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MECHANISM OF INTERFERON ACTION

Stability and Translation of Simian Virus-40 Early mRNA Coding for T-Antigen is Comparable in Untreated and Interferon-treated Monkey Cells

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SUMMARY. The effect of interferon treatment on the translation and the stability of simian virus 40 (SV40) early mRNA coding for T-antigen was examined in *ts*A-infected monkey kidney BSC-1 cells at 40°. Neither the translation nor the stability of SV40 early mRNA was altered by interferon under cellular conditions where the synthesis of reovirus polypeptides was significantly inhibited by interferon. SV40 early mRNA decayed with a half-life of about 3 hours as measured by T-antigen synthesis; the decay rate was indistinguishable between untreated and interferon-treated cells.

Interferons (IFN) inhibit the replication of a wide range of animal viruses (1). In many animal virus-host systems including reovirus-infected mouse fibroblast (2,3) and monkey kidney (3) cells the primary level of gene expression inhibited by IFN is the translation of viral mRNA into protein. However, in the case of simian virus 40 (SV40)-infected monkey cells treated with IFN prior to infection, a marked reduction in the accumulation of hybridizable SV40 early RNA is well documented (4-7). The synthesis of SV40 early polypeptides, T and t antigens, is also inhibited in monkey cells treated with IFN prior to infection (4,6,8,9) although in SV40-transformed mouse cells neither T nor t antigen synthesis is affected by IFN (8,10).

The inhibition of SV40 early polypeptide synthesis observed in monkey cells treated with interferon prior to infection could be due to a reduction in accumulation of early RNA as a result of either decreased synthesis or increased degradation of RNA, and/or to an inhibition in the actual trans-

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lation of functional early mRNA available in the cytoplasm. An IFN-mediated endonuclease has recently been described which could account for the reduction in SV40 early RNA levels by an increased RNA degradation in cells treated prior to infection (11-14). However, analysis of the kinetics of decay of SV40 early RNA in both nuclear and cytoplasmic fractions by pulse-chase and hybridization techniques did not reveal any difference in RNA half-life between untreated and IFN-pretreated cells (7). By contrast to the reduction in SV40 early RNA accumulation observed in cells IFN-treated prior to infection (4-7), recent experiments suggested that SV40 early RNA synthesis is unaffected when IFN treatment is initiated after virion uncoating in the case of both SV40 *wt*- and *ts*A-infected cells (K.A. Daher, S.M. Kingsman, and C.E. Samuel; also 5,6,9). The studies presented here were therefore undertaken to determine the effect of IFN-treatment on the translation, and functional stability, of SV40 early mRNA coding for T-antigen under conditions where SV40 early RNA synthesis is apparently unaffected by IFN. We have used the lytic infection of African green monkey kidney cells with a *ts*A mutant of SV40 at the elevated temperature. Under these conditions the replication of SV40 is restricted to the early stages (15) and early RNA is overproduced because of the inability of the *ts*A mutant T-antigen to inhibit early transcription (16,17).

MATERIALS and METHODS

Confluent monolayer cultures of BSC-1 cells were infected at 40° with SV40 tsA_{30} (5 pfu/cell) or reovirus type 3 Dearing strain (1000 particles/cell) as previously described (7,18). In the case of SV40-infected cultures, actinomycin D (1 μg/ml, Calbiochem) was added at 36 hr post-infection; in the case of reovirus-infected cultures, actinomycin D (1 μg/ml) was added at 1 hr post-infection. Cells were pulse-labeled for 30 min at the times indicated with 25 μCi of [^{35}S]methionine per ml (New England Nuclear, 800-1100 Ci/mmole), extracts prepared by Nonidet P-40 lysis at pH 9, and proteins immunoprecipitated by the indirect *Staphylococcus aureus* protein A procedure and analyzed by NaDodSO$_4$-polyacrylamide gel electrophoresis as previously described (8). Autoradiography was performed with the enhancer, EN^{3}HANCE^{TM} (New England Nuclear). SV40 anti-T immune serum from hamsters bearing SV40-induced tumors was provided by Dr. J. Cole (National Cancer Institute, Bethesda, Md.); antiserum to reovirus capsid polypeptides was prepared in hamsters injected intraperitoneally with UV-inactivated purified virions (0.5 mg emulsified in Freund's complete adjuvant; an identical booster dose

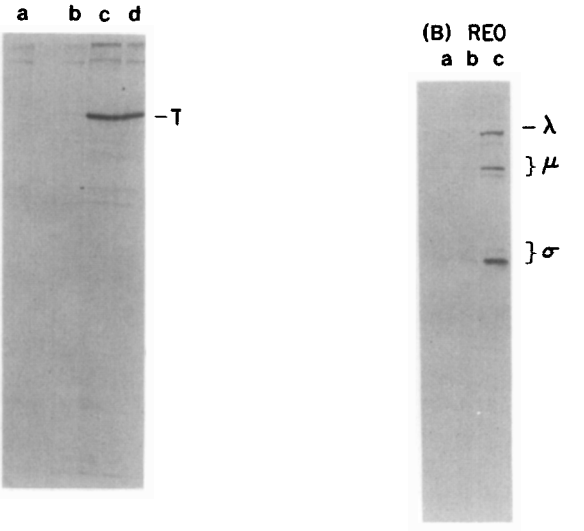

Figure 1. Effect of time of interferon treatment on the synthesis of viral proteins in SV40-infected and reovirus-infected BSC-1 cells. (A) Proteins precipitated from extracts of SV40 *ts*A-infected cells with SV40 anti-T immune serum. Cultures were labeled for 30 min beginning at 36 hr post-infection. (a) untreated, uninfected; (b) IFN-treated for 62 hr, from 26 hr before infection to 36 hr after infection; (c) IFN-treated for 19.5 hr, from 16.5 to 36 hr after infection; and (d) untreated, infected. (B) Proteins precipitated from extracts of reovirus-infected cells with antiserum to reovirus capsid polypeptides. Cultures were labeled for 30 min beginning at 12 hr post-infection. (a) IFN-treated for 62 hr before infection; (b) IFN-treated for 18.5 hr before infection; (c) untreated, infected.

was administered 5 weeks later and ten days later the animals were bled by cardiac puncture). Interferon treatment was with 600 units of IFN/ml for the period of time indicated at 40°. In the experiments presented, human leukocyte IFN (1.9 x 10^6 units/mg) provided by Dr. K. Cantell (Helsinki, Finland) was utilized; similar results have been obtained with NDV-induced monkey kidney IFN. Sendai virus-induced human leukocyte (buffy coat) IFN and NDV-induced monkey kidney IFN are almost equally active on heterologous monkey and human cells as on the homologous cell species (19).

RESULTS and DISCUSSION

As shown in Fig. 1A, the *de novo* synthesis of SV40 T-antigen as measured in a 30-min pulse at 36 hr post-infection was significantly inhibited by IFN when IFN treatment was started 26 hrs before infection (lane b). By contrast, when IFN treatment was started at 16.5 hr after infection there was no significant affect on T-antigen synthesis in IFN-treated (lane c) as compared to untreated (lane d) cultures in a 30-min pulse at 36 hr post-infection. The 19.5 hr during which the BSC-1 cells were exposed to IFN (16.5

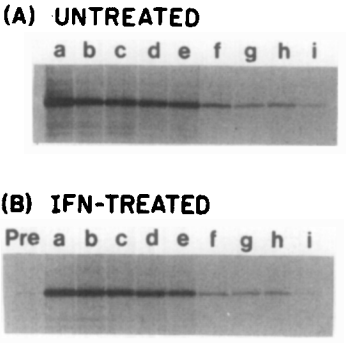

Figure 2. Decay of T-antigen synthesis in untreated and IFN-treated SV40-infected BSC-1 cells following the addition of actinomycin D. Actinomycin D was added at 36 hr post-infection; cultures were then pulse-labeled for 30 min beginning at the following times after addition of the actinomycin: (a) 0; (b) 0.5 hr; (c) 1 hr; (d) 2 hr; (e) 3 hr; (f) 6 hr; (g) 9 hr; (h) 12 hr; and (i) 25 hr. Equal volumes of [^{35}S]methionine-labeled extract were precipitated with anti-T antiserum. Extracts were prepared from (A) untreated cells; (B) IFN-treated cells treated at 26 hr before infection (pre) or at 16.5 hr after infection (a-i) until 36 hr after infection.

to 36 hours post infection) is sufficient time for the maximal induction of the two enzymes that are believed to play a role in the antiviral action of interferon, a 2',5'-oligoadenylate synthetase and a protein kinase (20-22). Indeed, when cultures were infected with reovirus (Fig. 1B) rather than SV40 (Fig. 1A), the synthesis of reovirus polypeptides was maximally inhibited by IFN after 18.5 hr of treatment (Fig. 1B). These results suggest that the rate of translation of SV40 early mRNA coding for T-antigen is not significantly affected by IFN under conditions where the translation of reovirus mRNA is extensively inhibited.

To investigate whether the stability of SV40 early mRNA differs between untreated and IFN-treated BSC-1 cells, we determined the rate of T-antigen synthesis in *ts*A-infected cells at varying times following the addition of 1 μg/ml actinomycin D to the medium at 36 hr post infection. Actinomycin D at this concentration completely inhibits ongoing RNA synthesis and significantly affects the processing of nuclear transcripts to yield cytoplasmic mRNAs (23). Fig. 2 shows the amount of T-antigen detected in immunoprecipitates prepared from extracts of untreated (Fig. 2A) and IFN-treated (Fig. 2B)

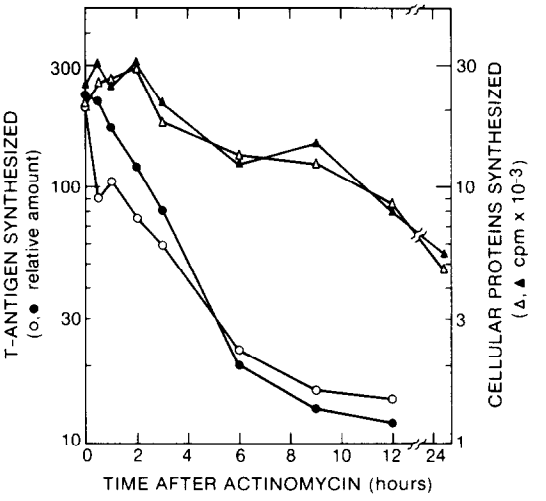

Figure 3. Decay of T-antigen synthesis and overall cellular protein synthesis in untreated and IFN-treated cells following the addition of actinomycin D. SV40-infected cultures were pulse-labeled for 30 min at the indicated times following the addition of actinomycin D to the culture medium at 36 hr post-infection. T-antigen synthesis (circles) was quantitated by immunoprecipitation, $NaDodSO_4$-polyacrylamide gel electrophoresis, and densitometer scanning of a series of films of varying exposure times. Overall cellular protein synthesis (triangles) was quantitated by determination of hot trichloroacetic acid-insoluble ^{35}S-labeled material in whole cell extracts. Untreated (open symbols); IFN-treated from 16.5 to 36 hr after infection (closed symbols).

BSC-1 cells pulse-labeled for 30 min at 0, 0.5, 1,2,3,6,9, 12 and 25 hours following the addition of actinomycin D. In order to quantitate the rate of decay of T-antigen synthesis, autoradiograms were scanned with a densitometer and the peak areas corresponding to T-antigen were excised and weighed. Because the response of the film is linear over a rather limited exposure range, multiple exposures were obtained with varying times and the measurements scaled to a common base. The results for each culture condition, untreated and IFN-treated cells, are shown in Fig. 3. The SV40 mRNA coding for T-antigen had a half-life of about 3 hr; there was no significant difference in the decay characteristics of SV40 early mRNA between IFN-treated and untreated cells. The actinomycin D treated cells maintained a relatively constant over-all pattern of cellular protein synthesis for 12 hr as determined by $NaDodSO_4$-polyacrylamide gel electrophoresis of whole cell extracts (results not shown). The half-life for over-all cellular protein synthesis estimated from incorporation of [^{35}S]methionine into hot trichloroacetic acid-

insoluble material was about 9 hr, and was comparable in untreated and IFN-treated cells (Fig. 3).

The half-life of functional SV40 early mRNA as measured by T-antigen synthesis in the presence of actinomycin D (about 3 hr) is somewhat shorter than the value for early RNA obtained in a recent study by pulse-chase and hybridization techniques (7). By these techniques, about 80% of the SV40 early RNA present in the cytoplasm decayed with a half-life of about 4 hr (7); this RNA may represent T-antigen mRNA as T-antigen mRNA is three to four-fold more abundant than small t mRNA in SV40 early lytic infection (24). The fact that the decay of SV40 early RNA is indistinguishable between IFN-treated and untreated cells as determined by both hybridization (7) and protein synthesis (Fig. 2) methods, and the fact that the IFN-mediated 2',5'-oligo A-dependent endonuclease specifically cleaves at sequences which are not unique to viral mRNAs (25), indicates that an enhanced degradation of SV40 early RNA by an IFN-mediated RNase probably does not play a major role in the antiviral action of IFN in SV40 lytic infections. Our results reported in this communication confirm and extend those of Mozes and Defendi (9) which establish that SV40 T antigen synthesis is sensitive to IFN treatment initiated before, but not after, infection of monkey cells with *ts*A virions at the nonpermissive temperature.

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