Effect of Actinomycin D on the Synthesis of Viral Antigen in Cercopithecus Kidney Cells Infected with SV 40

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Little is known about the individual stages of SV 40 virus development in Cercopithecus monkey kidney cells. This study was concerned with the phase of transcription for proteins essential for synthesis of virus specific protein. It was assumed that these processes would require DNA-dependent RNA synthesis and could, therefore, be inhibited by actinomycin D (1). Rapp et al. demonstrated the complete inhibition of virus specific protein synthesis by actinomycin D. In their experiments the drug was added after virus adsorption and remained in the medium until the end of the experiment. However, with the compound in contact with the cells throughout the experiment there was marked cytotoxicity and it was difficult to interpret the significance of repression of virus antigen synthesis (2, 3). Exposure of the cells to actinomycin D for only short periods at various times after infection would be more likely to give information about the time of transcription during one single growth cycle of SV 40, and simultaneously circumvent cell damage.

Primary Cercopithecus kidney cells (GMK) and BS-C-1 cells were grown on coverslips (7 x 30 mm.) in stoppered tubes containing Hanks solution with 0.5 % (w/v) lactalbumin hydrolysate and calf serum (5 % (v/v) for GMK and 20 % (v/v) for BS-C-1). For inoculation the growth medium was removed from the coverslip cultures (approximately 8 x 10^4 cells/coverslip) and the virus suspension (strain vac/111/L or strain 777) diluted in medium was added. The multiplicity was 1-5 to 5. (For percentage of infection see controls in Figs 1, 2). To obtain a reasonably uniform course of infection, virus was allowed to adsorb for 2 hr. Subsequently the coverslips were washed 3 times in Hanks solution at 37° and were then transferred into tubes containing 5 ml. medium supplemented with 2 % (v/v) SV 40 antiserum (titre 1/320 against 10^3 TCID50). This concentration of antiserum was sufficient to inactivate both unadsorbed virus and the progeny virus released from the cells (4).

Fifty hr after inoculation the coverslip cultures were fixed for fluorescent antibody studies (5). Nuclear viral antigen was localized with a fluorescein isothiocyanate-labelled serum from a Cercopithecus monkey repeatedly immunized with SV 40 virus (Hygiene Institut der Universitat Freiburg). This antiserum did not react with the induced complement fixing antigen. The infected cells in 80 microscope fields in treated and untreated cultures were counted and the percentage of infected cells calculated. In the experiments described here 1 µg./ml. actinomycin D (Lyovac-Cosmegen, Merck, Sharp and Dohme) was added for either ½ hr or 5 hr at various times after infection and then removed by washing the coverslip cultures 3 times in Hanks solution at 37°. The cultures were then reincubated until they were fixed for immunofluorescence. In control experiments with uninfected cells no cell damage caused by actinomycin D was observed.

Pulse treatment with actinomycin D during one growth cycle of SV 40 revealed three periods of sensitivity during which the virus infected cells responded in different
Fig. 1. Effect of actinomycin D treatment on the synthesis of viral capsid protein in SV40-infected GMK cells at various times after infection during a one step growth cycle. Virus protein measured by immunofluorescence 50 hr after infection. - - - - - , 1 μg./ml. actinomycin D for 1/2 hr; - - - - - , 1 μg./ml. actinomycin D for 5 hr at various times after inoculation; ■, duration of application. Percentage infected cells in untreated control cultures 50 hr after infection: 26%.

Fig. 2. Effect of actinomycin D treatment at various times after infection (1 μg./ml. for 1/2 hr) on the synthesis of viral capsid protein in SV40-infected GMK and BS-C-1 cells. - - - - - , GMK (SV40 VAC(111)); - - - - - , BS-C-1 (SV40 777). Percentage infected cells in untreated control cultures 50 hr after infection: GMK 3%, BS-C-1 4-6%.
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ways to the action of the antibiotic. Administration of the drug for 30 min. in SV40-infected GMK cells during the early period of infection doubled the number of cells producing capsid protein compared with untreated infected cultures (Fig. 1). On the other hand, pulse treatment with actinomycin D between 10 and 30 hr after infection resulted in a decrease in the number of fluorescing cells to approximately 70% of the number in untreated infected controls. This may have indicated the period of transcription of the viral genome, e.g. for early enzymes (6). There was no detectable effect when actinomycin D was given later than 30 hr after infection (Fig. 1, solid line). Actinomycin D treatment for 5 hr soon after infection did not enhance the number of fluorescing cells (Fig. 1, dashed lines). On the other hand, pulse treatment for 5 hr led to extensive reduction of the number of fluorescing cells, especially between 30 and 35 hr after infection. The reduction caused by actinomycin treatment until 25 hr after infection was less extensive, perhaps because cell functions active during this period were not inhibited. From 30 to 35 hr after infection transcription processes occurred which were essential for the appearance of viral protein (further studies are in progress). Actinomycin treatment later than 35 hr after infection had no effect. Thus, in experiments with actinomycin D the time of addition of the antibiotic as well as the duration of treatment were important. The enhancement of the number of fluorescing cells could be obtained with various titres of the infecting virus (Fig. 1, 2). The increase to twice the amount could be shown in the continuous cell line BS-C-1 too (Fig. 2).

Actinomycin D acts on transcription by blocking the DNA-dependent RNA polymerase (1). The observation that actinomycin treatment at an early period of the eclipse phase may enhance rather than suppress the influence of SV40 infection is therefore particularly interesting. This phenomenon is not understood at present. We tentatively suggest the following hypothesis, which assumes competition between cellular and viral m-RNAs for ribosomes. Pulse treatment with actinomycin in the early period of infection inhibits the production of cellular RNA. However, upon removal of the drug the host cell resumes nucleic acid synthesis several hours later (7). This observation and the fact that the first expression of the viral genome, the stimulation of thymidine kinase activity, does not occur earlier than 10 hr after infection (6) suggest that the viral DNA, unlike cellular DNA, is not affected by actinomycin during this early period after infection. As soon as transcription of the viral DNA starts, more ribosomes than in untreated cells are rendered available because of the lack of cellular m-RNA. Therefore more viral genomes can be readily translated than in untreated infected cells in which a competition for ribosomes might occur.

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