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Transient Inhibition of Polyoma Virus Synthesis by Sendai Virus (Parainfluenza I)

II. Mechanism of the Interference by Inactivated Virus[1]

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The mechanism of the transient inhibition of polyoma virus synthesis by beta-propiolactone-inactivated Sendai virus was studied. Polyoma virus early functions did not appear to be affected, although deoxyribonucleic acid (DNA) and structural protein synthesis were inhibited 60 and 35%, respectively. The inhibition of macromolecular synthesis was not sufficient to account for the 90% inhibition of infectious progeny formation. Encapsidation of polyoma DNA into mature virions appears to be completely inhibited after superinfection by beta-propiolactone-inactivated Sendai virus. Ultraviolet irradiation of live or beta-propiolactone-inactivated Sendai virus preparations abolishes the interfering capacity, indicating that a functional Sendai virus ribonucleic acid molecule is the interfering component.

Several investigators have examined viral interference systems in which the interfering virus was inactivated or functionally nonreplicating. It was previously demonstrated that poxvirus deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) synthesis was inhibited in cells infected with frog virus 3 that was inactivated by heat, ultraviolet or gamma irradiation, or grown at temperatures nonpermissive for frog virus 3 (1, 54). These studies indicated that a structural component of the virion was responsible for the inhibition. Baluda (3, 4) and Bratt and Rubin (7) demonstrated homologous interference with ultraviolet-inactivated Newcastle disease virus, and it was also reported that strains of poliovirus, rendered incapable of replicating by temperature-sensitive conditions or the presence of guanidine, interfered with replicating poliovirus (50). Homologous viral interference by defective or incomplete virus particles containing a subgenomic RNA molecule has also been reported (24, 25, 27, 35, 36, 51, 53). Evidence that purified virion components could induce interference was shown by studies on the adenovirus fiber antigen. Pereira (47) demonstrated the inhibition of adenovirus, vaccinia, and poliovirus by a substance produced in HeLa cells infected with adenovirus and that the inhibitory material could be neutralized by anti-adenovirus sera. Khoobyarian and Fischinger (34) reported interference by heat-killed adenovirus, and Levine and Ginsberg (37) subsequently showed that the purified adenovirus fiber antigen could not only interfere with virus replication but also inhibit DNA, RNA, and protein synthesis in uninfected cells.

An enormous literature exists describing the effects of viral infection on host cell functions and macromolecular synthesis. Reports by several investigators suggest that a component of the vesicular stomatitis virus is capable of eliciting inhibitory effects on cellular DNA, RNA, or protein synthesis (26, 56, 58). Host cell DNA synthesis has also been shown to be inhibited by nonreplicating frog virus 3 (39) or ultraviolet-inactivated Sendai virus (19). Inhibition of protein synthesis in HeLa cells infected with ultraviolet-irradiated vaccinia virus was reported by Moss (45) and host protein and RNA synthesis was demonstrated by Holland (23) to be inhibited rapidly after infection with poliovirus, even under conditions preventing viral RNA replication. Nonreplicating poliovirus inhibits host RNA and protein synthesis; however, it apparently does not inhibit events of Mengo virus (40) or SV5 (10) infection.

The accompanying communication describes the inhibition of polyoma virus synthesis elicited by superinfection with beta-propiolactone-inactivated Sendai virus. The interference was found to be transitory and not mediated by interferon. This communication describes a study to elucidate the mechanism of the interference with polyoma virus synthesis by inactivated Sendai virus.

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MATERIAL AND METHODS

Tissue culture. Primary mouse embryo cell cultures were used throughout these studies and prepared as described previously (33). Cultures were grown in Eagle (15) minimal essential medium (MEM) supplemented with 5% fetal calf serum (FCS). Radioisotopic incorporation experiments were performed by using MEM supplemented with 5% dialyzed FCS.

Viruses. Polyoma virus, originally obtained from Sarah Stewart, National Institutes of Health, was the wild-type strain. Sendai virus was supplied by Barbara B. Knowles, Wistar Institute. Propagation of the viruses and the inactivation of Sendai virus with 0.025% beta-propiolactone were performed as described in the accompanying paper. When ultraviolet light (UV) was used as the inactivating agent, Sendai virus preparations were exposed to a germicidal lamp (General Electric, 15-w, G 15TB) for varying amounts of time at a distance of 10 cm. The preparations were continually mixed during UV exposure by means of a magnetic stirrer.

Virus purification and quantitation. Polyoma virus was purified as described previously by Consigli et al. (11). Quantitation of virus particle concentration was accomplished by hemagglutination (HA) by using washed guinea pig red blood cells for both polyoma and Sendai viruses. The infectivity of polyoma virus preparations was determined by the plaque assay (plaque-forming units; PFU) by using primary mouse embryo cell cultures (11).

Experimental procedure. Mouse embryo cell cultures were infected with polyoma virus at a multiplicity of 100 PFU per cell. After adsorption for 1.5 hr, the monolayers were washed three times with phosphate-buffered saline (PBS), and fresh medium was placed on the cells. Infection of cell cultures with Sendai virus (2,000 HA units per 60 by 15 mm culture) was accomplished in an identical fashion, except that the adsorption period was shortened to 30 min. It was also necessary in certain experiments to save the medium that was on the cells prior to Sendai infection and replace it on the cells after the adsorption period to maintain cultural conditions and for accurate quantitation of polyoma virus synthesis. Uninfected cultures were mock-infected under identical conditions with Eagle MEM (serum-free).

Selective salt extraction of polyoma DNA from infected cultures. At various times after infection, cultures were pulsed for 1 hr with ^{3}H-thymidine (4 μCi/ml) 2 hr prior to harvesting. The radioactive medium was removed and the monolayers were washed twice with PBS and then incubated for an additional hr in unlabeled medium to deplete the cellular pool of ^{3}H-thymidine phosphate. Cells were lysed by the addition of sodium dodecyl sulfate (SDS), and polyoma viral DNA was selectively extracted with 1 M NaCl (22). Supernatant fluids containing viral DNA were dialyzed overnight at 4 C against tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (0.01 M Tris, *p*H 7.5; 0.01 M ethylenediaminetetraacetate [EDTA]), and radioisotopic incorporation was determined. Pellets were dissolved in 1 M NaOH for assay of protein content and radioisotopic incorporation as an index of polyoma-induced synthesis of host cell DNA.

Fractionation of DNA by centrifugation in ethidium bromide-cesium chloride. DNA preparations, obtained by selective salt extraction of infected cells or from purified polyoma virus particles (48), were further separated into component I (supercoiled) and component II (nicked circular and linear) DNA by equilibrium centrifugation in ethidium bromide-cesium chloride density gradients (52).

Incorporation of radioisotopic precursors into polyoma virus. Polyoma-infected cultures were maintained in Eagle MEM containing either ^{3}H-thymidine or ^{3}H-valine (3 μCi/ml) from 2 hr until 40 hr after polyoma infection. Parallel cultures were superinfected with inactivated Sendai virus at different times (2, 10, and 24 hr). Radioactive medium, which was removed prior to Sendai infection, was replaced on the cultures after the Sendai virus adsorption period. All cultures were harvested after 40 hr of infection, and the polyoma virus was purified. Fractions collected from cesium chloride density gradients were assayed for radioisotopic incorporation and HA activity. Fractions from the cesium chloride gradients containing ^{3}H-thymidine-labeled virus were, respectively, pooled and dialyzed against Tris-hydrochloride buffer (0.01 M, *p*H 7.5) to remove the cesium chloride. The DNA from these purified polyoma particles was released by alkaline degradation of the viral capsids as previously described by Perry et al. (48).

Prelabeling of cellular DNA. Mouse embryo cells were seeded and grown to monolayer (three generations) in Eagle MEM containing ^{3}H-thymidine (1 μCi/ml). This procedure was shown by autoradiography to label 90 to 95% of the cells in the nuclei. Two hours prior to the start of the experiment, radioactive medium was removed; the cells were washed once with PBS and incubated in unlabeled medium. Polyoma-infected cultures, that had been superinfected with inactivated Sendai virus 2 hr after polyoma infection, were monitored for prelabeled host DNA degradation. At various intervals, medium was removed and saved, cells were lysed with SDS, and the low-molecular-weight DNA was solubilized by selective salt extraction. The amount of radioactivity present in the medium, salt-soluble extract, and cell pellet was determined and expressed as percentage of the total radioactivity incorporated during prelabeling.

Incorporation of prelabeled host DNA into purified polyoma virus progeny. Mouse embryo cells, that were prelabeled with ^{3}H-thymidine as described above, were infected with polyoma virus and, 2 hr later, were superinfected with inactivated Sendai virus. These cultures were maintained in Eagle MEM and harvested 48 hr after polyoma infection. Polyoma virus was purified, and fractions collected from cesium chloride gradients were assayed for radioisotopic incorporation and HA activity.

Fluorescence microscopy. The synthesis of polyoma structural proteins or polyoma-induced tumor antigen was qualitatively assayed by the indirect immunofluorescence technique (33). The stained preparations

were observed with a Leitz fluorescence microscope and scored for nuclear fluorescence. Polyoma "T" antiserum, prepared in hamsters, was kindly supplied by Graham Wilcox of this laboratory.

Transformation assay. Transformation of primary mouse embryo cells by polyoma virus and the effect of inactivated Sendai virus on this phenomenon was investigated by using the agar suspension culture method described by Macpherson and Montagnier (41).

Sequential addition of inactivated Sendai virus, 5-fluoro-deoxyuridine, or actinomycin D. Parallel cultures of polyoma-infected cells were respectively treated at different times by the addition of 5-fluorodeoxyuridine (FUdR, 10^{-4} M) or actinomycin D (act D, 2.5 μg/ml), or were superinfected with inactivated Sendai virus. All cultures were harvested 48 hr after polyoma infection and assayed for the synthesis of polyoma virus (PFU). For comparison, a normal polyoma virus growth curve was performed in conjunction with these studies.

Isotopically labeled Sendai virus. Sendai virus, labeled in the RNA, was prepared in 10-day-old fertile eggs that were prelabeled with ^{3}H-uridine (200 μCi per egg) 24 hr prior to Sendai virus inoculation. Virus was purified by two cycles of differential centrifugation (600 × *g* for 15 min, and 30,000 × *g* for 1 hr) and pelleting through a 5 to 20% linear sucrose gradient (80,000 × *g*, 3 hr).

Quantitative assays and radioisotopes. Protein content was assayed by the method of Lowry et al. (38). Radioactivity was determined in a Beckman model LS-233 liquid scintillation counter. Radioisotopes used in these studies were thymidine-*methyl*-^{3}*H* (specific activity, 10 to 20 Ci/mmole) and L-^{3}H-valine (10 to 20 Ci/mmole), and were purchased from Schwarz BioResearch, Orangeburg, N.Y. Actinomycin D was a gift from Merck, Sharp & Dohme, Rahway, N.J., and FUdR was a gift from Hoffman-La Roche Inc., Nutley, N.J.

RESULTS

The accompanying paper described the inhibition of polyoma virus synthesis by beta-propiolactone-inactivated Sendai virus. The interference was not mediated by interferon and was transient in nature, in that apparently normal synthesis of polyoma virus occurred after a 48-hr period. A relationship between the times of infection by the two viruses was demonstrated, and it was shown that infection by inactivated Sendai virus must take place immediately before or within a 16-hr period after polyoma infection to manifest maximum inhibition. Preliminary pulse experiments to determine the rates of macromolecular synthesis in polyoma-infected cells that were superinfected with inactivated Sendai virus revealed a marginal decrease in the DNA synthetic rate. No significant differences were observed in the incorporation of radioisotopic precursors into either RNA or protein.

DNA synthesis. Since radioisotopic incorporation experiments revealed a nominal decrease in total DNA synthesis in polyoma-infected cells that were superinfected with inactivated Sendai virus, it was of interest to determine if this inhibition was specifically affecting either viral or host DNA synthesis. Polyoma-infected mouse cells that were superinfected with inactivated Sendai virus were pulse labeled with ^{3}H-thymidine at regular intervals throughout the infection period, and polyoma virus DNA was selectively extracted by the Hirt (22) procedure. Sendai-treated cultures were still found to synthesize DNA soluble in 1 M NaCl, although at a rate only 40% that of normal polyoma-infected control cultures at 24 hr (Fig. 1). Salt-insoluble DNA, used as an index of host cell DNA synthesis, was synthesized at a reduced rate (60% at 21 hr) when compared to control polyoma-infected cultures (Fig. 2). Therefore, the polyoma-induced synthesis of host cell DNA still occurred, although the level of induction appeared to be less.

Polyoma DNA was further fractionated into component I and component II DNA by isopycnic centrifugation in an ethidium bromide-cesium chloride density gradient. Polyoma super-

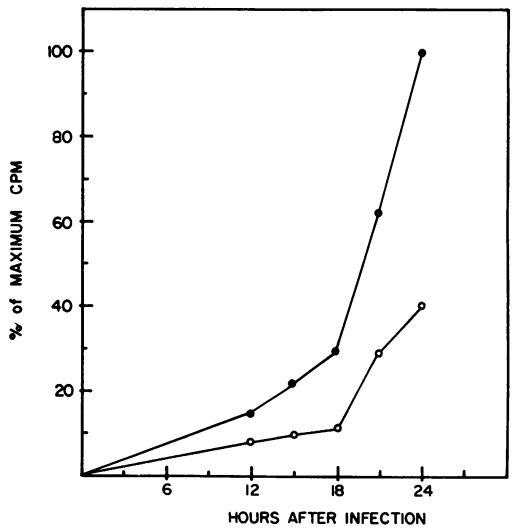

FIG. 1. *Effect of inactivated Sendai virus on polyoma DNA synthesis. Polyoma-infected control cultures (●) or cultures that had been superinfected (2 hr) with inactivated Sendai virus (○) (2,000 HA units per culture) were pulsed with ^{3}H-thymidine (3 μCi/ml) for 1 hr, 2 hr before harvesting. At the end of 1 hr, the cells were washed once with PBS and then incubated for an additional hour in unlabeled media, at which time the cultures were lysed by the addition of SDS, and the viral DNA was extracted with 1.0 M NaCl according to the method of Hirt (22).*

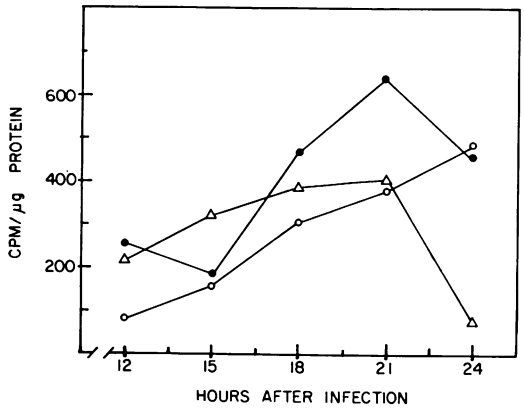

FIG. 2. *Effect of inactivated Sendai virus on the polyoma-induced synthesis of host cell DNA. Uninfected (△), polyoma control (●) or polyoma-infected Sendai virus-superinfected (○) cultures were pulsed with ^{3}H-thymidine (3 μCi/ml) for 1 hr, 2 hr prior to harvesting. At the end of the pulse period, cultures were washed once with PBS and then incubated for an additional hour in unlabeled medium, at which time the cells were lysed by the addition of SDS, and the viral DNA was extracted with 1.0 M NaCl. After centrifugation, the pellet was dissolved in 1.0 N NaOH, and the specific activity was determined as an index of host cell DNA synthesis.*

coiled DNA was synthesized in both the untreated and inactivated Sendai-treated cultures (Fig. 3). The quantity of polyoma supercoiled DNA (component I) was determined by summing the radioactivity in those fractions composing the component I peak. Superinfection by inactivated Sendai virus prevented the synthesis of supercoiled, component I polyoma DNA by factors of 80% (18 hr), 60% (21 hr), and 40% (24 hr).

Synthesis of polyoma antigens. Polyoma-specific antigen synthesis in infected cultures that were superinfected with inactivated Sendai virus was determined by specific immunofluorescence. Synthesis of polyoma virus-induced tumor antigen occurred in cells that were superinfected with inactivated Sendai virus (Table 1). Polyoma structural proteins were also synthesized in considerable amounts, although a moderate degree of inhibition (35%) was found.

Incorporation of radioisotopic precursors into purified polyoma virus. In light of the previous findings that both polyoma virus DNA and proteins were synthesized after superinfection by inactivated Sendai virus, even though infectious progeny formation was inhibited 90% under identical conditions, it was necessary to consider the possibility that defective virus particles were being formed. Polyoma virus was purified from cultures that had been superinfected with inactivated Sendai virus at different times after polyoma infection. Virus was labeled with either ^{3}H-thymidine or ^{3}H-valine throughout the infection period. Incorporation of ^{3}H-thymidine into the DNA of purified polyoma virus was completely inhibited if the cultures were superinfected early (2 hr) in the infection cycle with inactivated Sendai virus (Fig. 4). As the time of superinfection was delayed, a corresponding increase in the amount of ^{3}H-thymidine incorporated was found. In an identical experiment in which ^{3}H-valine was substituted for the ^{3}H-thymidine, label was shown to be incorporated into purified incomplete virus in substantial amounts regardless of the time of superinfection by inactivated virus. This observation, that incomplete virus particles are assembled, is consistent with the immunofluorescence data (Table 1) which demonstrated that polyoma structural proteins are synthesized after superinfection by inactivated Sendai virus.

Characterization of the DNA in purified virions assembled after Sendai superinfection. Fractions composing the complete virus peak in the ^{3}H-thymidine incorporation experiment (Fig. 4) were pooled, and the virus particles were subjected to alkaline degradation to release the polyoma DNA (28, 48). A comparison of DNA encapsidated after Sendai superinfection to that from virus particles synthesized during a normal infection was made by ethidium bromide-cesium chloride density gradient centrifugation of the released DNA (Fig. 5). The results demonstrated that the DNA made and encapsidated during superinfection by inactivated Sendai virus was of a supercoiled nature similar to that found in normal polyoma virus particles. Relative ratios of component I to component II DNA were similar in both control and Sendai-treated preparations. Again the sequential increase in amounts of viral DNA correlates well with the time of Sendai superinfection. The 2-hr superinfection system was not used in this experiment since ^{3}H-thymidine label was not incorporated into virions (Fig. 4).

Degradation and encapsidation of prelabeled host DNA. A study was initiated to investigate the possibility that Sendai superinfection of polyoma-infected cells was causing degradation of host cell DNA thereby allowing this DNA to be available for pseudovirion production. Cell cultures that had been prelabeled with ^{3}H-thymidine were infected with polyoma virus and superinfected with inactivated Sendai virus 2 hr later. The results (Table 2) show no significant difference in the solubilization of prelabeled DNA into the media or into cellular DNA fragments soluble in 1 M NaCl as a consequence of Sendai virus superinfection. In addition (Table 2), pseudovirion production was drastically inhibited by superinfection as

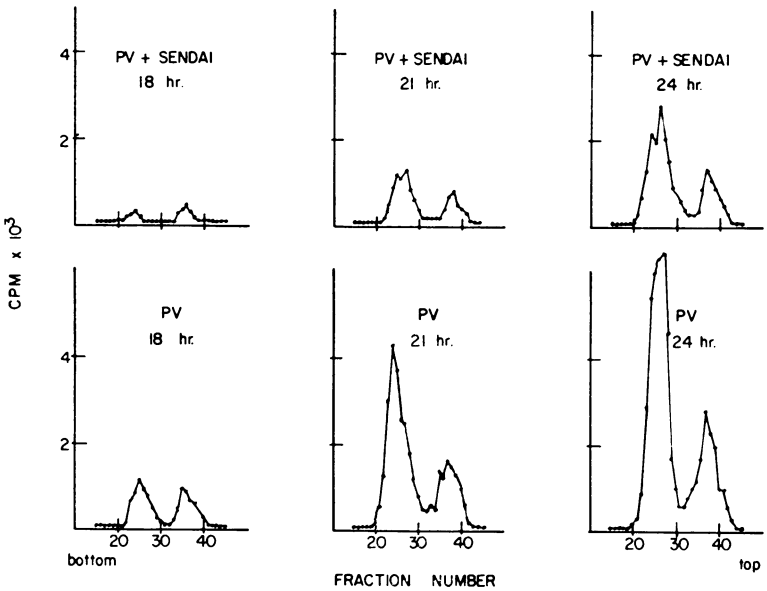

FIG. 3. *Polyoma-infected cultures that were superinfected with inactivated Sendai virus 2 hr after polyoma infection were pulsed with ^{3}H-thymidine for 1 hr and chased for an additional hour in unlabeled medium. The cells were then lysed with SDS, and the viral DNA was extracted with 1 M NaCl. This salt-soluble DNA was fractionated by isopycnic centrifugation in an ethidium bromide-cesium chloride density gradient. Fractions collected from the bottom of the tubes were assayed for radioactivity.*

measured by the encapsidation of prelabeled host cell DNA into purified polyoma virions. Previous data (Fig. 4 and 5) also indicated that host DNA synthesized during infection is not packaged, since ^{3}H-thymidine was not incorporated into virus particles if superinfection took place 2 hr after polyoma infection (Fig. 4, 2 hr). When superinfection was performed at later times, there was no apparent increase in the relative quantity of component II DNA encapsidated (Fig. 5).

Sequential addition of FUdR, act D, and inactivated Sendai virus to polyoma-infected cultures. An attempt was made to identify the event in the polyoma replication cycle that was inhibited by inactivated Sendai virus and to correlate this event with other temporal relationships of polyoma replication. Times of DNA and RNA synthesis in polyoma-infected cultures were determined by the sequential addition of FUdR and act D, respectively. These drugs, along with inactivated Sendai virus, were added sequentially at different times to parallel cultures of polyoma-infected mouse embryo cells. All cultures were harvested at 48 hr, assayed for PFU activity, and compared to a normal polyoma growth curve. Inactivated Sendai virus must be added prior to polyoma DNA synthesis (FUdR curve) or RNA synthesis (act D curve) to elicit an inhibitory effect on the synthesis of polyoma virus (Fig. 6).

Transformation. Since it was demonstrated in the previous experiment that Sendai virus must be added during the early events of polyoma replication to effect its inhibition on viable progeny formation, it was of interest to investigate transformation as an additional early viral function. Results (Table 3) based on colony formation in soft agar did not demonstrate any effect on cell transformation by polyoma virus due to superinfection by inactivated Sendai virus. As shown previously, other early viral functions (i.e., tumor antigen and polyoma-induced host cell DNA synthesis) were not drastically inhibited by Sendai virus superinfection.

Infection of mouse cells with radioactive Sendai virus. Studies were initiated to monitor the fate of inactivated Sendai virus in polyoma-infected mouse cells, in an attempt to gain further insight into the mechanism of inhibition. Preliminary experiments showed that, when polyoma-infected cultures were superinfected with ^{3}H-uridine-labeled inactivated Sendai virus 2 hr after polyoma infection, approximately 15% of the radioactivity was found associated with the nuclear fraction within 2 hr, and this amount increased to 30% by 10 hr. No significant radioactivity could be detected in the ribonucleoprotein pellet.

Studies were performed to determine the time required for the mouse cell to uncoat the inacti-

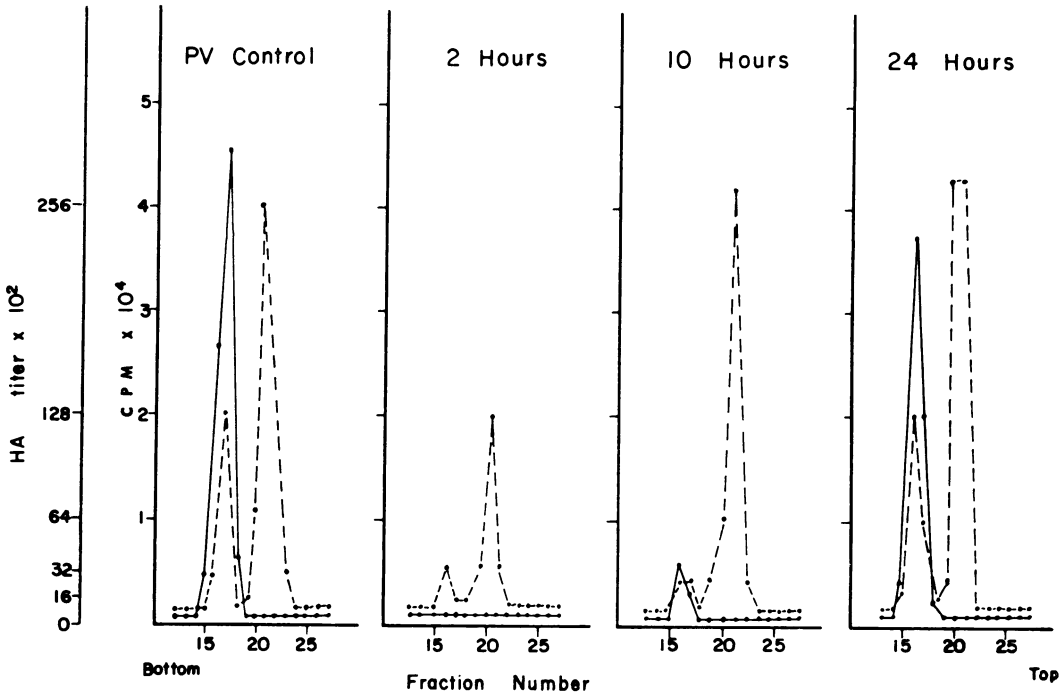

FIG. 4. *Effect of inactivated Sendai virus on the incorporation of ³H-thymidine into purified polyoma virus particles. Polyoma-infected cultures were maintained in ³H-thymidine (3 μCi/ml) from 2 to 40 hr postinfection. At the indicated times, parallel cultures were superinfected with inactivated Sendai virus (2,000 HA units per culture). Cultures were harvested at 40 hr, and the polyoma virus was purified by centrifugation in cesium chloride. Radioactivity (——) and HA activity (-----) were assayed.*

TABLE 1. *Effect of inactivated Sendai virus on the synthesis of polyoma structural and induced tumor antigens*

Conditions	Structural antigen synthesis[a]		Tumor antigen[b]	
	Nuclear fluorescence[c] (%)	Antigen synthesis (%)	Nuclear fluorescence[c] (%)	Antigen synthesis (%)
Uninfected	0.0	0.0	0.0	0.0
PV control	47.0	100.0	45.8	100.0
PV + Sendai[d]	31.0	65.6	46.0	100.0

[a] Cover-slip cultures were harvested 30 hr after polyoma infection.

[b] Cover-slip cultures were harvested 24 hr after polyoma infection.

[c] Minimum of 300 cells counted.

[d] Superinfection by inactivated Sendai virus (equivalent of 2,000 HA units per culture) was performed 2 hr after polyoma infection. PV, Polyoma virus.

vated Sendai virus and release the RNA in a form sensitive to exogenous ribonuclease. Cell lysates were prepared by freeze-thawing, and acid-soluble radioactivity was determined before and after ribonuclease treatment. The RNA from ³H-uridine-labeled Sendai virus was sensitive to ribonuclease within 1 hr after addition and almost totally ribonuclease sensitive 3 hr after addition of Sendai virus to polyoma-infected cells (Table 4).

Effect of UV irradiation of the interfering capacity of Sendai virus. Since live and beta-propiolactone-inactivated Sendai virus caused inhibition of polyoma virus synthesis, it was of interest to determine if the ability of Sendai virus to interfere with polyoma virus synthesis could be reduced by UV irradiation. UV irradiation of live or beta-propiolactone-inactivated Sendai virus preparations resulted in a loss of interfering ability (Table 5). This loss of the interfering capacity due to UV irradiation was seen prior to any detrimental effect on Sendai structural proteins as determined by HA activity of the virus preparations (Table 5). Excess UV irradiation abolished both HA and interfering capability.

DISCUSSION

Findings described in the preceding paper revealed that the inhibition of polyoma synthesis

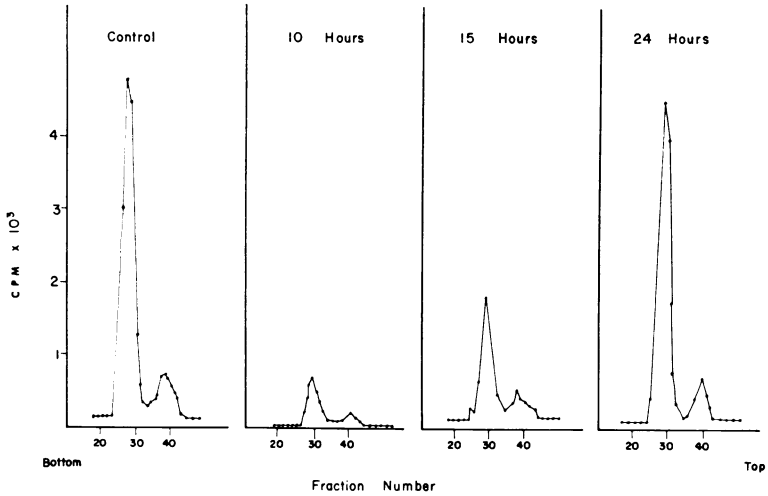

FIG. 5. *Effect of inactivated Sendai virus on the polyoma DNA that is encapsidated after superinfection. Fractions comprising the complete virus peak in the ^{3}H-thymidine incorporation experiment were pooled, and the particles were subjected to alkaline degradation to release the packaged DNA. DNA preparations were centrifuged in an ethidium bromide-cesium chloride density gradient. Fractions were collected from the bottom of the tubes, and radioactivity was determined.*

TABLE 2. *Effect of inactivated Sendai virus on the degradation of prelabeled host cell DNA and the synthesis of pseudovirions*

Conditions[a]	Hr post-infection	% of total counts/min in the medium	% of total counts/min (cells) soluble in 1.0 M NaCl	Counts/min in purified virus[b]
PV-infected control	5	2.2		1,748
	10	4.5		
	24	15.1	11.0	
	30	20.1	11.0	
PV infected + Sendai	5	3.6		<100
	10	5.9		
	24	16.7	11.0	
	30	16.2	14.0	

[a] PV, Polyoma virus.

[b] Polyoma-infected, prelabeled cultures that had been superinfected with inactivated Sendai virus 2 hr after polyoma infection were harvested at 48 hr, and the polyoma virus was purified. Counts per minute were compared to the virus purified from polyoma-infected prelabeled cultures without Sendai.

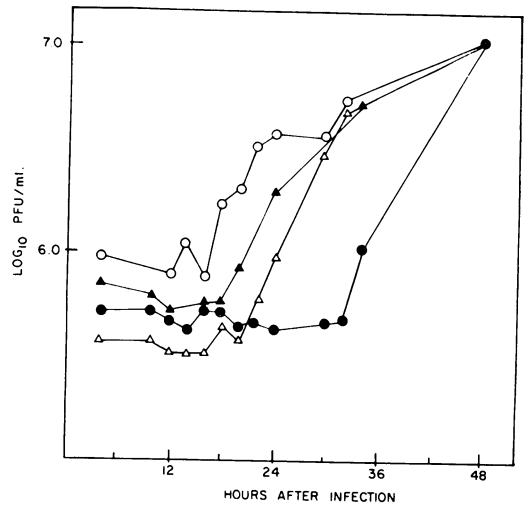

FIG. 6. *Sequential addition of FUdR (10^{-4} M), actinomycin D (2.5 μg/ml), and inactivated Sendai virus. Inhibitors or virus were added at different times to parallel cultures of polyoma-infected cells. All cultures were harvested at 48 hr and assayed for PFU activity. A growth curve is shown for comparison (●) along with the FUdR (△), act D (▲), and Sendai (○) curves.*

by beta-propiolactone-inactivated Sendai virus was not due to the production of interferon and was dependent upon the time of superinfection by the inactivated Sendai virus. A maximum 90% level of inhibition was manifested when superinfection occurred in the initial 16-hr period after polyoma infection. The interference was transient in that normal production of polyoma virus progeny occurred after further incubation of the cultures beyond the normal observation period of 48 hr. It was further demonstrated that the inactivated Sendai virus was not capable of virus-specific RNA or protein synthesis.

The inhibition of polyoma synthesis by beta-propiolactone-inactivated Sendai virus takes place at a step after the adsorption, penetration, and uncoating (32) events of polyoma replication, since interference is seen when Sendai virus is added many hr after these early events have occurred. Additional early events subsequent to virus uncoating were also investigated and did not appear to be inhibited. Polyoma-specific tumor antigen was synthesized to the same extent in control as well as in superinfected cultures (Table 1), and the cell transformation capability of polyoma virus was not affected by inactivated Sendai virus (Table 3). Another early polyoma virus function, the induction of host cell DNA synthesis, was not inhibited, judging from the steady rise in the synthetic rate and the elevated level of DNA synthesis at 24 hr (Fig. 2). Even though induction appeared to take place, the synthetic rates observed were significantly less than those of the control-infected cultures. This observation was consistent in all of our experiments concerned with DNA synthesis. Rates of ^{3}H-thymidine incorporation were decreased when measured in whole cultures or when DNA was fractionated into viral and host fractions by selective salt extraction and isopycnic centrifugation in an ethidium bromide-cesium chloride density gradient (Fig. 1, 2, and 3). This general, but not drastic, decrease in DNA synthesis due to superinfection by inactivated Sendai virus became less as time went on, thus giving the appearance of a delaying effect on polyoma DNA synthesis. Inhibition of cellular DNA synthesis by Newcastle disease virus was reported by Ensminger and Tamm (16), and Fuchs and Kohn (19) described a transient inhibition of DNA synthesis in HeLa cells by inactivated Sendai virus. Although these viruses are very similar, different mechanisms of inhibition were described. Newcastle disease virus (16) apparently inhibited protein synthesis required for initiation of DNA synthesis, whereas inactivated Sendai virus (19) interfered with the endogenous synthetic pathway of thymidine. Regardless of the mechanism responsible for the nominal inhibition of DNA synthesis

TABLE 3. *Effect of inactivated Sendai virus on the transforming ability of polyoma virus*

No. of cells seeded	No. of colonies[a]	
	PV control	PV + Sendai[b]
1.0×10^5	5	5
1.0×10^6	18	12
2.0×10^6	26.5	17.5
5.0×10^6	59.5	56
1.0×10^7	134.5	122

[a] Average of two plates, determined by the agar overlay method (41). Uninfected mouse cells plated at the above densities did not demonstrate colonies. PV, Polyoma virus.

[b] Sendai virus added 2 hr after polyoma infection.

TABLE 4. *Determination of Sendai virus uncoating by ribonuclease sensitivity of ^{3}H-uridine-labeled virus in polyoma-infected cultures*

Time of harvest	% of total counts/min degradable by ribonublease[a]
1.0	9.0
3.0	79.0
Control virus	0.0

[a] Ribonuclease treatment was 50 μg/ml for 30 min.

TABLE 5. *Effect of Sendai virus inactivated with beta-propiolactone, ultraviolet irradiation, or a combination of both on the synthesis of infection polyoma virus progeny*

Conditions[a]	Time of harvest (hr)	PFU/ml	% Inhibition	HA titer of Sendai prepn[b]
PV control	4	6.2×10^4		
PV control	48	1.6×10^6	0	
PV + live Sendai	48	1.6×10^5	90	2,560
PV + BPL Sendai	48	2.4×10^5	85	2,560
PV + UV Sendai (1 min)	48	1.6×10^5	90	2,560
PV + UV Sendai (5 min)	48	1.1×10^6	32	1,280
PV + BPL-UV Sendai (1 min)	48	3.3×10^5	80	2,560
PV + BPL-UV Sendai (2.5 min)	48	1.3×10^6	16	2,560
PV + BPL-UV Sendai (5 min)	48	1.2×10^6	24	1,280

[a] Superinfection with the Sendai virus preparations was performed 2 hr after polyoma infection in all cases. Times indicated represent minutes of UV exposure carried out after inactivation with 0.025% beta-propiolactone. PV, Polyoma virus; BPL, beta-propiolactone.

[b] HA titer of all Sendai virus preparations prior to inactivation procedures was 2,560.

seen in these studies, it does not appear to account for the 90% inhibition of infectious progeny formation that is observed since substantial amounts of polyoma viral DNA are made during Sendai virus superinfection.

Polyoma virus structural protein synthesis was inhibited to an even lesser extent (35%) than the viral DNA. The fact that both polyoma DNA (Fig. 1 and 3) and proteins (Table 1) are synthesized is a further indication that the interference is not due to the production of interferon (29, 42). Neither does the inhibition seem to be due to interference similar to that described for adenovirus (20, 37) or for frog virus 3 (1), since viral macromolecular synthesis is inhibited in these interference systems.

Purification of radioisotopically labeled polyoma virus demonstrated that ^{3}H-thymidine, which was present throughout the infection period, did not appear in the purified virus grown under conditions of superinfection (Fig. 4, 2 hr), even though previous data showed that sufficient quantities of DNA were synthesized. Identical experiments using ^{3}H-valine revealed incorporation of label into purified virus, primarily into incomplete virions. As expected, delay of superinfection allowed progressively increasing amounts of both DNA and protein label to appear in the complete peak. These observations indicated that superinfection by inactivated Sendai virus prevents the encapsidation of polyoma DNA into mature virus particles, whereas assembly of polyoma capsomeres (12) into incomplete virions is less drastically inhibited.

Experiments were performed to investigate the possibility that pseudovirion production was increased due to Sendai virus superinfection. This possibility seemed to be a viable hypothesis since Sendai virus and other similar viruses have been shown to induce DNA degradation and chromosome pulverization (2, 30, 46). Such fragmentation of host cell DNA into small pieces which could compete successfully with viral DNA for virion proteins might account for the decrease in production of infectious polyoma virus. A similar case, in which a decrease in polyoma viral DNA synthesis results in successful competition for encapsidation by host cell DNA and subsequent reduction in infectious virus, has been described by Basilico (5). However, results show this not to be the case in this system. Prelabeled host cell DNA appears to be fragmented and solubilized in the same manner under conditions of Sendai virus superinfection; however, pseudovirion production is also inhibited as much, if not more, than infectious progeny formation (Table 2). The possibility that host DNA made during infection was degraded and encapsidated also appears to be ruled out since the DNA from virions synthesized during superinfection did not contain increased quantities of component II DNA (Fig. 6). These results again lend credence to the hypothesis that superinfection by inactivated Sendai virus specifically inhibits the encapsidation of DNA into mature virus particles. Both polyoma viral DNA and host cell DNA, which have been shown to be present in considerable quantities after Sendai virus superinfection, fail to be packaged into complete virus particles 48 hr after infection.

Experiments to determine the exact time of action of the inactivated Sendai virus indicated that superinfection must take place prior to polyoma DNA (FUdR sensitive) or RNA (act D sensitive) synthesis (Fig. 6). However, it cannot be verified that the Sendai-sensitive event is occurring prior to these events, since the time necessary for the inactivated Sendai virus to exert its effect is not known and may vary from a few minutes to several hours. The data indicate that Sendai virus is not suppressing early viral functions. However, recent interest in viral DNA-protein complexes (6), host cell histone synthesis (21, 57), and the possible role of host histone in DNA complexes and subsequent maturation functions (17) suggest the possibility that this might be a likely area for the inhibition by Sendai virus since DNA maturation appears to be influenced.

Studies aimed at elucidating the mechanism of the inhibition by inactivated Sendai virus failed to detect Sendai virus RNA associated with polysomes; however, a moderate amount of the input Sendai RNA appeared to localize in the nucleus of the cell. Other investigators have also shown a nuclear localization of Sendai virus RNA (9) and RNA synthesis (8). This at least places the Sendai virus in a strategic position to effect the inhibition since polyoma virus replicates in the nucleus. Further studies revealed that the Sendai virus RNA is uncoated 1 hr after infection and is completely susceptible to ribonuclease 3 hr after it is added to the cells. The failure to demonstrate an association between Sendai virus RNA and cell ribosomes does not rule out the possibility that the viral RNA is functioning as a messenger. As reported in the accompanying publication, inactivated Sendai virus did not synthesize any detectable RNA or protein. However, other investigators demonstrated the synthesis of Sendai virus-specific nonstructural proteins by ultraviolet or beta-propiolactone-inactivated Sendai virus (44) and suggested that the virion RNA coded for early viral proteins (i.e., replicase), and the complementary RNA, which could not be syn-

thesized by the inactivated virus, coded for viral structural proteins.

The data is consistent with the supposition that a component of the Sendai virion is eliciting the interference. The fact that ultraviolet irradiation eliminates the inhibitory capacity (Table 5) of live or beta-propiolactone-inactivated Sendai virus suggests that a biologically functional RNA molecule is necessary. Virion protein involvement is not indicated since the amounts of UV treatment that abolished interference had no detrimental effect on hemagglutinating activity which was used as an index of protein damage. The presence of functional RNA molecules and the reduction of interfering capacity by UV irradiation has been reported in interference phenomenon by incomplete virions of vesicular stomatitis and Sendai viruses (27, 51). A likely hypothesis concerning the mechanism of the inhibition of polyoma virus synthesis by beta-propiolactone-inactivated Sendai virus involves the localization of the Sendai virus RNA in the cell nucleus where it selectively inhibits polyoma viral functions necessary for the encapsidation of DNA into mature virus particles by either competing for cell ribosomes or binding to enzymes or other substrates required for polyoma functions.

Although it is felt that the beta-propiolactone-inactivated Sendai virus genome is the inhibitory component of the virion, more complex details of the inhibitory mechanism may be involved. Since it has been reported that inactivated Sendai virus is capable of specific protein synthesis (i.e., replicase), the possible function of such proteins in the interference should be considered. Other investigators (43, 53) have also described the role of viral polymerase proteins in interference phenomena.

The specific inhibition of polyoma maturation could result from a difference in localization of synthesis for different viral proteins as has been demonstrated for vesicular stomatitis virus (49, 55) or to a selective inhibition of a given ribosomal population. Doyle and Holland (14) observed greater inhibition of certain VSV proteins after poliovirus infection, and Freda and Buck (18) described a reciprocal interference system between Mengo virus and vaccinia virus in which vaccinia early, but not late, proteins were synthesized. Katz and Moss (31) demonstrated inhibition of the assembly and maturation of vaccinia virus by rifampin, and Dales and Silverberg (13) observed the synthesis of only empty reovirus capsids after superinfection by vaccinia virus.

The demonstration in this study that a component of the beta-propiolactone-inactivated Sendai virion specifically inhibits the maturation of polyoma virus in mouse cells may, in the future, provide a unique insight into the complexities of DNA tumor virus replication as well as interference phenomena.

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