UV Irradiation Analysis of Complementation Between, and Replication of, RNA-Negative Temperature-Sensitive Mutants of Newcastle Disease Virus

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Random UV irradiation-induced lesions destroy the infectivity of Newcastle disease virus (NDV) by blocking downstream transcription from the single viral promoter. The nucleocapsid-associated polypeptides most likely to be involved in RNA synthesis are located at the extreme ends of the genome: *NP* and *P* are promoter proximal genes, and *L* is the most distal gene. We attempted to order the two temperature-sensitive (*ts*) RNA-negative (RNA^-) mutant groups of NDV by determining the UV target sizes for the complementing abilities of mutants A1 and E1. After UV irradiation, E1 was unable to complement A1, a result compatible with the A mutation lying in the *L* gene. In contrast, after UV irradiation, A1 was able to complement E1 for both virus production and viral protein synthesis, with a target size most consistent with the E mutation lying in the *P* gene. UV-irradiated virus was unable to replicate as indicated by its absence in the yields of multiply infected cells, either as infectious virus or as particles with complementing activity. After irradiation, *ts* mutant B1 Δ P, with a non-*ts* mutation affecting the electrophoretic mobility of the P protein, complemented E1 in a manner similar to A1, but it did not amplify the expression of Δ P in infected cells. This too is consistent with irradiated virus being unable to replicate despite the presence of the components needed for replication of E1. At high UV doses, A1 was able to complement E1 in a different, UV-resistant manner, probably by direct donation of input polypeptides. Multiplicity reactivation has previously been observed at high-multiplicity infection by UV-irradiated paramyxoviruses. In this case, virions which are noninfectious because they lack a protein component may be activated by a protein from irradiated virions.

As a paramyxovirus, Newcastle disease virus (NDV) employs the negative-strand virus strategy. The first virus-specific synthetic event in an infected cell is the production of mRNA (plus strand). With the translation of these mRNA's, replication (the synthesis of genome-sized plus-strand RNA and then complete negative-strand RNA) begins. Transcription from these progeny genomes amplifies viral mRNAs and consequently viral proteins.

Genetic analysis of NDV is hampered by the peculiarities of negative-strand RNA viruses, which contain a completely covalently linked genome (reviewed by Bratt and Hightower [4]). Recombination has not yet been demonstrated (11, 19, 21). If recombination did exist, it might easily be obscured by the high rate of mutation (42, 24) or by the tendency of NDV to form multiploid particles containing complementing genomes (11, 20, 23, 28).

UV irradiation has been used as an effective tool to circumvent some of these problems.

Irradiation of NDV inactivates infectivity with single-hit kinetics (5, 7, 32, 39) and blocks mRNA transcription (7). Recent irradiation studies have shown that the NDV genes are sequentially transcribed from a single promoter (9), as previously shown for Sendai virus (18) and vesicular stomatitis virus (VSV) (1, 2). Genes for the three proteins associated with viral nucleocapsids (6, 10, 40), and thought to be involved in NDV-specific RNA synthesis, appear widely separated on the genome; those for the NP and P proteins are least sensitive to irradiation and thus closest to the promoter, whereas that for the L protein is as sensitive as infectivity and thus furthest from the promoter (9).

Two of the five complementation groups of NDV temperature-sensitive (*ts*) mutants isolated by Tsipis and Bratt (42), groups A and E, have defects affecting RNA synthesis (42; M. E. Peebles, L. L. Rasenas, and M. A. Bratt, submitted for publication). As yet, no definite as-

signment of viral proteins to these groups has been made. We postulated that the UV sensitivity of a particular *ts* mutant's ability to complement another unirradiated *ts* mutant should be determined by the distance between the required gene and the promoter. If some or all of the NDV genes from UV-irradiated virus were able to complement NDV *ts* mutants, the relative order of these mutant genes might be determined. UV irradiation has successfully been used to order amber mutants of phage T4 (25) and RNA-negative (RNA⁻) *ts* mutants of Sindbis virus (17). Surviving genes of UV-irradiated VSV have been shown to complement group II and IV mutants (14, 15).

UV-inactivated NDV at high multiplicity can produce greater than predicted levels of infected cells or virus (16, 29). This multiplicity reactivation could be due to cooperation among, or repair of, damaged virus or, alternatively, to a nongenetic phenomenon involving the donation of a required protein(s) from damaged virus to an otherwise potentially infectious virus lacking that protein (4).

We found that UV-irradiation-damaged genomes of NDV were unable to replicate. However, irradiated A1 was still able to complement E1, but irradiated E1 was no longer able to complement A1. Target sizes were comparable to those of the P protein (*E* gene) and the L protein (*A* gene). At high radiation doses, A1 was able to complement E1 in a UV-resistant, multiplicity of infection (MOI)-dependent manner. This complementation probably reflects protein transfer from input irradiated virions and provides a possible explanation for multiplicity reactivation.

MATERIALS AND METHODS

Cell cultures. Primary and secondary chicken embryo cell cultures were maintained in the standard medium described by Hightower and Bratt (26) at 39.5°C in a 5% CO₂ atmosphere. For yield experiments, secondary cultures were used 24 h after plating in 35-mm tissue culture dishes. For plaque titrations, secondary cultures were used as they reached confluency (24 to 48 h after plating) in 60-mm tissue culture dishes.

Virus stocks. Wild-type virus, AV-WT, was previously cloned from the Australia Victoria (1932) strain of NDV (3). AV-WT was the parent for the series of *ts* mutants isolated by Tshipis and Bratt (42). The major *ts* mutants used in this report were A1 and E1, complementing mutants with defects in RNA synthesis (42). Another *ts* mutant, B1ΔP, spontaneously arose during recloning of mutant B1. B1 has a defect in the gene for the HN protein (38). B1ΔP differs from B1 in having a second, non-*ts* mutation which alters the migration of protein P without altering its plating efficiency, virus-specific RNA synthesis, or ability to complement A1 and E1.

Virus stocks were grown in the allantoic sac of 10-

day-old embryonated hen eggs at 36°C. Allantoic fluid, harvested after the death of the majority of embryos (48 to 64 h), was concentrated, purified as described previously (8, 43), and stored at -70°C.

Plaque assays. Titration of infectivity was performed as described previously (3). Plates were incubated at permissive temperature (37.5°C).

Complementation experiments. Confluent cell monolayers were inoculated with virus at 4°C, washed, and incubated with media at a nonpermissive temperature of 41.8°C (42).

The various multiplicities used are described in the figure legends. The 9.5-h yield of each virus alone (Y_S) was subtracted from the yield of multiple infections (Y_M). The results are presented as the ratio of the $Y_M - Y_S$ values of crosses of complementing mutants, where one parent was treated with UV irradiation before infection, to $Y_M - Y_S$ of a similar cross, where neither parent was irradiated.

UV irradiation. Two milliliters of virus diluted in standard buffer (0.01 M Tris, pH 7.4, 0.1 N NaCl, and 0.002 M EDTA) was placed in an uncovered 60-mm tissue culture dish and constantly agitated during exposure to UV irradiation from a Sylvania G15T8 germicidal lamp at a distance of 78 cm.

Quantification of infected cell proteins. After the medium was removed from infected cultures at 9.5 h, the cultures were washed with Hanks balanced salts solution (HBSS; GIBCO Laboratories) and labeled for 30 min with 20 μCi of [³⁵S]methionine per ml (New England Nuclear Corp.) as described by Hightower et al. (27). Cultures were washed with HBSS and lysed in gel sample buffer (0.125 M Tris-hydrochloride, pH 6.8, 20% glycerol, 10% 2-mercaptoethanol, 6% sodium dodecyl sulfate [SDS], and 0.0001% bromphenol blue). Equal portions were electrophoresed at a constant 30 mA on 10% polyacrylamide gels (SDS-PAGE) by the method of Laemmli (30). The gels were dried and exposed to Kodak Royal X-Omat film. The autoradiograms were scanned with an Ortek densitometer, and the areas under peaks were calculated with a Wang digitizer.

RESULTS

Complementation between A and E mutants. Mutants of group A complement mutant E1 at nonpermissive temperature, producing 50 to 2,000 times greater yields than the sum of the single-infection yields (42). At permissive temperature, A1 makes small plaques distinguishable from E1's AV-WT-like large plaques. Using this phenotype facilitated the identification of the progeny of each mutant in mixed infections. The yields of crosses between A1 and E1 always contained both parents (Table 1); thus, complementation functions in both directions.

UV irradiation of one partner of A1 × E1. UV irradiation inactivated the infectivity of A1, E1, and AV-WT with identical single-hit kinetics (Fig. 1A).

To determine the complementing ability of UV-irradiated A1 or E1, cells were infected with the irradiated parent at an MOI of 1 (preirradiation) and with the nonirradiated parent at an MOI of 5. Virtually all of the cells were infected

TABLE 1. Relative yields of A1 and E1 in complementary crosses

MOI (E1) (A1)	A1 and E1 yield ^a					
	0		1		5	
	PFU/ml	Total PFU/ml	%		Total PFU/ml	%
			E1	A1		E1 A1
0		1.5×10^1			4.1×10^2	
1	6.0×10^1	2.2×10^5	46	54	4.5×10^5	78 22
5	3.0×10^2	2.2×10^5	46	54	3.6×10^5	70 30

^a Yields from these infections were titrated for infectivity at 37.5°C. A1 makes predominantly small plaques and E1 makes predominantly large plaques.

with the nonirradiated virus. In addition, many were infected with a single virion from the irradiated parent. This procedure was used in a series of A1 $\times$ E1 crosses in which either A1 or E1 had been subjected to increasing doses of UV irradiation. The nonpermissive temperature yields from such a mixed infection are plotted in Fig. 1B. In the series of crosses in which E1 was the irradiated parent (A1 $\times$ E1_{UV}), the curve for yield reduction closely followed the loss of E1

input infectivity (Fig. 1A); the target size for the A gene, which E1 must provide for A1, was apparently the same as the target size for infectivity. However, in E1 $\times$ A1_{UV}, A1's ability to complement E1 appeared much more resistant to UV irradiation than its infectivity; the target size for the E gene, which A1 must provide E1, was much smaller (approximately four times) than that for infectivity.

AV-WT could be substituted for either irradi-

FIG. 1. UV sensitivity of infectivity and complementing ability. (A) Infectivity remaining after irradiation of AV-WT (★), A1 (+), and E1 (×). (B) Yields from A1 (MOI = 5) $\times$ E1 (MOI = 1)_{UV} (○) and from E1 (MOI = 5) $\times$ A1 (MOI = 1)_{UV} (●). In this and all subsequent figures, the yields from single infections (Y_S) have been subtracted from the yields of multiple infections (Y_M) but are included (A_S ; E_S) on the left side of the figure (when they fall within the figure) to give an idea of background levels. When A_S or E_S values do not fall within the figure, they are designated $A_S \downarrow$ or $E_S \downarrow$.

FIG. 2. Yields of E1 and A1 from $A1 \times E1_{UV}$ and $E1 \times A1_{UV}$. Nonirradiated parent, MOI = 5; irradiated parent, MOI = 1. Large (E1) and small (A1) plaque components of yields were counted. E1 ($\square$) and A1 ($\circ$) plaque components of $A1 \times E1_{UV}$ yields; E1 ($\blacksquare$) and A1 ($\bullet$) plaque components of $E1 \times A1_{UV}$ yields; infectivity remaining after irradiation of A1 (+) or E1 ($\times$). Fraction of each mutant in nonirradiated $A1 \times E1$ yield is 1.0.

ated A1 or irradiated E1 in these crosses (not shown). The difficulty with using AV-WT is that high single-infection yields at low UV doses must be subtracted from the mixed-infection yields. The *ts* mutants did not have this problem since they produced little background yield at nonpermissive temperature.

Relative yields of A1 and E1. The yields from $A1 \times E1_{UV}$ and $E1 \times A1_{UV}$ could be further analyzed by plaque size to determine which mutant was produced under these conditions. In Fig. 2, the A1 and E1 components of the yields are compared. In the $A1 \times E1_{UV}$ yield, both A1 and E1 declined at similar rates. This indicates that only those A1-infected cells coinfecting with surviving infectious E1 were able to produce virus. In contrast, the E1 component of $E1 \times A1_{UV}$ yield decreased at a rate similar to that of the total yield (Fig. 1B), whereas the yield of A1 declined at a rate similar to that of its remaining input infectivity, indicating that it was not repaired to an infectious state at a detectable rate even though it was able to complement.

Analysis of $E1 \times A1_{UV}$ yield for A1-type complementing activity. Infectious A1 quickly disappeared from the yield of $E1 \times A1_{UV}$ with increasing irradiation. In this circumstance $A1_{UV}$ might conceivably be able to replicate, producing noninfectious virions. These noninfectious A1 virions might then be able to complement E1 as had the parental irradiated A1 and would thus potentially be detected by this complementing activity. Cells were coinfecting with yields from $E1 \times A1_{UV}$ and either A1 or E1. Within the $E1 \times A1_{UV}$ yield, the relative representation of E1 complementing ability increased, whereas the representation of A1 complementing ability decreased, at a rate similar to that of the inactivation of input infectivity (Table 2). Therefore, the only A1-type complementing activity present in these yields was the yield from the remaining infectious A1.

Viral protein synthesis in infected cells. To determine whether the relative yields from $A1 \times E1_{UV}$ - and $E1 \times A1_{UV}$ -infected cells reflected cellular events, viral protein synthesis was analyzed in these cells. An experiment similar to that in Fig. 1B was performed (Fig. 3A). These same cells were labeled with [35 S]methionine for 30 min immediately after removal of the yield media. The cell lysates were displayed by SDS-PAGE, and viral polypeptides NP plus P and M were quantified from the autoradiograms (Fig. 3B). The rate of inactivation of virus-specific protein synthesis was similar to the rate of inactivation of yield, indicating complementation at the synthetic level.

Relative contribution of gene products from irradiated and nonirradiated parents. Since A1 and E1 polypeptides have identical migration rates on SDS-PAGE, the experiment shown in Fig. 3 provided information on total viral proteins synthesized in these cells, but not the relative contributions of each parent. However, B1 Δ P, a recombined *ts* mutant B1 with a non-*ts* mutation affecting the migration of the P protein

TABLE 2. A1-type complementation activity in $E1 \times A1_{UV}$ yields

Yield from ^a :	Complemented with ^b :			
	A1		E1	
	PFU/ml	%	PFU/ml	%
$E1 \times A1_0$	1.3×10^3	100	7.8×10^2	100
$E1 \times A1_{0.32}^c$	2.7×10^3	207	1.8×10^2	23
$E1 \times A1_{0.64}^d$	2.4×10^3	184	10	1.2

^a Input = 8.8×10^3 PFU; MOI = 0.01.

^b MOI = 1.

^c Yield from $E1 \times A1_{UV}$ where A1 was UV irradiated for 0.32 min.

^d Yield from $E1 \times A1_{UV}$ where A1 was UV irradiated for 0.64 min.

FIG. 3. Comparative effects of UV irradiation of one parent on yields and viral protein synthesis in cultures multiply infected with A1 and E1. (A) UV inactivation of A1 (+) and E1 (x) infectivity. Yield of A1 (MOI = 5) x E1 (MOI = 1)_{UV} (O) and yield of E1 (MOI = 5) x A1 (MOI = 1)_{UV} (●). (B) Relative amount of viral polypeptides NP + P and M made in the same cells in the 30-min period after removal of the yield medium at 9.5 h postinfection. Proteins were quantified from electropherograms of polyacrylamide gels as described in Materials and Methods. From A1 x E1_{UV}: NP + P (◇), M (□). From E1 x A1_{UV}: NP + P (◆), M (■).

(Fig. 4), was used as the irradiated parent in an experiment like that shown in Fig. 3. Just as AV-WT could substitute for the irradiated parent, so too could B1ΔP, with the advantage that the inactivation rate of one protein, ΔP, could be measured. B1ΔP's ability to complement either A1 or E1 (Fig. 5A) was similar to that of the irradiated parents in Fig. 3A. Viral proteins synthesized in A1 x B1ΔP_{UV}-infected cells were inactivated at the same rate as A1 x E1_{UV} proteins or infectivity. However, in E1 x B1ΔP_{UV}-infected cells, viral protein synthesis declined at a rate similar to that of E1 x A1_{UV}

with the exception of ΔP. The synthesis of ΔP was inactivated at a rate similar to the rate of inactivation of infectivity. Since proteins labeled under these conditions represent products from amplified transcripts (products from primary transcripts are not detectable under the low-MOI conditions used here; M. E. Peebles and M. A. Bratt, unpublished data), these results are consistent with the ΔP being produced only from spared, infectious B1ΔP genomes. The UV inactivation rate of replication (and subsequent secondary transcription resulting in protein synthesis) appeared to be similar to that of infectivity. By this criterion, UV-irradiated B1ΔP is unable to replicate and amplify its transcription and translation, even though all the replication machinery necessary to replicate E1, and therefore presumably B1ΔP, is present.

Complementation target size. Table 3 presents D^0 values, the amount of UV irradiation required to reduce activity to 37% survival, calculated from inactivation curves similar to those in Fig. 1B. These values are compared with those previously calculated by Collins et al. (9) for the gene target sizes. The target size of complementing ability in A1 x E1_{UV} was similar to that of infectivity and the *L* gene. The target size of complementing ability in E1 x A1_{UV} was similar to that of the *P* gene. These results are most consistent with A1 providing the *P* gene product to E1 and are compatible with E1 providing the *L* gene product to A1.

Effects of high UV dose on yield from E1 x A1_{UV}. Inactivation of the ability of A1_{UV} to complement E1 appeared to be quite linear over the dose range examined (up to 1.3 min). To

FIG. 4. Migrational difference in P protein from mutant B1ΔP. Autoradiogram of 10% SDS-PAGE of AV-WT- and B1ΔP-infected cells labeled for 30 min with [³⁵S]methionine (20 μCi/ml) at 9.5 h postinfection.

Fig. 5. Yield and viral protein synthesis in cultures multiply infected with either A1 or E1 (MOI = 5) and B1ΔP (MOI = 1)_{UV}. (A) UV inactivation of B1ΔP infectivity (†). Yield of A1 × B1ΔP_{UV} (○) and E1 × B1ΔP (●). (B) Relative amount of viral proteins as in Fig. 5. From A1 × B1 P_{UV}: NP + P (◇), M (□). From E1 × B1ΔP_{UV}: NP + P (◆), M (■), and ΔP (▲).

determine whether this linear relationship continued at higher doses of UV radiation, A1 was irradiated up to 10.2 min, and the yield of E1 × A1_{UV} was examined. The inactivation of A1's complementing ability became resistant (the slope decreased) between 3 and 5 min and remained resistant through 10 min (Fig. 6). This resistance, as Deutsch and Brun (14) have suggested from their VSV studies, represents a UV-resistant target, possibly a protein. If this is true, increasing the MOI of A1_{UV} will increase the input protein and consequently increase the level of the UV-resistant plateau. In Fig. 6, A1_{UV} at an MOI of 25 resulted in a plateau level approximately 30-fold higher than an MOI of 1. It thus appears that the protein required for E1 could dissociate from A1_{UV} and associate with E1.

DISCUSSION

Replication. Even in the presence of complementing genomes, UV-irradiated genomes do not appear to be replicated (or repaired): (i) with increasing UV irradiation, the representation of infectious A1 or A1-type complementing activity in E1 × A1_{UV} yields decreases at the same rate as A1 input infectivity; and (ii) The amount of the ΔP marker protein synthesized from amplified genomes in E1 × B1ΔP_{UV} infected cells decreases with UV irradiation at the same rate as infectivity, whereas the other viral proteins decrease at a rate similar to the yield rate. A UV lesion appears to inactivate the ability of the B1ΔP genome to replicate and amplify viral protein synthesis and particle production even though all of the requirements for replication are present, since E1 does replicate. (This would not be the case with irradiated AV-WT alone or a *ts* mutant alone at permissive temperature, since a UV lesion would prevent complete transcription. As a result, the genome would not be supplied with all of the polypeptides required for replication.) Therefore, not only is transcription blocked by UV irradiation (7, 9), it is now clear that replication is also blocked by UV irradiation.

A gene. Several pieces of evidence suggest that the A gene codes for the L protein. (i) The L gene is the largest NDV gene and by target theory should represent the largest group of mutants, which it does (42). (ii) The noncytopathic (nc) mutants of NDV, which are uniformly deficient in RNA synthesis and accumulation of the L protein in infected cells (33), will complement E1, but not group A mutants, for RNA synthesis (34). Thus, the nc mutants appear to share defects in the L polypeptide and the A gene. (iii) The results of the UV-complementation experiments described here are also consistent with the A gene coding for the L protein. The UV target size for the A gene is the entire genome, similar to the conclusion of Col-

TABLE 3. Comparison of target sizes for complementation with target sizes for genes

Determination	D^0 (erg/mm ²) ^a	Determination	D^0 (erg/mm ²) ^b
Infectivity	91 ± 7.6	Infectivity	91
A1 × E1 _{UV}	104 ± 5.8	L	91
		HN	156
		M	267
		F	351
E1 × A1 _{UV}	416 ± 87	P	429
		NP	585

^a Average D^0 of infectivity assumed to be the same as that of Collins et al. (9). Averages of five to seven experiments.

^b From Collins et al. (9).

FIG. 6. High-dose UV irradiation and high-MOI effects on complementation. A1 was irradiated at various doses up to 10.2 min and used to infect, at a multiplicity of 1 or 25, cultures which were also infected with E1. E1 (MOI = 5) $\times$ A1 (MOI = 1)_{UV} ($\square$); E1 (MOI = 5) $\times$ A1 (MOI = 25)_{UV} ($\blacksquare$). Infectivity of A1 remaining after irradiation is also plotted (+).

lins et al. (9) concerning the *L* gene. However, it is possible that an entire genome target size might reflect a requirement for replication instead of a specific gene.

***E* gene.** After low-dose irradiation, both A1_{UV} and B1 Δ P_{UV} were able to make enough of the *E* gene product to complement the RNA-synthesizing step(s) of transcription or replication (or both) of E1. Their UV target size was most similar to that of the *P* gene. Because of the multiple steps involved in complementation, this target size may not be exact. The genes with the next-larger and next-smaller target sizes are *F* and *NP*, respectively (9). It is unlikely that the *E* gene would code for the *F* protein, since *F* is not involved in RNA synthesis. However, *NP* must be considered, since both *P* and *NP* are probably needed in RNA synthesis.

After high-dose irradiation, A1_{UV} still comple-

ments E1 in a UV-resistant, MOI-dependent manner. (The reverse is not true: E1_{UV} is unable to complement A1 in a UV-resistant way at a similar MOI; Peebles and Bratt, unpublished data.) The *E* gene product in the input A1_{UV} virions is probably this UV-resistant target and must, therefore, be able to dissociate from A1_{UV} in order to complement E1. The NP polypeptide is very firmly associated with the viral genomic RNA, an unlikely candidate for a diffusible agent. In fact, NP is the only virus polypeptide not removed from nucleocapsids by the high salt or in a CsCl gradient (37).

In addition, the *E* gene product must be required in very small amounts, perhaps enzymatically, since products from single genes (low-dose irradiation) and proteins from input virions (high-dose irradiation) provide enough for complementation. NP is required in large stoichiometric amounts to cover each 50S genome-sized RNA as it is produced in the cell. Requirements for *P* are not as clear, but much less *P* than NP is found in nucleocapsids, and the number of those which are functional or necessary is unclear. Furthermore, Deutsch et al. (15) found that only group II VSV mutants could be complemented by both a functional surviving gene and by a structural protein of UV-irradiated virus, similar to the E1 mutant of NDV described here. Group II mutants of VSV represent lesions in the NS gene (31, 35). Both the *P* polypeptide of NDV and the NS polypeptide of VSV are phosphorylated minor nucleocapsid-associated species (36, 40, 41), and both genes are penultimate to their promoters, just after the *NP* and *N* gene, respectively (1, 2, 9). It seems more likely, then, that the *E* gene of NDV represents the *P* protein, rather than the NP protein.

Multiplicity reactivation. Complementation by a low MOI of irradiated virus was due only to the infectious virions present before irradiation, since both the A1 $\times$ E1_{UV} and the E1 $\times$ A1_{UV} curves were straight lines intercepting the ordinate at 1.0. Increasing the MOI of the irradiated virus to 5 resulted in an apparent multihit curve with an extrapolated ordinate intercept of approximately 5 (data not shown). This reflects the initial infection of an average five infectious virions per cell and the survival of at least one virion per cell at low doses of irradiation. In complementation experiments in which the MOI of one parent was progressively decreased below 1 by dilution instead of irradiation, yields decreased with single-hit kinetics. The decrease precisely mimicked the UV inactivation of infectivity, regardless of which parent was diluted (data not presented). The single-hit kinetics again imply that a single infectious virion, and only a single infectious virion, is required for complementation.

Drake (16) and Kirvaitis and Simon (29) have demonstrated that high-MOI infections with UV-irradiated NDV result in more infected cells or a greater yield than expected from surviving infectivity. Recombination cannot explain this phenomenon, since it has not been found for paramyxoviruses (reviewed by Bratt and Hightower [4]). It is also unlikely that two viruses lethally irradiated in different parts of their genome could complement each other, producing complementing heterozygotes, since NDV is a single transcription unit: a lesion anywhere would destroy downstream genes (9). Potentially infectious but non-plaque-forming virus was detected by Granoff (20, 22). We have shown here that input virions can supply a UV-resistant function, probably a protein, possibly the P protein, resulting in complementation of an NDV mutant. This process was especially obvious when a high MOI of highly inactivated virus was used. It has previously been demonstrated that VSV proteins from high MOIs of highly UV-irradiated virus can complement *ts* mutants (12, 13, 15). These findings support the Bratt and Hightower (4) hypothesis: a lethally irradiated virus might be able to provide a necessary function to a virion which is noninfectious due to defective protein packaging rather than a defective genome. If this were the case, the inactivation of infectivity might be a deceptively low estimate of the remaining potentially infectious virus.

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