of ^3H -dThd into uninfected cells pulsed at the same time.

We examined mock-irradiated and irradiated infected cells 4 and 6 hr after infection, when overall DNA synthesis was markedly inhibited. Mock-irradiated NDV-infected cells showed heavily labeled S-phase cells and no label in non-S-phase cells (Fig. 1a). NDV-infected cells irradiated with 1,800 ergs/ mm^2 showed a uniform pattern of light labeling, characteristic of repair replication (Fig. 1b, reference 15). There were similar findings in mock-irradiated and irradiated mengovirus-infected cells (Fig. 1c and d).

Table 1 shows that grain counts were similar in control and mengovirus- or NDV-infected cells at UV doses of 1,800 ergs/ mm^2 and 225 ergs/ mm^2 . It should be emphasized that the cells did

not show any evidence of cytopathologic changes. Therefore, we consider that variation in grain counts resulting from self-absorption of tritium in cells of varying shape (i.e., thickness) was minimal. The minor difference in grain counts at a dose of 225 ergs/ mm^2 between mengovirus-infected and uninfected cells is not statistically significant at the 95% confidence level. If there is a small difference, it does not correlate at all with the almost 80% inhibition of overall DNA synthesis in the mengovirus-infected cells.

As both mengovirus and NDV cause inhibition of cellular protein synthesis soon after infection (11, 16) and the inhibition of cellular DNA synthesis may be related to this, we also examined repair replication in HeLa cells whose protein synthesis had been inhibited. Cells were irradiated and pulsed 2 hr after treatment with puromycin, an inhibitor of protein synthesis. Other techniques used were as described for experiments with virus-infected cells. Mock-irradiated cells treated with puromycin showed no labeling over the nuclei of non-S-phase cells (Fig. 1e). Cells irradiated with 1,800 ergs/ mm^2 showed a uniform pattern of light nuclear labeling (Fig. 1f). Grain counts showed that there was no difference between drug-treated and untreated cells at either dose of UV light (Table 1).

The autoradiographs of the cells that were either mock-irradiated or exposed to 225 ergs/ mm^2 were also used to estimate the per cent of cells in S phase (Table 2). In each case, 500 cells were counted to determine (i) the per cent of nuclei labeled above background in mock-irradiated cultures and (ii) the per cent of nuclei heavily labeled in irradiated cultures. All cell cultures,

TABLE 1. *Repair replication in virus-infected or puromycin-treated cells*

Expt	DNA synthesis ^a	UV (ergs/ mm^2)	Repair replication ^b
Newcastle disease virus-infected	34.9	225	67.2 $\pm$ 6.04
Uninfected control		225	64.3 $\pm$ 3.71
NDV-infected	26.0	1,800	44.7 $\pm$ 3.97
Uninfected control		1,800	45.3 $\pm$ 3.32
Mengovirus-infected	20.7	225	47.8 $\pm$ 6.08
Uninfected control		225	60.6 $\pm$ 5.05
Mengovirus-infected	11.6	1,800	64.1 $\pm$ 5.54
Uninfected control		1,800	66.6 $\pm$ 8.78
Puromycin-treated 200 $\mu\text{g}/\text{ml}$	28.8	225	52.6 $\pm$ 3.39
Untreated control		225	49.4 $\pm$ 3.68
Puromycin-treated 20 $\mu\text{g}/\text{ml}$	11.5	1,800	50.8 $\pm$ 3.85
Puromycin-treated 200 $\mu\text{g}/\text{ml}$	5.3	1,800	51.0 $\pm$ 3.76
Untreated control		1,800	52.6 $\pm$ 4.46

^a Values expressed as percentage of uninfected or untreated control.

^b Values expressed as number of grains per nucleus ($\bar{X} \pm$ standard error).

TABLE 2. *Effect of UV irradiation on number of cells in S phase after virus infection or puromycin treatment*

Expt	DNA synthesis ^a	Percentage of cells in S phase	
		No UV	UV (225 ergs/ mm^2)
Newcastle disease virus-infected	34.9	31.9	28.6
Uninfected		36.6	33.3
Mengovirus-infected	20.7	36.0	32.0
Uninfected		37.9	35.4
Puromycin-treated	28.8	35.2	31.8
Untreated		36.6	32.0

^a Values expressed as percentage of uninfected or untreated control.

FIG. 1. Repair replication in virus-infected or puromycin-treated cells. Cells were infected or treated for 2 hr with 20 μ g of puromycin per ml and either mock-irradiated or irradiated with 1,800 ergs of UV light per mm^2 . Subsequent pulsing with ^3H -thymidine and processing of cells for autoradiography were as described. Exposure was for 2 weeks at 4 C. Cells were then stained through the emulsion with Giemsa stain. In mock-irradiated cell cultures, cells in S phase show heavy nuclear labeling, whereas the remaining cells are unlabeled. In irradiated cultures, all cells show light nuclear labeling. (a) NDV-infected cells, mock-irradiated; DNA synthesis 3.8% of uninfected control. (b) NDV-infected cells, irradiated; DNA synthesis 3.8% of uninfected control. (c) Mengovirus-infected cells, mock-irradiated; DNA synthesis 11.6% of uninfected control. (d) Mengovirus-infected cells, irradiated; DNA synthesis 11.6% of uninfected control. (e) Cells treated with puromycin, mock-irradiated; DNA synthesis 11.5% of untreated control. (f) Cells treated with puromycin, irradiated. DNA synthesis 11.5% of untreated control.

whether virus-infected, puromycin-treated, or controls, showed slightly fewer cells in S phase after irradiation. The difference in percentage of S-phase cells between irradiated and nonirradiated cell cultures is not statistically significant at the 95% confidence level and can be attributed to our inability to recognize cells in very early or late S phase that ordinarily would be lightly labeled during the 1-hr pulse. The small difference in percentage of cells in S phase between NDV-infected and uninfected cell cultures is statistically significant only at the 95% confidence level and does not correlate with the severe inhibition of overall DNA synthesis. The difference between mengovirus-infected and uninfected cells is not statistically significant at the 95% level. Thus, although both mengovirus and NDV markedly reduced the amount of DNA synthesized per cell during S phase, this effect was not associated with a marked reduction in the number of cells in S phase.

Our finding that puromycin causes no inhibition of repair replication essentially agrees with Cleaver's finding that cycloheximide causes only a 20% inhibition of repair replication when overall DNA synthesis is close to 90% inhibited (5).

The main result of this study is that repair replication of cellular DNA proceeds normally in cells infected with NDV or mengovirus or treated with puromycin when overall DNA synthesis is severely inhibited. Furthermore, infection with either virus or treatment with puromycin does not damage DNA early in infection to stimulate repair replication. This is not surprising, as it is known that cellular DNA is not degraded early in infection, even though its synthesis is inhibited (8).

The finding that repair replication is not inhibited by virus infection or inhibitors of protein synthesis, in spite of severe inhibition of semi-conservative DNA replication, correlates with the earlier finding that DNA chain elongation is normal and that there is no precursor block in DNA synthesis under these circumstances (8, 12). Both repair replication and semiconservative replication involve processes that utilize the actions of DNA polymerases and ligases. These enzyme activities determine rate of chain elongation in the absence of a precursor block. Our results show that in neither type of replication do these enzyme activities appear to be inhibited. This supports the hypothesis that rate of chain

elongation is not primarily affected in the inhibition of DNA synthesis by NDV and mengovirus infection or by inhibition of protein synthesis (8, 12).

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