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UV Inactivation of the Biological Activity of Defective Interfering Particles Generated by Vesicular Stomatitis Virus

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UV inactivation of vesicular stomatitis virus and its defective interfering (DI) particles was measured in order to obtain the target size for interference. In the case of DI particles whose genomes mapped at the 5′ end of the virion RNA, this target size corresponded to the entire DI particle RNA molecule regardless of whether it amounted to 10, 30, or 50% of the viral genome. These data were interpreted as demonstrating that both termini of the DI particle RNAs were required for their replication and for interference with virion RNA replication. The unique heat-resistant DI particle, with an RNA molecule corresponding to the 3′ half of the viral genome, exhibited an inactivation target size of approximately 42% of its RNA molecule with respect to both homotypic and heterotypic interference. Unlike other DI particles, this particle interfered with virion primary transcription. The unusual inactivation target size of the heat-resistant DI particle was interpreted as being a compromise between the requirements for replication of its genome and those for interference with virion primary transcription.

During undiluted passages of vesicular stomatitis virus (VSV) in cell tissue culture, defective interfering (DI) particles are generated in addition to complete virions (5, 6, 9). Generally, the DI particles interfere specifically with the intracellular infectious cycle of homologous virions. Moreover, they contain all of the viral proteins, but only a portion of the viral genome (8). The amount of retained virion RNA has been shown to vary in DI particles produced from infections by VSV mutants (24, 26, 28) and wild-type isolates of different origins (15). The majority of DI particle genomes were derived from the 5′ terminus of the viral RNA and contained part of the L-protein cistron (32). In contrast, the genome of the DI particle generated by a heat-resistant (HR) mutant (24, 26) was exceptional. This one originated from the 3′ half of the viral RNA and contained all VSV protein cistrons except that of the L protein (15, 32, 34). Detailed studies of the terminal nucleotide sequences of DI particle RNAs indicated that the two ends were complementary and, in some cases, capable of self-annealing into panhandle-like structures (14, 20, 22, 23). The presence of inverted terminal complementary sequences suggested that identical promoter nucleotide regions exist in both the positive and negative RNA strands during replication of DI particle genomes (23, 28a). Thus, the replicative ability of these genomes may require the conservation of some terminal sequences at both ends. Because of the close relationship between DI particle RNA replication and interference (19, 21), these requirements may apply to both biological activities. This hypothesis can be tested by measuring UV inactivation kinetics of the interfering abilities of DI particles containing genomes of various sizes. The use of UV irradiation to measure the target size for DI particle interference and replication is based on the block of nucleic acid transcription beyond the site of a UV lesion (1, 2, 17). Since UV lesions on an RNA molecule are caused by the generation of pyrimidine dimers at sites where these nucleotide residues are adjacent (18), the method is applicable only if the distribution of these nucleotides is at least approximately random. This seems to be the case for VSV RNA, as indicated by the successful application of UV inactivation kinetics in determinations of gene order (1, 2).

A single UV lesion in a DI particle RNA would prevent transcription of its 5′ terminus into the complementary positive RNA strand. If the complementary nucleotide sequences serve as an affinity site on the positive strand for the RNA polymerase, a failure to transcribe the 5′ end of DI particle RNA would result in abortive replication of the DI particle genome. Thus, the target size for DI particle replication and interference should always correspond to the entire DI particle RNA. On the other hand, if conservation of the two ends was not required, the UV inactivation data might identify a constant nucleotide sequence in all DI particle genomes required for interference and replication. Such

a sequence could not exceed the size of the smallest DI particle RNA presently available, namely that of the tsG31 DI particle. Previous work on UV inactivation of DI particles was of a qualitative nature and revealed that much greater energies were required for DI particle inactivation than for virion inactivation (7, 10). We have quantitatively determined the target sizes of DI particle RNAs which have different molecular weights and genetic loci. We found that UV inactivation followed single-hit kinetics and that the target sizes of the RNAs, which mapped at the 5′ end of the viral genome, corresponded to their respective molecular weights. However, the HR DI particle RNA, which mapped at the 3′ end of the viral genome, followed inactivation kinetics corresponding to approximately half the size of the molecule. We also demonstrated that, unlike other DI particles, this one interfered with primary transcription by the virion, and we concluded that the target size reflected a compromise between requirements for DI particle replication and requirements for interference with virion primary transcription.

MATERIALS AND METHODS

Cells and viruses. Baby hamster kidney cells (BHK-21 clone 13 [16]) were grown as monolayers either in 32-ounce (960-ml) prescription bottles for large-scale viral preparations, in 2-ounce (60-ml) square flint glass bottles for plaque assays, or in 35-mm plastic tissue culture plates for UV irradiation experiments. The media and conditions of growth were as described previously (15, 32).

Virus isolates of the Indiana serotype of VSV were originally provided by C. R. Pringle (Glasgow wild type, tsG31, tsG11), A. S. Huang (Hu), and L. Prevec (HR) and were described previously (10, 15, 26–28). In addition to these, the Concan isolate of the New Jersey serotype of VSV was obtained from L. Prevec (26).

Purification of virions, DI particles, and particle-bound RNA. Viral inocula were prepared by sevenfold clonal purification followed by a single enrichment step as described by Stampfer et al. (36). The virion progeny was then purified from the supernatant medium by ultracentrifugation at 50,000 × *g* for 120 min. The virion pellet was resuspended overnight at 4°C in a small volume of 3E buffer (0.12 M Tris-acetate, pH 7.2, 0.06 M sodium acetate, 0.003 M disodium EDTA). Resuspended pellets were then subjected to a 5-min centrifugation at 3,000 × *g* to remove cellular debris. The supernatant fluid was then layered on linear 10 to 35% (wt/vol) sucrose gradients in 3E buffer and centrifuged at 4°C and 40,000 × *g* for 100 min. The light-scattering zone was removed with a Pasteur pipette and stored in small samples at −70°C until required. Radioactively labeled virions were prepared in the same manner except that the infected cells were overlaid with medium containing 10 μCi of [5,6-^{3}H]uridine per ml, 10 μCi of [2-^{3}H]adenosine per ml, 10 μCi of [5-^{3}H]cytidine per ml, and 10 μCi of [5-^{3}H]guanosine per ml; the specific activities of these isotopes were 14 to 41 Ci/mmol, and they were obtained from New England Nuclear Corp., Boston, Mass. Particle-bound RNA was isolated by phenol extraction as previously described (37).

DI particles were purified from infections with inocula which generated an excess of DI particles over virions (usually a third undiluted passage of the specified virus isolate). The purification procedure was similar to that employed for virion isolation with the following modifications: 15 to 30% (wt/vol) sucrose gradients in 3E buffer were used in order to obtain a better separation between DI particles and virions. The light-scattering zone, which contained DI particles, was removed from the gradient tube and dialyzed against 3E buffer overnight at 4°C to remove the sucrose. The DI particles were repurified on a 10 to 35% sucrose gradient in 3E buffer at 110,000 × *g* for 60 min and then examined for contamination with virions by a plaque assay (32). If the contamination exceeded 10^5 PFU/ml per optical density unit at 260 nm as measured by a Cary 14 spectrophotometer (Applied Physics Corp., Monrovia, Calif.), the last step was repeated until the virion contamination was less than this amount. The level of contamination was estimated to be approximately 1 PFU of virions in 10^4 biologically active DI particles. This estimate was based on the finding that 1 optical density unit of virions corresponded to approximately 10^9 PFU and on the assumptions that the intensity of light scattering of the DI particles was proportional to their sizes and that the ratio of biologically active to nonactive DI particles was the same as in virion preparations.

UV irradiation of virion and DI particles. Purified virion or DI particle solutions in 3E buffer containing sucrose were used directly in the UV irradiation experiments. The extent of UV inactivation was not affected by the presence of sucrose. Since EDTA in the 3E buffer caused cell detachments in plaque and interference assays, this ion was chelated by adding $MgCl_2$ to a concentration of 0.005 M before UV inactivation. Inactivations were carried out in 35-mm tissue culture plates (Falcon Plastics, Oxnard, Calif.) with 1-ml volumes. The solutions were exposed to a G15T8 germicidal lamp (Sylvania Electric Products, Inc., Salem, Mass.) at a distance of 60 cm for variable times depending on the required light energies. The UV energies were determined with a YSI model 65A radiometer (Yellow Springs Instrument Co., Yellow Springs, Ohio).

Assays for biologically active virions and DI particles. The biological activity of UV-irradiated virions was determined by a standard plaque assay (32). The biological activity of DI particles was determined by assaying their interfering ability. BHK cells in 60- or 35-mm tissue culture plates were infected with 5 PFU of virions per cell in the presence or absence of a DI particle solution (32). After 18 h at 39°C, the virion progenies from both types of infections were determined by plaque assays. Interfering ability was expressed as the $\log_{10}$ decrease in the viral yield from mixed infections as compared with the yield from the infection by virions alone. The relative concentrations of active DI particles in the UV-irradiated solutions were determined by comparison with stan-

dard dilution curves (see below).

Assay for DI particle interference with virion primary transcription. Confluent monolayers of BHK cells in 60-mm tissue culture dishes were pretreated for 1 h with media containing 5 μg of actinomycin D (Calbiochem, La Jolla, Calif.) per ml and then infected with 1 to 5 PFU of purified New Jersey virions per cell, either alone or in a mixture with the appropriate DI particles. The concentration of DI particles was capable of reducing the yield of homotypic virions by two orders of magnitude when used under similar conditions. After absorption, the cells were washed with sterile saline, overlaid with media containing 5 μg of actinomycin D per ml and 50 μg of cycloheximide per ml, and then incubated at 37°C. At the appropriate times after infection, the cells were washed with sterile saline and covered with 2 ml of 3E buffer containing 1% sodium dodecyl sulfate; this was followed by the addition of 3 ml of phenol-cresol mixture (37). The cytoplasmic RNA was then isolated as previously described (37). To determine the amount of virion transcription products, a sample of the cytoplasmic RNA was annealed (15) to a radioactively labeled New Jersey virion RNA probe (0.1 μCi/μg of RNA). From the RNase-resistant radioactive counts, the relative concentrations of viral mRNA's in the sample and in the total preparation were calculated.

Primary transcription by the Indiana serotype of VSV in the presence and absence of DI particles was determined by radioactively labeling the newly generated mRNA species. This was accomplished by overlaying the cells immediately after infection with medium containing 15 μCi of [^{3}H]uridine (40 Ci/mmol; New England Nuclear Corp.) per ml in addition to cycloheximide and actinomycin D. The amount of accumulated mRNA was determined by adding 2 ml of 3E buffer containing 1% sodium dodecyl sulfate followed by cold 10% trichloroacetic acid to the rinsed monolayer and counting the radioactivity in the filtered precipitate (15).

RESULTS

Determination of the relative concentrations of DI particles surviving UV irradiation. Unlike virions, the biological activity of DI particles cannot be determined by a plaque assay. Since the biological activity of interest was DI particle interference, a relationship between this ability and the concentration of active DI particles must be derived. An examination of data in the literature indicated that interference within the range of zero to three orders of magnitude was not linear with respect to concentration of wild-type DI particles from Indiana, Cocal, or New Jersey VSV infections (3, 25). Similarly, interference within the range of zero to two orders of magnitude exhibited some deviation from linearity (Fig. 1). The downward curvature of the line in Fig. 1 suggested that the potential for interference was not fully expressed at high DI particle concentrations. This may have been due to the loss of some DI particles which entered uninfected cells. The data in Fig. 1 were obtained with cells infected with 5 PFU of virions per cell. According to the Poisson distribution, under these conditions the number of uninfected cells would be 0.67%. Because of the competitive nature of DI particle interference, an increase in virion plaque-forming units per cell would have required an unrealistically high concentration of DI particles in order to obtain interference by two orders of magnitude. In view of this, the relative concentrations of survivors after UV irradiation were determined by using an empirical dilution curve, like that shown in Fig. 1, for each DI particle preparation. The UV-irradiated samples were tested for their interfering abilities as described above, and the concentrations of active DI particles were calculated from their respective standard dilution curves. Inherent in these determinations was the assumption that the presence of UV-inactivated DI particles in the solution did not affect the interfering ability of the survivors.

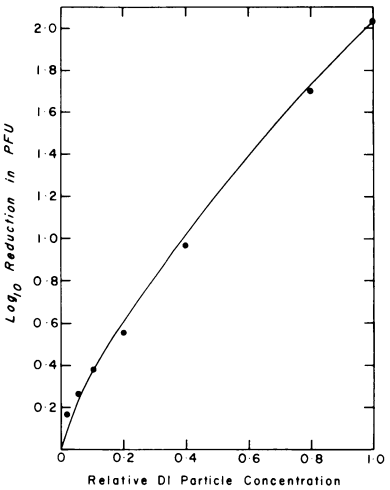

FIG. 1. *Typical dilution curve used for determining the relative concentrations of DI particles remaining biologically active after UV irradiation. BHK cells in 35-mm tissue culture plates were infected with 5 PFU of standard virions per cell in the presence or absence of varying amounts of wild-type (Hu) DI particles. Samples were withdrawn at 18 h after infection and were assayed for plaque-forming ability (see text). Results are plotted as the* log_{10} *reduction in the yield of infectious particles from cells coinfected with DI particles and virions as compared with cells infected with virus alone versus relative concentration of the DI particle solution. In the absence of DI particles, the yield of virions was approximately* 10^9 *PFU/ml. The relative concentration of the undiluted DI particle solution was arbitrarily set at 1.*

UV inactivation curves of DI particles with genomes of various sizes and cistronic contents. Of the four DI particle types used in

these studies, three (Hu, tsG11, and tsG31 DI particles) contained genomes which mapped at the 5′ terminus of the virion RNA, and the fourth (HR DI particles) had a genome mapping at the 3′ terminus of the viral genome (15). The RNAs of tsG11 and HR DI particles were comparable in size and equal to about 50% of the virion RNA (24, 32), whereas tsG31 and Hu DI particles contained 10 and 30% of the viral genome, respectively (11, 15). UV light inactivation curves of these DI particles and of the Glasgow wild-type virion, from which the tsG mutants were originally derived (27), are shown in Fig. 2. The linear relationship is reflected by the values of the regression line parameters (r^2), which in all cases were very close to 1.00 (Fig. 2). This indicated that the inactivation followed single-hit kinetics. In addition to Glasgow wild-type virion inactivation, Fig. 2 shows inactivation of tsG11, Hu, HR, and tsG31 DI particles. The slopes of the inactivation curves (Table 1) were used to calculate the target size required for interference, assuming that the entire virion RNA was required for infectivity. The target sizes relative to the virion RNA are also shown in Table 1. In addition, Table 1 compares the sizes of DI particle genomes with the size of virion RNA based on the following RNA molecular weights: virion, 3.8×10^6 (30); tsG31 DI particles, 4.3×10^5 (15); Hu DI particles, 1.1×10^6 (11, 15); tsG11 DI particles, 1.9×10^6 (32); and HR DI particles, 2.1×10^6 (24). Table 1 also shows the percentages of the genomes required for activity, as derived from the other data in the table. With the exception of the HR DI particle, the target size for biological activity corresponded to the entire DI particle genome.

UV inactivation kinetics of HR DI particle interference and HR virion infectivity. Aside from the unique genetic locus of its RNA, the HR DI particle has the unusual ability of interfering heterotypically with New Jersey virions of the Concan subgroup (26, 29). A comparison of UV light inactivation kinetics of the homotypic and heterotypic interfering abilities of this DI particle was also made. As Fig. 3 shows, the two activities followed very similar inactivation curves, with slopes of 4.5×10^{-4} $mm^2 \cdot erg^{-1}$ for homotypic interference and 4.1×10^{-4} $mm^2 \cdot erg^{-1}$ for heterotypic interference. These results suggested that the two types of interference may have similar mechanisms, as reflected by the linear inactivation kinetics and by the target size corresponding to approximately one-half of the particle RNA.

The greater resistance to UV irradiation of the HR DI particles as compared with the tsG11 DI particles, which contain genomes of similar sizes, might have been inherent in the two different virion isolates from which the DI particles were generated. Unusual nucleotide sequences in the RNA of the HR virion could have resulted in radiobiological properties different from those of the wild-type virion. Therefore, the HR virion was subjected to UV irradiation, and the results were compared with inactivation data of the Glasgow virion. As Fig. 3 shows, the infectivity of the Glasgow and HR virions followed essentially the same inactivation kinetics. Thus, the high resistance of the HR DI particles to UV

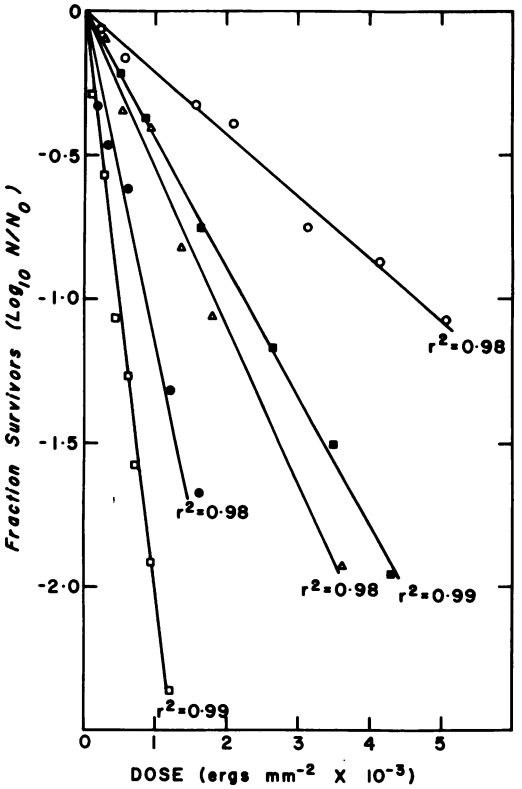

FIG. 2. *UV inactivation kinetics of the biological activities of virions and DI particles with genomes of various sizes. Purified virions or DI particles in 1.0 ml of 3E buffer containing 0.005 M $MgCl_2$ were placed in 35-mm tissue culture plates and exposed to various doses of UV light. The fraction of surviving virions was determined by plaque assays. The interfering ability of irradiated DI particles was assayed as described in the text, and the fraction of survivors was determined from standard dilution curves like the one shown in Fig. 1. Linear regression statistics were employed to determine the rate of inactivation. The r^2 value represents the linear regression coefficient, where $r^2 = 1$ for a perfect straight line. Symbols: □, Glasgow virion; ●, tsG11 DI particle; △, Hu DI particle; ■, HR DI particle; ○, tsG31 DI particle.*

TABLE 1. *Size of virion and DI particle RNAs required for biological activity*

Particle	Rate of inactivation ($\times 10^4$ mm^2·erg^{-1})[a]	Relative genome size necessary for biological activity[b]	Relative size of particle genome[c]	% of genome required for biological activity[d]
Glasgow virion	−19.2	1.00	1.00	100
tsG31 DI	−2.2	0.11	0.11	100
Hu DI	−5.5	0.29	0.29	100
tsG11 DI	−11.7	0.61	0.50	122
HR DI	−4.5	0.23	0.55	42

[a] Rates of inactivation were determined for each particle from a linear regression line (Fig. 2).

[b] Inactivation rates of DI particles were normalized with respect to that of the virion (arbitrarily set at 1) in order to obtain the genome sizes necessary for biological activity.

[c] Molecular weights of DI particle genomes were normalized with respect to that of virion RNA (arbitrarily set at 1). Molecular weights were obtained from references 11, 15, 24, 30, and 32 (see text).

[d] Ratio of relative genome size necessary for biological activity to relative size of particle genome.

inactivation was not derived from the parental virion.

Effect of HR and wild-type (Hu) DI particles on primary transcription by New Jersey virions. The ability of the HR DI particle to express its genomic content (4) and particularly to transcribe its RNA into mRNA's even in the absence of helper virus (12) suggested the possibility that this particle may interfere also at the level of virion primary transcription. Since New Jersey virion RNA has very few nucleotide sequence homologies with the Indiana virion RNA (31, 33), primary transcription by the New Jersey virions could be assayed in the presence of HR DI particle transcription by annealing with a radioactively labeled probe of New Jersey virion RNA. At the appropriate times after infection, cytoplasmic RNA was isolated as described above, and a sample of the isolated RNA was annealed with 30,000 cpm of the probe. The size of the sample was sufficiently small to correspond to the linear portion of the saturation annealing curve. Under these conditions, the amount of annealed radioactive counts was proportional to the concentration of nonradioactive complementary cytoplasmic RNA in the sample. With the aid of this assay, the accumulation of viral primary transcripts was measured at 0 to 6 h after infection (Fig. 4). Cells infected with the virion alone were compared with cells coinfected with virion and HR DI particles. The concentration of accumulated viral mRNA was expressed in terms of ^{3}H counts of the virion RNA probe to which the total cytoplasmic viral mRNA would anneal. As expected, no annealing occurred between this probe and HR DI particle transcripts in cytoplasmic extracts obtained from cells infected with this particle alone. A comparison of the accumulated virion mRNA's in the presence and in the absence of HR DI particles indicated that cumulative interference with viral primary transcription during 6 h postinfection amounted to approximately 50%.

To determine whether this inhibition might have been due to the intracellular presence of Indiana proteins or to an exclusion phenomenon, the effect of wild-type (Hu) DI particles on primary transcription by New Jersey virions was also measured. The concentration of Hu DI par-

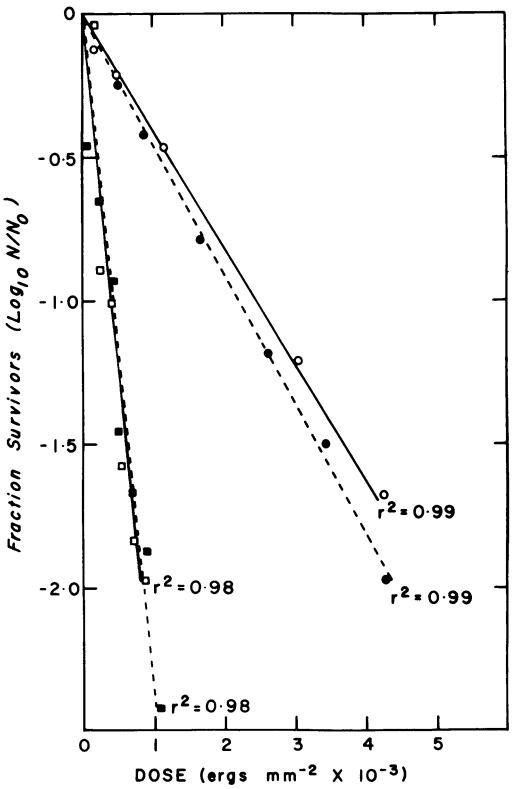

FIG. 3. *Effect of UV irradiation on the homotypic and heterotypic interfering abilities of the HR DI particle and on the infectivities of HR and Glasgow virions. UV inactivations and biological activity assays were performed as described in the legend to Fig. 2. Homotypic interference assays (●) were performed with Glasgow Indiana virions (multiplicity of infection, 5), whereas heterotypic interference assays (○) used New Jersey virions (multiplicity of infection, 6). The UV inactivation of Glasgow Indiana virions (■) was compared with that of HR Indiana virions (□).*

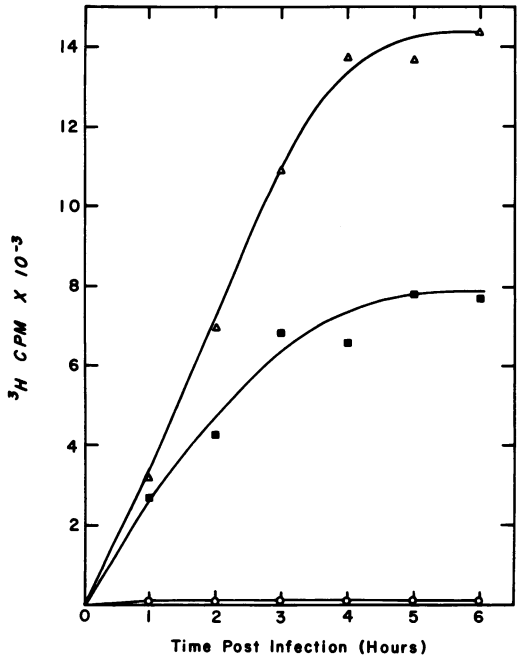

FIG. 4. *Primary transcription by New Jersey virions in the presence and absence of Indiana HR DI particles. BHK cells in 60-mm tissue culture plates were pretreated with 5 μg of actinomycin D per ml for 30 min. The cells were then infected with New Jersey virions (multiplicity of infection, 5) in the presence and absence of HR DI particles or with HR DI particles alone. After infection, the cells were covered with 5 ml of medium containing actinomycin D and 50 μg of cycloheximide per ml. The HR DI particle solution reduced the yield of infectious virions by 2.5 $\log_{10}$ units. At hourly intervals up to 6 h postinfection, intracellular RNAs were extracted and annealed to ^3H-labeled New Jersey virion RNA (0.1 μCi/μg of RNA), as described in the text. The relative concentrations of accumulated transcription products from 4×10^6 cells are expressed as the total ^3H counts per minute of virion RNA which could be made RNase resistant after annealing. The results were corrected for nonspecific annealing by subtracting ^3H counts per minute of virion RNA rendered RNase resistant after annealing with intracellular RNA from mock-infected cells. Symbols: △, New Jersey virions; ■, New Jersey virions and HR DI particles; ○, HR DI particles.*

ticles in this experiment was comparable to that of the HR DI particles in the previous experiment. The results appear in Fig. 5, which shows primary transcription in cells infected with virion alone and primary virion transcription in cells coinfected with virions and DI particles. The data demonstrate that Hu DI particles did not influence primary transcription by New Jersey virions.

Effect of HR and wild-type (Hu) DI particles on primary transcription by Indiana virions. The accumulation of primary transcripts in cells infected with Indiana virions in the presence and absence of the homotypic HR and wild-type (Hu) DI particles was also measured. In the mixed infection, no distinction was made between transcripts originating from the virion RNA and transcripts originating from the HR DI particle RNA. The purpose of these experiments was to establish whether a general inhibitory effect on primary transcription could be detected. The newly synthesized primary transcripts were labeled with [^{3}H]uridine and assayed by trichloroacetic acid precipitation of radioactive counts. The accumulation of trichloroacetic acid-precipitable transcripts in the time interval of 0 to 6 h postinfection is plotted in Fig. 6. A comparison of primary transcription by Indiana virions in the presence and absence of Hu DI particles indicated that this DI particle had no effect on mRNA synthesis by the virion under these conditions. Moreover, the wild-type

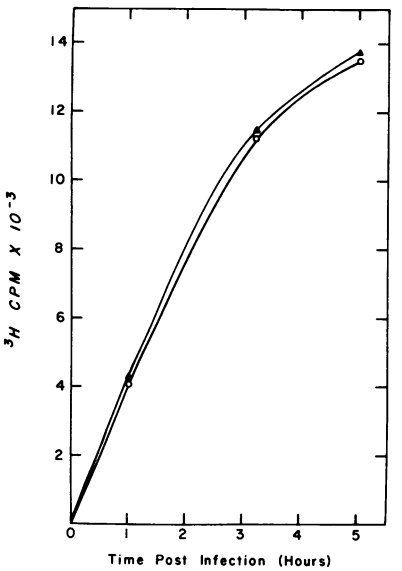

FIG. 5. *Primary transcription by New Jersey virions in the presence and absence of wild-type (Hu) Indiana DI particles. The experimental conditions were the same as described in the legend to Fig. 4, except that wild-type Indiana DI particles (Hu) were substituted for the HR DI particles. The Hu DI particle solution reduced the yield of homotypic virions by 2.7 $\log_{10}$ units and that of the heterotypic New Jersey virions by 1.7 $\log_{10}$ units in BHK cells. Cells infected with Hu DI particles alone showed no accumulation of mRNA above that of mock-infected cells. Symbols: ▲, New Jersey virions and Hu DI particles; ○, New Jersy virions.*

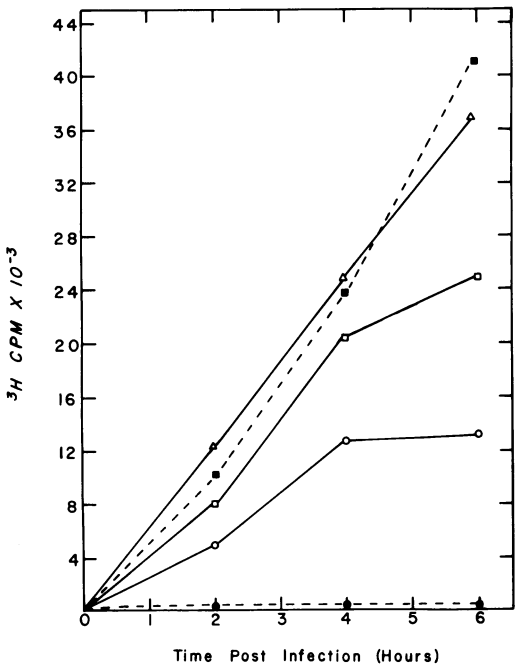

FIG. 6. *Primary transcription by Indiana virions in the presence and absence of DI particles. The conditions of infection were as described in the legend to Fig. 4, except that the infection medium also contained 5 μCi of [^{3}H]uridine per ml. The concentrations of intracellular primary transcripts at the indicated times were determined by precipitation of radioactively labeled intracellular RNA with 10% trichloroacetic acid as described in the text. The data were corrected for host-directed RNA synthesis by subtracting acid-insoluble radioactivity obtained from mock-infected cells. Symbols: △, Glasgow Indiana virions alone; ○, HR DI particles alone; ●, Hu DI particles alone; □, virions and HR DI particles; ■, virions and Hu DI particles.*

DI particles alone did not exhibit any appreciable transcriptional ability. These results are in agreement with previous reports (9, 21). On the other hand, in cells coinfected with virion and HR DI particles, the accumulation of mRNA species was considerably decreased despite the fact that the HR DI particle RNA itself was being transcribed. The inhibitory effect on the virus was probably much stronger than apparent from the data in Fig. 6, since the contribution of HR DI particle transcription was not subtracted from the data representing the coinfection.

DISCUSSION

A comparison of RNA species synthesized after viral infection in the presence and absence of DI particles suggested to several investigators that replication of DI particle RNAs took place at the expense of virion RNA synthesis (21, 35). It was concluded that interference resulted from a successful competition of DI particle RNA with virion RNA templates for the replicase, which was available in a limited supply. This limited supply seemingly occurs early in viral infection, since addition of DI particles late in the infectious cycle eliminated interference but not replication of DI particle RNA (36). Thus, although DI particle replication does not require interference (13, 36), apparently interference cannot take place without replication of DI particles. This conclusion was fully confirmed by the data in this paper. UV irradiation of DI particle RNA prevented the transcription of nucleotide sequences beyond the lesion toward the 5′ end of the molecule. Thus, the 3′ end of newly synthesized positive strands would not contain the sequences complementary to the 5′ end of the original DI particle RNA. The inability of a DI particle RNA with a single UV lesion to interfere with viral infection is interpreted as being the result of its inability to generate positive RNA strands with a proper polymerase binding site at their 3′ termini. This interpretation would explain the inactivation slopes of all DI particles, except that of the HR DI particle (Fig. 2 and Table 1).

Holland et al. have published preliminary data of UV and nitrous acid inactivation kinetics of a DI particle comparable in the size of its genome to the Hu DI particle investigated in this paper (7). They concluded that the target size of this particle was slightly higher than 10% of the virion target size and, although smaller, was comparable to the total DI particle RNA size (7). The simplification of DI particle concentration determinations through the use of an empirical dilution curve, as shown in Fig. 1, rather than the use of the laborious amplification procedure (7) may have resulted in greater accuracy of our data. The conditions of the inactivation experiments were, in fact, dictated by the requirement of a maximum attainable accuracy in the assay for biologically active DI particles. For these reasons, DI particle concentrations which exhibited a relatively high degree of interfering ability before irradiation were selected. However, because of the competitive nature, interference decreases at high multiplicities of infection of the virion (3). On the other hand, the absolute requirement for a helper virus demanded selection of a multiplicity of infection high enough not to leave an appreciable fraction of cells free of virions. The compromise between these two conflicting considerations led to the conditions described above. Unfortunately, under these conditions interference and DI particle concentrations did not follow a completely linear relationship, as described by Bellet and Cooper

(3).

The inactivation target size obtained with the HR DI particle did not correspond to the entire RNA molecule and required an interpretation different than that applied to the inactivation data with other DI particles. After eliminating the trivial possibility of an anomaly in the HR mutant virion itself (Fig. 3), several explanations can be considered. The possibility of the existence of an internal nucleotide sequence similar or identical to that of the 5′ terminus could explain the small inactivation target size. The presence of such a nucleotide sequence would, however, predict the generation of DI particles which would contain an RNA approximately half the size of the HR DI particle genome and would map in the 3′ half of the virion RNA. So far, we have not been able to isolate such a particle from cells coinfected with virions and partially inactivated HR DI particles.

However, it is more likely that the UV inactivation kinetics of the HR DI particle were influenced by the ability of this particle to carry out its own transcription and, in the process, to interfere with virion primary transcription. It should be noted that this is the first case in which a VSV DI particle has been shown to interfere with primary transcription. On the other hand, several investigators have reported no effect by DI particles on virion primary transcription (9, 21), and it is likely that the genomes of the particles investigated mapped at the 5′ end of the viral RNA. Our data with the Hu DI particle (Fig. 5) confirmed these observations. If the interfering ability of the HR DI particle is determined by interference with primary transcription as well as with virion RNA replication, the UV inactivation curve may represent a compromise between two different target sizes. The inactivation kinetics may therefore be pseudo-first order. Even though the interference with primary transcription was only twofold, subsequent amplification of this initial effect may be expressed in a much greater reduction of viral yields. This could also imply a continuous interference with secondary transcription. However, at present no experimental evidence on the extent of this effect is available. The data presented in this paper emphasize the complex nature of the HR DI particle interference and offer at least one possible explanation for the anomalous target size of this particle RNA. Since replication of DI particles has been uncoupled from interference by a careful timing of the addition of DI particles to infected cells (35) and since interference with primary transcription can be studied in vitro, experiments to measure the target sizes separately have been designed and are under consideration.

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