ACTINOMYCIN AND THE DIFFERENTIAL SYNTHESIS OF REOVIRUS AND L CELL RNA

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Received April 1, 1965

Reovirus differs from other small, RNA-containing animal viruses in that its nucleic acid is double-stranded (Gomatos and Tamm, 1963a). This unusual feature lends added interest to the biochemical events associated with its replication. Analysis of these events is complicated by the continued synthesis of cell RNA and protein in infected L-929 cells (Gomatos and Tamm, 1963b). Investigations of the poliovirus-infected HeLa cell, another complex system, have been facilitated by the observation that actinomycin blocks cell RNA synthesis but not virus formation (Reich et al., 1961; Shatkin, 1962; Darnell, 1962). It was reported earlier that a 10% yield of reovirus was formed in L cell cultures containing 2γ actinomycin/ml (Gomatos et al., 1962), a concentration ten-fold greater than that necessary to suppress profoundly the synthesis of cell RNA (Reich et al., 1961). It seemed possible that actinomycin, at an appropriate concentration, might also be used to inhibit L cell RNA production without affecting reovirus replication. This report describes the production of maximum yields of reovirus at an actinomycin level which inhibits L cell RNA formation by 90%.

Materials and Methods

L-929 cells were grown in suspension culture in Eagle's medium (Eagle, 1959) containing 2% fetal bovine serum. Stock virus was prepared in monolayer cultures from a seed of reovirus type 3 kindly provided by Dr. D. Axelrod, and infectious virus was measured by plaque assay. Both

procedures have been described (Gomatos et al., 1962).

For infection, cells were concentrated to 5×10^6/ml and infected in suspension with an input multiplicity of approximately 20 PFU/cell. After adsorption for 2 hours at 37°, unadsorbed virus was removed by washing with growth media, and the cells were resuspended at a population density of $2\text{-}2.5 \times 10^5$/ml. RNA synthesis was measured by incorporation of uridine-2-C^{14} (2×10^{-6}M, specific activity = 24 mc/mm) into alkaline hydrolysates (.3N KOH, 16-18 hours, 37°C) of cells extracted twice with 5% perchloric acid at 4°C (Schmidt and Thannhauser, 1945). The orcinol method was used to determine total RNA (Mejbaum, 1939).

Results

In L cells infected in suspension culture with a high multiplicity of reovirus, an increase in infectious virus titer can be detected 8-9 hours after infection (Figure 1). Maturation is rapid between 8 and 14

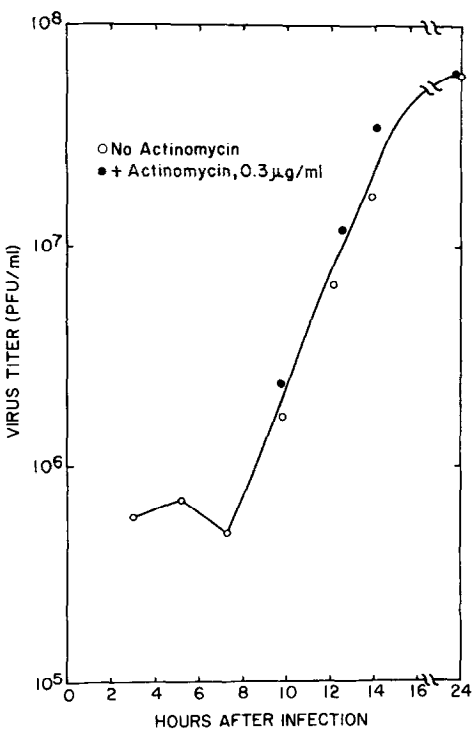

Fig. 1. Time course of reovirus replication.

hours and is complete by 24 hours after infection. Virus replication is not affected by the addition of 0.3 μg actinomycin/ml at 2-1/2 hours after infection. Concentrations of 1 μg/ml or greater reduce the 24-hour yield of infectious virus to 3-12% of that obtained in the absence of inhibition (Table 1). However, even at a concentration of 5 μg/ml the titer increased from ca. 1 PFU/cell at 5-1/2 hours to 8 PFU/cell at 24 hours after infection.

TABLE 1

Effect of Actinomycin on Reovirus Replication and L Cell RNA Synthesis

Actinomycin μg/ml	RNA cpm/μg	% RNA Inhibition	Virus Titer PFU/ml	% Virus Inhibition
0.0	103	--	2.5×10^7	--
0.1	42	58	2.8×10^7	--
0.2	23	77	2.4×10^7	--
0.3	21	79	4.9×10^7	--
0.4	15	85	4.7×10^7	--
0.5	11	89	2.3×10^7	--
1.0	8	92	3.0×10^6	88
2.0	6	94	6.6×10^5	97
5.0	2	98	1.5×10^6	94

Virus titers determined 24 hours after infection. At 5-1/2 hours after infection the titer was 1.7×10^5 PFU/ml.

Cell RNA synthesis, in contrast to reovirus production, is markedly inhibited by 0.5 μg actinomycin/ml (Table 1). Suspension cultures of growing L cells were exposed for 4 hours to the indicated drug concentrations, and uridine-2-C^{14} was then added for one hour. The cells were chilled, centrifuged to remove the growth medium, and the amount of radioactivity incorporated into cell RNA was determined. Synthesis

of RNA was suppressed 58% at 0.1 μg/ml. A progressive inhibition occurred with increasing actinomycin concentrations until at 5 μg/ml synthesis was 2% of that in the culture containing no antibiotic. Exposure to actinomycin for periods longer than 4 hours reduced RNA synthesis further. The small amount of RNA which is formed in uninfected cells in the presence of 0.3-.5 μg actinomycin/ml sediments as 4S soluble RNA in a sucrose density gradient, and radioactive ribosomal RNA is not synthesized.

The rate of RNA synthesis in L cells is not altered following reovirus infection, and virus-directed RNA is masked by cell RNA synthesis (Gomatos and Tamm, 1963b), (Figure 2). The data presented above indicate that virus-directed RNA would be detected in infected L cells which were treated with 0.3-0.5 μg actinomycin/ml to inhibit cell RNA synthesis. Cultures of infected L cells received 0.3 μg actinomycin/ml at 2-1/2 hours after infection and uridine-2-C^{14} at 2-3/4 hours. Similar additions were made to uninfected cells which had been treated in an identical way but without the addition of virus. At the indicated time intervals, samples were removed and acid-precipitable, alkali-soluble radioactivity was determined. Uninfected cells incorporated uridine into RNA for 2 hours after antibiotic addition but at a decreasing rate, and there was little net increase in

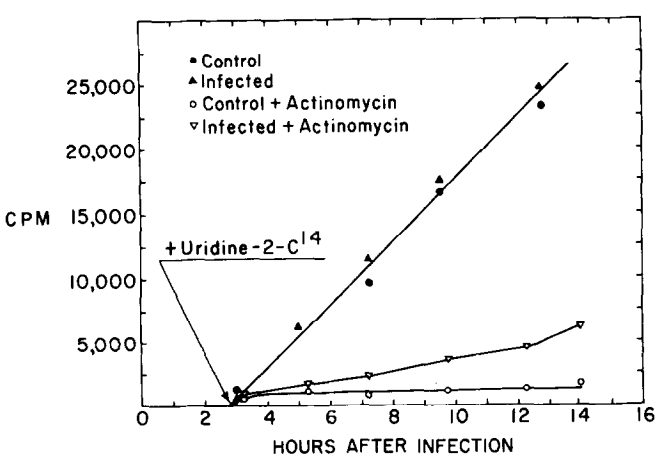

Fig. 2. Uridine-2-C^{14} incorporation into RNA.

radioactivity during the next several hours. Infected cells continued to synthesize RNA and a five-fold increase in radioactivity occurred between 5-1/2 and 14 hours after infection. Synthesis of virus-directed RNA was observed 5-7 hours after infection although the titer of infectious virus did not increase during this interval. The possibility remains that virus was formed but its presence was obscured by the input virus which remained. Although infectious virus increased at a logarithmic rate 8-14 hours after infection, virus-directed RNA synthesis was linear during this interval. A similar finding was previously reported for poliovirus-infected HeLa cells (Shatkin, 1962). The significance of this difference may be clarified by a study of the chemical and physical nature of the reovirus-directed RNA which is synthesized in infected cells. Such a study is possible with infected cells exposed to an appropriate actinomycin concentration.

Summary

The addition of 0.5 μg actinomycin/ml to L-929 cell cultures inhibits RNA synthesis 90%; under these same conditions reovirus replication is unaffected.

Acknowledgment

The expert technical assistance of Mr. R. Ruhl is gratefully acknowledged

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