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Inhibition of the Initiation of Cellular DNA Synthesis After Reovirus Infection

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The synthesis of cellular DNA was measured in synchronized L cells after reovirus infection. Initiation of the synthetic phase of the cell cycle was completely inhibited in cells infected 8 h before the beginning of DNA synthesis.

The inhibition of cellular DNA synthesis after reovirus infection appears to be selective for the replicative, but not the transcriptive function of cellular DNA (7, 11). This inhibition is not related to detectable modifications of the integrity of the DNA template, activity of specific enzymes involved in DNA synthesis, or the levels of certain DNA precursor pools (5, 13). Recent evidence (3, 10, 13) relating the time of the beginning of the inhibition of DNA synthesis to the multiplicity of infection strongly suggests that the inhibition process is specifically related to a virus-specific function and is not the result of cell necrosis. Inhibition of DNA synthesis by reovirus is reflected in a decrease in the number of DNA chain initiation events (R. Hand and I. Tamm, 1973, Abstr. Annu. Meeting Amer. Soc. Microbiol., p. 211, 1973), but not in the rate of DNA chain growth (5, 8, 9). This report shows that synchronized L-cells fail to enter the DNA synthetic phase (S phase) of the cell cycle when they have been infected with reovirus for 8 h.

Suspension cultures of strain L-929 mouse fibroblasts and the Dearing strain of type 3 reovirus were used in all experiments. The growth and maintenance of cell cultures and the production, purification, and assay of virus have been described previously (13). The procedure for synchronization of cells in suspension culture was similar to that described by Littlefield (12). Every 72 h cells were centrifuged and resuspended to a concentration of 5×10^5 cells/ml in fresh prewarmed basal Eagle medium containing 10% fetal calf serum. An excessive decrease in the pH of the growth medium due to high cell concentration was prevented by growing cells in flasks which were not sealed airtight and by reducing the sodium bicarbonate concentration from 2.2 g/liter to 1.5 g/liter. Greater than 70% of the cells were synchronized by this method as determined by mitotic index, and cells maintained under these conditions remained synchronized from one medium change to the next. These cells entered the S phase 8 h after resuspension in fresh medium. A synchronous wave of cell division occurred approximately 24 h after resuspension resulting in a twofold increase in cell number. Thus, the block appeared to occur during the early G-1 phase of the cell cycle.

Since the inhibition of DNA synthesis begins approximately 8 h after reovirus infection in asynchronous cultures at a multiplicity of 10 PFU/cell (7), synchronous cultures were infected using 10 PFU/cell immediately after resuspension in fresh medium, 8 h before entry into the S phase. At selected time intervals after infection, samples were removed from infected and control cultures. Each sample was pulse labeled for 30 min with [^{3}H]thymidine (0.5 μCi/ml). At the end of the labeling period the DNA was extracted by the method of Colter et al. (2). The quantity of DNA in each sample was determined by the method of Burton (1) and the radioactivity was measured by liquid scintillation counting.

DNA synthesis in control cells began approximately 8 h after resuspension in fresh medium (Fig. 1). The duration of linear incorporation of thymidine into DNA of control cells corresponded to that determined for the S phase of L cells (12). In contrast to the control, reovirus infection completely prevented the entry of synchronized cells into the S phase of the cell cycle. A significant background level of thymidine incorporation into DNA was detected in control and infected cultures before 8 h postinfection. This incorporation should be a function of DNA synthesis that was occurring in the asynchronous fraction of the cell population of each culture. The inhibition of this incorporation between 7 and 9 h after infection provided a

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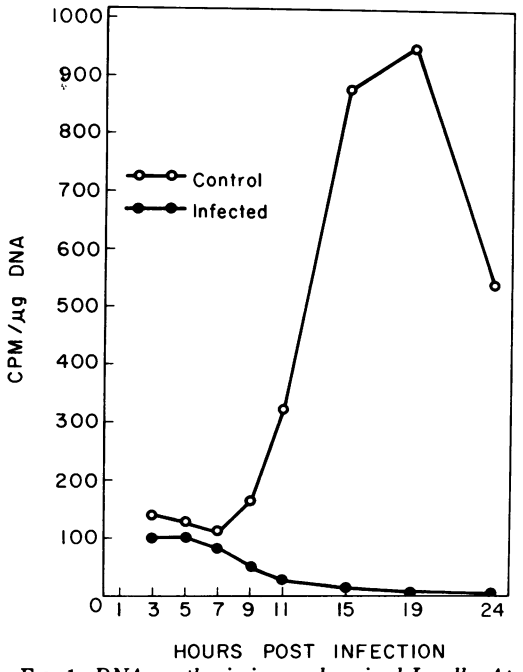

FIG. 1. *DNA synthesis in synchronized L cells. At time 0 synchronized cells were resuspended in fresh prewarmed medium and infected with 10 PFU/cell (closed circles), or uninfected (open circles). At the times indicated 2.5 × 10⁷ cells were removed from each culture and pulse-labeled with [³H]thymidine. The specific activity of each sample was then determined.*

convenient internal control in the experiment since inhibition of DNA synthesis occurred at this time in asynchronous cultures (7).

It is clear that the initiation of the S phase of the cell cycle is prevented when sychronous cell cultures are infected with low multiplicities of reovirus 8 h before the beginning of DNA synthesis. An earlier report (6) demonstrated partial inhibition of DNA synthesis when synchronized cells were infected with reovirus 4 or 6 h before the beginning of the S phase. Recent studies have confirmed these observations (manuscript in preparation). However, the occurrence of events early in the S phase which are relatively insensitive to reovirus infection (6) appears not to be the case. If reovirus infection occurs early enough (8 h before initiation of the S phase), cellular DNA synthesis can be completely prevented. The results presented here must also be considered in light of the method of synchrony used which provides an uninterrupted entry into the S phase, thus exposing the sequence of events preceding the initiation of DNA synthesis to the inhibitory effects of reovirus infection. It has been shown that less than 10% of the cells in reovirus-infected suspension cultures exhibit detectable cytopathology by 8 h after infection (4). For this reason, it is unlikely that the inhibitory effects seen in these experiments were the indirect result of cell necrosis.

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