Relationship Between the Ribonucleic Acid-Synthesizing Capacity of Ultraviolet-Irradiated Newcastle Disease Virus and Its Ability to Induce Interferon

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Ultraviolet (UV)-irradiated Newcastle disease virus which has lost its infectivity but has the capacity to induce interferon also has the capacity to induce ribonucleic acid (RNA) synthesis both in vitro and early in infection in vivo. With large doses of UV irradiation, RNA-synthesizing capacity and interferon-inducing capacity are lost in parallel. Limited amounts of base-paired RNA associated with a transcriptive intermediate are involved in this RNA synthesis. These findings suggest the possibility that the single-stranded RNA of the UV-irradiated virus induces interferon by serving as a template for the synthesis of base-paired RNA. UV irradiation of the virus breaks down viral RNA but at a rate which is too slow to be a major cause of the loss of RNA-synthesizing capacity. Evidence is presented which suggests that less of the template RNA of the UV-irradiated virus is copied and that the product which is synthesized is smaller than that synthesized by nonirradiated virus.

In recent years there has been increasing interest in the question of whether ultraviolet (UV)-inactivated Newcastle disease virus (NDV) has the ability to induce virus-specific ribonucleic acid (RNA) synthesis. Interest in this otherwise esoteric question is based on two observations related to interferon induction. The first is that NDV is a good inducer of interferon in cultured cells of its natural host (chick embryo cells) only after UV irradiation of the virus (8, 17, 24); the second is that double-stranded RNA is the best exogenous inducer of interferon (9, 10, 16). These findings raised the question of whether the single-stranded RNA of the UV-irradiated virion can itself induce interferon (14). Alternatively, the crippled virus might possess the ability to induce the synthesis of base-paired RNA which could in turn serve as an interferon inducer (19).

Several studies on the RNA-synthesizing capacity of UV-irradiated NDV have been carried out, but with conflicting results (13, 14, 19). At the heart of this controversy is the small amount of base-paired, virus-specific RNA detectable in cells infected with infectious virus (5, 25) and the small amount of any type of virus-specific RNA

(single-stranded or base-paired) in cells infected by UV-irradiated virus (13, 14, 19).

We have chosen the more direct approach of analyzing the innate RNA-synthesizing capacity of UV-irradiated virus. This approach is now possible because the synthesis of RNA complementary to the template RNA of the virion, catalyzed by the RNA polymerase contained in the virion, can be measured in vitro (18). By using this system, we have asked whether UV-irradiated NDV possesses the ability to synthesize RNA and, in particular, base-paired RNA. In the course of these studies, we have also compared the effects of UV irradiation on viral infectivity, viral RNA, and interferon-inducing capacity. We also examined the products of the in vitro RNA polymerase reaction when UV-irradiated and nonirradiated virus were employed. As indicated in a preliminary publication (Clavell, Dore, and Bratt, *Bacteriol. Proc.*, p. 215, 1971), the results of these studies have allowed us to conclude that UV-irradiated NDV, capable of inducing interferon, possesses the ability to synthesize RNA and that with more extensive irradiation these properties are lost in parallel. Finally, by using the procedures described by Robinson (*Virology*, *in press*) for detecting virus-specific RNA synthesis

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within 90 min of Sendai virus infection, we have been able to detect NDV-specific RNA synthesis in cells early in infection by both UV-irradiated and nonirradiated virus. Although not providing conclusive proof, these results suggest the possibility that the single-stranded RNA of the UV-irradiated virus does not itself induce interferon but does so by serving as a template for the synthesis of limited amounts of base-paired RNA.

MATERIALS AND METHODS

Virus. Cloned NDV-HP (Israel, HP, 1935) was grown, concentrated, purified, and assayed for infectivity as previously described (3, 6; Clavell and Bratt, *in preparation*). The final stage of purification consisted of centrifugation through an isopycnic sucrose-deuterium oxide gradient (12, 18). All virus preparations contained 1.0 to 1.6 mg of NDV protein (21) and 10^{10} to 5×10^{10} plaque-forming units PFU/ml.

^{32}P -labeled virus was prepared in embryonated eggs as previously described (4, 12) and subjected to the same purification procedures.

Cells. Primary and secondary chick embryo cell cultures were prepared as previously described (3, 4).

RNA polymerase reaction. Reagents and procedures were similar to those described previously (18). Reaction mixtures contained the indicated concentrations of the following components: 50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 7.3; 5 mM magnesium acetate; 3 mM dithiothreitol (DTT); 0.1 M NaCl; 0.7 mM each of adenosine triphosphate (ATP), uridine triphosphate (UTP), and cytidine triphosphate (CTP); 0.01 mM ^3H -guanosine triphosphate (GTP; 1,160 counts per min per pmole); 0.8 mg of Triton N101 per ml; and 100 μg of viral protein per ml. Unlabeled triphosphates were obtained from P.L. Biochemicals, Inc., and ^3H -GTP was from New England Nuclear Corp.

In all cases, reaction mixtures were incubated at 32 C. Reactions were terminated either by cooling to 4 C with the addition of 5 volumes of 0.08 M sodium pyrophosphate or, in the experiments where the reaction products were analyzed by velocity sedimentation, by the addition of sodium dodecyl sulfate (SDS) to 1%. Samples were assayed for trichloroacetic acid-precipitable ^3H -guanosine monophosphate (GMP) either before or after further fractionation. This was accomplished by the addition of 1 mg of yeast RNA per ml as a carrier and the addition of trichloroacetic acid to 10%. Precipitated samples were collected on membrane filters (Millipore Corp., Bedford, Mass.), dried, and counted in a scintillation cocktail consisting of toluene and Omnifluor (New England Nuclear Corp.) in a Packard scintillation counter.

Intracellular RNA synthesis. All cultures were treated with 20 μg of actinomycin D (courtesy of Merck Sharpe & Dohme, Rahway, N.J.) per ml for 45 min. Some of the cultures were then infected at 4 C for 45 min with 200 PFU per cell of nonirradiated virus or an equivalent amount of virus which had been UV-irradiated for 3 min. After being washed, these cultures

and uninfected cultures were labeled in the presence of the same concentration of actinomycin D with 250 μCi of ^3H -uridine per ml (25 Ci/mmol, New England Nuclear Corp.). Half of the cultures were, in addition, treated with 50 μg of cycloheximide (Actidione, Calbiochem) per ml for 45 min prior to infection and during the whole incubation period. This amount of cycloheximide is sufficient to suppress protein synthesis under these conditions by more than 95%. After incubation for 2 or 6 hr, the cultures were harvested and their RNA was extracted in cold phenol (4).

RNA fractionation procedures. Samples were layered on 10 to 30% linear sucrose gradients (18) containing 0.5% SDS and centrifuged at 22 C for 3.5 hr at $180,000 \times g$ in a Spinco SW 41 rotor. Fractionation beginning with the top of the gradient was done with an ISCO fractionator (Instrumentation Specialties Co.) which measured optical densities. The fractionator was coupled with an ISCO fraction collector which allowed collection of 30 fractions from each 12-ml gradient. Samples were trichloroacetic acid-precipitated and counted as described above. Ribonuclease resistance was measured by incubating samples in a mixture of 50 μg of pancreatic ribonuclease (Schwarz BioResearch, Inc.) per ml and 25 units of T1 ribonuclease (Calbiochem) per ml at 37 C for 1 hr in $1 \times \text{SSC}$ (0.15 M NaCl; 0.015 M sodium citrate) containing 0.25% SDS.

Cold phenol-extracted (4) chick embryo cell RNA was always added as a marker. In some experiments, ^{32}P -labeled viral RNA species were also added as a marker. Reproducibility of the location of 4, 18, and 28S cellular RNA (not shown in the figures) and 50S viral RNA [Under the sedimentation conditions employed here, NDV RNA always sediments as a 50S rather than 57S molecule as previously described (4, 12).] was always within 0.5 fractions, which corresponds to approximately 1 S unit.

Interferon. Interferon was measured by yield reduction of vesicular stomatitis virus (VSV). Primary chick embryo cells were infected at a multiplicity equivalent to 5 PFU/cell with either nonirradiated or UV-irradiated NDV. Culture fluids were removed 24 hr later and, to inactivate any residual virus, they were heated to 60 C for 1 hr. These samples were then added to secondary cultures of chick embryo cells. After 18 hr, these cultures were infected at a multiplicity of 3 PFU/ml by the Indiana strain of VSV (courtesy of A. S. Huang). After 24 hr, culture fluids were analyzed for VSV yields by plaque formation. Yield reductions were calculated by dividing the yields of cultures treated with interferon by the yields from untreated cultures. Maximal yield reductions of 670-fold were obtained from fluids of cultures infected with NDV irradiated for between 40 sec and 3 min. The fraction of maximal interferon-inducing ability was calculated by dividing this number into the values for yield reduction at various times.

UV irradiation. A Sylvania germicidal lamp (G15T8) was used at a distance of 55 cm as previously described (7, 20). Under constant voltage, this provided radiation energy of 16 ergs per mm^2 per sec.

RESULTS

The experiment shown in Fig. 1 was done to determine whether UV-irradiated NDV has the ability to synthesize RNA *in vitro* and to compare the effects of UV irradiation on this activity with effects on infectivity and interferon-inducing capacity. Virus irradiated for increasing amounts of time was analyzed for these three properties. As previously described, inactivation of infectivity follows single-hit kinetics (7, 20). Under these conditions, 4 sec of irradiation is sufficient to reduce infectivity to 37% (the surviving fraction

FIG. 1. Comparison of the effects of UV irradiation on NDV infectivity, RNA-synthesizing capacity, and interferon-inducing capacity. RNA-synthesizing capacity was measured in two experiments during *in vitro* incubation for 1 hr. The fraction of maximum activity was obtained by dividing the radioactivity incorporated in reactions employing UV-irradiated virus by the amount incorporated in reactions employing nonirradiated virus. Radioactivity incorporated in a reaction mixture containing no virus was subtracted prior to calculation of these fractions. In one experiment, the value subtracted was 1.3% of that catalyzed by nonirradiated virus and 31% of that catalyzed by virus irradiated for 5 min. In the other experiment, the values were 1.4% for the nonirradiated virus and 20% for virus irradiated for 10 min.

which, according to the Poisson distribution, is obtained when an average of one lethal hit has been received by each infectious unit). The rate of inactivation of RNA-synthesizing capacity is much slower but also follows single-hit kinetics. Initially, 40 sec of irradiation is sufficient to reduce RNA-synthesizing capacity to 37%, but this rate of inactivation eventually slows down to less than one-tenth of this rate. As previously reported (8, 17, 24), interferon-inducing capacity initially increases, remains constant, and then is reduced with further irradiation. With periods of irradiation greater than 3 min, the interferon-inducing capacity appears to be reduced in parallel with RNA-synthesizing capacity.

RNA breakdown by UV light. Gandhi and Burke have suggested that the loss of interferon-inducing capacity found after extensive UV irradiation might be the result of breakage of the viral RNA (13). To determine whether breakage of viral RNA results from UV irradiation of the virus, ³²P-labeled NDV was subjected to UV irradiation for increasing periods of time. The viral RNA was then fractionated by velocity sedimentation (Fig. 2). The RNA of nonirradiated virus sediments as a sharp peak at 50S. The material at the top of the gradient probably consists of phospholipid or the 4S RNA found in various amounts in preparations of ³²P-labeled NDV (12). Increasing amounts of UV irradiation cause the viral RNA to sediment progressively more slowly than 50S. This suggests that the RNA is broken by UV irradiation, although the possibility that its slower sedimentation is only the result of alterations in secondary structure has not been ruled out.

The rate of conversion of 50S RNA to more slowly sedimenting species is plotted along with the effects of UV irradiation on RNA-synthesizing capacity and interferon-inducing capacity (Fig. 3). It is obvious that the rate of conversion is considerably slower (requiring almost 20 min for conversion of all but 37% of the 50S RNA to more slowly sedimenting species) than the rate of inactivation of the other properties. After 5 min of irradiation, 80% of the viral RNA still sediments as a 50S molecule, whereas only 10% of the RNA-synthesizing capacity remains. We conclude from this that, although UV irradiation may break down viral RNA, this rate of breakdown is too slow to be the major cause of the inactivation of either RNA-synthesizing capacity or interferon-inducing capacity.

Comparison of products from RNA polymerase reactions employing UV-irradiated and nonirradiated virus. The products and characteristics of the *in vitro* RNA polymerase reaction employing nonirradiated virus have been extensively ana-

FIG. 2. Breakdown of viral RNA by UV irradiation. NDV which had been ^{32}P -labeled was irradiated for various periods of time, solubilized by the addition of SDS to 1%, and fractionated by velocity sedimentation.

lyzed (Clavell, Dore, and Bratt, *in preparation*). Having shown that UV-irradiated virus also has the ability to synthesize RNA *in vitro*, it was therefore of interest to compare the products made by UV-irradiated virus with those made by nonirradiated virus.

Virus irradiated for increasing periods of time was used in the RNA polymerase reaction, and the products were fractionated by velocity sedimentation in sucrose gradients (Fig. 4). When nonirradiated virus is used, ^3H -GMP is incorporated into several species of RNA. These include a high-molecular-weight species, which in this case sediments around 42S, and a large amount of RNA which sediments at 16S. In addition, there appear to be significant amounts of trichloroacetic acid-precipitable tritiated material which sediments considerably slower than 16S. The nature of this slowly sedimenting material is unclear. It appears likely that at least some of it is an artifact of the procedures employed since even unincubated reaction mixtures contain some of this material. However, because at least some

FIG. 3. Comparison of the UV light-induced breakdown of viral RNA with loss of RNA-synthesizing capacity and interferon-inducing capacity. The data for RNA breakdown were taken from the experiments shown in Fig. 2. Values were determined by calculating the fraction of total counts which continued to sediment with S values greater than 40S after various periods of UV irradiation. No decrease in total counts was found. Other data were carried over from Fig. 1.

of this material remains trichloroacetic acid-precipitable, even after phenol extraction and two cycles of alcohol precipitation, the possibility that it is a significant product of the reaction cannot be eliminated.

Figure 4 also shows that UV irradiation of the virus markedly reduces the total amount of incorporation (as seen previously in Fig. 1) and alters the nature of the RNA species synthesized. The synthesis of the high-molecular-weight species is actually reduced by UV irradiation at about the same rate as total RNA synthesis (Fig. 1). Even after 5 min of irradiation, the high-molecular-weight species are still significantly above background, and the total amount of label incorporated into RNA species greater than 30S is about 8 to 10% of the amount incorporated in reactions employing nonirradiated virus. An additional effect of irradiation is that the high-

FIG. 4. Velocity sedimentation analysis of the *in vitro* reaction products using virus irradiated for 0 to 5 min. Incubation of the 0.5-ml reaction mixtures was carried out for 1 hr.

molecular-weight species from reactions employing virus irradiated for 3 and 5 min sediment around 50S rather than 42S as do the high-molecular-weight species from reactions employing nonirradiated virus.

Another effect of increasing UV irradiation is that the amount of 16S RNA synthesized decreases more rapidly than the synthesis of either total or high-molecular-weight RNA. The reduction in amount of 16S RNA synthesized appears to be accompanied by a shift to the synthesis of RNA species which are progressively smaller than 16S.

Kinetic studies on the RNA polymerase reaction employing nonirradiated virus have suggested that the high-molecular-weight product, which contains partially base-paired RNA, serves as a transcriptive intermediate for the synthesis of the 16S RNA (18; Clavell, Dore, and Bratt, *in preparation*). To characterize the species involved in the synthesis of RNA by UV-irradiated virus and to compare the kinetics of labeling of these species with those of nonirradiated virus, the experiment shown in Fig. 5 was done. Polymerase reactions employing nonirradiated virus and virus irradiated for 3 min were incubated for 30-

FIG. 5. Velocity sedimentation and ribonuclease-resistance analysis of 30- and 75-min reaction products employing nonirradiated and UV-irradiated virus. ^{32}P -labeled viral RNA ($\circ$) was added as a marker. After fractionation, samples were divided in two; prior to trichloroacetic acid precipitation, one portion of each was treated with ribonuclease. Counts given are for total trichloroacetic acid-precipitable ^3H counts/minute ($\bullet$) and ribonuclease-resistant ^3H counts/minute ($\blacktriangledown$).

and 75-min periods. Panels 5A and B show the results obtained for nonirradiated virus incubated for 30 and 75 min, respectively. It can be seen that the transcriptive intermediate increases by a factor of 2 between 30 and 75 min, but it sediments in each case around 42S, which is considerably slower than the added marker of ^{32}P -labeled 50S viral RNA. During the period between 30 and 75 min, the intermediate appears to decrease in ribonuclease resistance from 90 to 75%. During this period, the 16S RNA increases by a factor of 5 while remaining almost completely ribonuclease sensitive.

The products of 30- and 75-min reactions employing virus irradiated for 3 min are shown in panels 5C and D, respectively. Again, it can be seen that the total amount of RNA synthesized is considerably less than that synthesized by nonirradiated virus, that synthesis of 16S RNA has been almost completely eliminated, and that concomitantly there has been a shift to the synthesis of more slowly sedimenting species. It can also be seen that, during the period between 30 and 75 min, the transcriptive intermediate appears to

change only from complete to 90% ribonuclease resistance. In addition, it can be seen that the transcriptive intermediate has not shifted in *S* value but instead continues to sediment in the 50S region with the marker of ^{32}P -labeled RNA extracted from virions. This failure of the transcriptive intermediate of UV-irradiated virus to decrease in sedimentation constant suggests that less of the template is being copied or that less of the newly synthesized RNA complementary to the template remains attached to it.

In vivo RNA synthesis. The fact that UV-irradiated NDV has a virion polymerase which has the ability to synthesize RNA *in vitro* suggested the possibility that similar virus-specific RNA synthesis, perhaps also catalyzed by the virion polymerase, might occur after infection of chick embryo cells. Robinson has recently shown that such virus-specific RNA can be detected within the first 90 min of infection by nonirradiated Sendai virus (Robinson, Virology, *in press*). Most of the RNA synthesized within this period is probably catalyzed by the polymerase which has recently been shown to be contained in Sendai virions (22, 23) since much of this RNA synthesis can occur in cells treated with the inhibitor of protein synthesis, cycloheximide.

The experiment illustrated in Fig. 6 shows that NDV-specific RNA synthesis can be detected early in infection by both nonirradiated and UV-irradiated virus in untreated and cycloheximide-treated cells. Panel A shows the pattern obtained when cells infected with nonirradiated virus are labeled between 0 and 6 hr after infection with

^3H -uridine in the presence of 20 μg of actinomycin D per ml and in the presence and absence of cycloheximide. As in the case of Sendai virus infection (Robinson, Virology, *in press*), NDV-specific RNA synthesized during infection in the presence of cycloheximide is limited to those RNA species which are complementary to, and sediment slower than, the 50S RNA of the virus (4). In the absence of cycloheximide, however, all of those species normally seen late in infection (4) are found. Similar results, but with lower amounts of ^3H -uridine incorporated, were obtained when RNA was extracted 2 hr after infection of similarly treated cells.

Panel 6B also shows that during the labeling period of from 0 to 2 hr ^3H -uridine incorporation significantly above background has occurred in cycloheximide-treated cells infected with UV-irradiated virus. An identical pattern was found when no cycloheximide was present. Panel 6C contains data for untreated and cycloheximide-treated cells labeled from 0 to 6 hr after infection by UV-irradiated virus. The labeling pattern and total ^3H -uridine incorporation in these cultures is practically identical to that seen in panel B for a 2-hr labeling period. The fact that the patterns obtained with labeling periods of 0 to 2 hr and 0 to 6 hr suggests either that this RNA is turning over or that incorporation is essentially complete within 2 hr. Preliminary experiments support the latter hypothesis. Thus, UV-irradiated virus is capable of synthesizing virus-specific RNA early in infection. That the patterns obtained in the presence and absence of cycloheximide are almost

FIG. 6. RNA synthesis early in infection by nonirradiated and UV-irradiated NDV in the presence and absence of cycloheximide. In all cases, ^{32}P -labeled viral RNA was added as a marker ($\circ$). (A) Cultures infected with non-irradiated NDV and labeled between 0 and 6 hr postinfection in the presence ($\blacksquare$) and absence ($\bullet$) of cycloheximide. (B) Uninfected cultures labeled for 6 hr in the presence of cycloheximide ($\blacktriangle$) and a similarly treated culture labeled between 0 and 2 hr after infection with virus irradiated for 3 min ($\blacksquare$). (C) Cultures labeled in the presence ($\blacksquare$) and absence ($\bullet$) of cycloheximide between 0 and 6 hr after infection with virus irradiated for 3 min.

identical strongly suggests that all of this ^3H -uridine incorporation is catalyzed by the virion polymerase brought in at the time of infection.

DISCUSSION

The results presented here indicate that UV-inactivated NDV loses its infectivity much more rapidly than its ability to synthesize RNA and that UV-irradiated virus which is capable of inducing interferon still possesses the ability to synthesize RNA *in vitro* and to induce RNA synthesis in infected cells as well.

Previous studies were unable to resolve the question of whether UV-irradiated virus induces RNA synthesis in infected cells (13, 14, 19). As stated in the introduction, a major barrier was the low level of detectability in this system. In addition, in each of the earlier studies [including the one which reported detection of NDV-specific RNA in cells infected with UV-irradiated virus (19) and the others which were unable to detect any (13, 14)], the time chosen for observation was in the range of 4 to 7 hr postinfection. Although this is clearly the best time for detecting NDV-induced RNA synthesis in normal infection (5, 15), it might be predicted that if UV-irradiated virus were to induce any RNA synthesis it would do so early in infection. We have now been able to demonstrate that such synthesis does occur within 2 hr of infection. Although this RNA is consistently and significantly above background, it is still near the threshold of detectability. For this reason, it has been impossible so far to analyze this material in detail and to determine whether any of it is base-paired. It seems likely, however, that some base-paired RNA is involved in this early RNA synthesis as it is in the *in vitro* reaction and in NDV-specific RNA synthesis late in infection (5).

It is clear that the synthesis of RNA *in vitro* is catalyzed by the polymerase contained in the virus particle, which has been shown previously to catalyze the synthesis of RNA complementary to the template RNA of the virion (18). The finding that ^3H -uridine is incorporated into RNA early in infection by UV-irradiated virus, and that this incorporation occurs even in the absence of protein synthesis, suggests that this incorporation is also catalyzed by the virion polymerase. Although there is as yet no conclusive proof of an absolute requirement for the action of the virion polymerase early in normal infection, the arguments which led to the search for such enzymes (1, 18) provide a compelling hypothesis for such a requirement.

Nevertheless, the presence of this polymerase in the virion, its ability to function both *in vitro* and *in vivo*, and the parallel loss of RNA-syn-

thesizing capacity and interferon-inducing capacity raise doubts about the conclusion that the single-stranded RNA of UV-inactivated NDV is the inducer of interferon when this virus is introduced into cultured chick embryo cells (14), a conclusion based on inability to detect virus-specific RNA synthesis after infection by UV-irradiated virus. The same doubts must also be raised about the conclusion that the single-stranded RNA of the virion is the interferon inducer when NDV infects L cells (2, 11), for, in that system also, the virion polymerase may function early even though no viral replication occurs. Identical doubts were raised by Friedman almost 2 years before the existence of the NDV polymerase was known (R. M. Friedman, in the discussion of reference 2). Therefore, the finding that UV-irradiated virus possesses the ability to synthesize RNA and that, as in the case of non-irradiated virus, this synthesis is mediated through a transcriptive intermediate which is partially base-paired supports the hypothesis of Huppert et al. (19) that the single-stranded RNA of the UV-irradiated virus may serve as a template for the synthesis of base-paired RNA which, in turn, might serve as an interferon inducer.

Our one reservation lies in the question of the extent to which the transcriptive intermediate is base-paired *in vivo*. Preliminary evidence with VSV suggested that much of the apparent ribonuclease resistance of the *in vitro* transcriptive intermediate of that virus may be an artifact of the use of SDS (Alice Huang, *personal communication*). Preliminary results with NDV in our own laboratory suggest that not only transient SDS treatment, but also the process of alcohol precipitation, greatly enhances the ribonuclease resistance of this species. We have found that the *in vitro* transcriptive intermediates of both non-irradiated and UV-irradiated virus undergo this enhancement of ribonuclease resistance. It is clear, however, that these intermediates produced *in vitro* have at least a limited amount of ribonuclease resistance which is attributable to naturally occurring base pairing. On the addition of either alcohol or SDS, this becomes much more extensive. Since we do not know the nature of the structure of the transcriptive complex, which may still be associated with the nucleocapsid in the *in vitro* reaction (22), nor whether a similar association occurs *in vivo*, no final conclusions can be made about the extent of base pairing *in vivo*. Nor do we understand the role of treatment with SDS or alcohol in increasing the ribonuclease resistance of the *in vitro* intermediate. The possibility is being considered that these treatments, which are known to denature protein, increase ribonuclease resistance by enhancing base pairing

through the removal of proteins which normally prevent such pairing. In spite of these reservations, it is possible that even a limited amount of base-paired RNA *in vivo* could be enough to serve as an inducer of interferon.

If both nonirradiated and UV-irradiated virus are capable of inducing the synthesis of a limited amount of base-paired RNA which in turn may be required for interferon induction, we are left with the still unanswered question of why the virus must be inactivated to induce interferon in this system. The principal argument in the past has been that the virulence of NDV infection and the inhibition of host cell macromolecular synthesis prevent the virus from inducing this host-coded protein—interferon. However, the fact that macromolecular synthesis is not usually inhibited until around 8 hr after infection (5) and that even avirulent strains of NDV must be UV-inactivated to induce interferon (8, 24) raises doubts about this explanation. Alternatively, however, it must be recognized that the nature of the RNA species synthesized by UV-irradiated virus is indeed different from those induced by nonirradiated virus and that protein presumably coded for by these presumptive messengers (4) may be altered or not synthesized as a result of irradiation. This could render the virus incapable of inducing those changes in the cell which either specifically or nonspecifically prevent interferon induction.

The mechanism by which UV irradiation destroys RNA-synthesizing capacity is not yet known. It seems unlikely, however, that the primary effect is directly on the polymerase since UV irradiation of the virus not only reduces RNA-synthesizing capacity but also radically alters the products which are synthesized. This strongly suggests that the major effect is on the template RNA of the virion rather than on the enzyme itself. Potential effects on the template include breakage, alteration of secondary structure, or changes in nucleotide bases. The demonstration that the RNA of the virus sediments more and more slowly with increasing amounts of UV irradiation suggests that breakage does, in fact, take place. However, the rate of conversion of 50S RNA to smaller species is considerably slower than the rate of inactivation of RNA-synthesizing capacity. Thus, although breakage of the RNA may play a role, this is not the principal cause of the loss of RNA-synthesizing capacity. It seems more likely that inactivation of RNA-synthesizing capacity results mainly from changes in the nucleotide bases of the RNA which reduce its ability to serve as a template for the polymerase. Consistent with this interpretation is the finding that the transcriptive intermediate formed by UV-irradiated virus appears to have less comple-

mentary RNA associated with it as indicated by the failure of these transcriptive intermediates to sediment more slowly than the single-stranded RNA of the virus. The fact that the products released from these transcriptive intermediates are smaller than the 16S molecules released from the intermediates for nonirradiated virus also suggests that smaller regions are being copied.

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ADDENDUM IN PROOF

A. Meager and D. C. Burke (*personal communication*) have found similar effects of UV irradiation on virion RNA and on RNA-synthesizing capacity of NDV.

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