JOURNAL OF VIROLOGY, July 1971, p. 74-80
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Vol. 8, No. 1
Printed in U.S.A.

Transcription of the Influenza Ribonucleic Acid Genome by a Virion Polymerase

II. Nature of the In Vitro Polymerase Product

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Received for publication 12 April 1971

The properties of the ribonucleic acid (RNA) synthesized by the influenza (WSN) virion polymerase have been investigated. Most of the product RNA is synthesized in association with virion RNA species from which it can be released by heat treatment as single-stranded, ribonuclease-sensitive polynucleotides (average molecular weight, 2-hr sample, about 10^5 daltons). At least 95% of the product is complementary to the viral RNA species. On the basis of the molar ratio of the RNA species isolated from a ^{3}H-uridine-labeled virus preparation, it was calculated that the WSN genome consists of seven pieces of RNA with a sum molecular weight of about 5×10^6 daltons.

Animal ribonucleic acid (RNA) viruses which possess virion-associated polymerase enzymes represent virus groups whose members lack an infectious virion RNA (1, 2, 7, 10, 13, 17, 18). If the chief function of an RNA-dependent RNA polymerase recently detected in several myxovirus strains (7) is to make RNA species which can initiate the process of infection (such as messenger RNA), then one might expect some of the polymerase product RNA to be complementary to the virion genome.

Having recently established optimal conditions for demonstrating in vitro activity of the influenza polymerase (5), we undertook the present study to answer the following questions. (i) Is the product RNA associated with the virion RNA? (ii) Do the product species represent complementary, identical, or irrelevant polynucleotide sequences in relation to the virion RNA? (iii) What is the time-course of product synthesis and is the viral genome conserved during the polymerase activity?

To answer these questions, kinetic experiments were set up employing ^{3}H-uridine-labeled virus to follow the fate of the viral RNA and α-^{32}P-uridine triphosphate (UTP) to monitor product synthesis. Suitable annealing tests were employed to investigate the properties of the product RNA. In addition, data will be presented showing that the size of the influenza genome has been previously underestimated.

MATERIALS AND METHODS

Viruses and cells. Preparation of ^{3}H-uridine-labeled influenza WSN (A_0) virus from primary chick embryo fibroblasts has been described previously (5).

Enzyme reaction conditions. The conditions for monitoring the synthesis of ^{32}P-product RNA by ^{3}H-uridine-labeled influenza WSN virus preparations have been described in an accompanying paper (5). Reaction mixtures contained α-^{32}P-UTP (8 mCi per μmole) to label the product species.

Purification of virion and product RNA from reaction mixtures. The procedures employed for isolating ^{3}H-labeled viral RNA and ^{32}P-labeled product RNA from reaction mixtures have been described (Bishop and Roy, J. Mol. Biol., *in press*). The procedure involved termination of the reaction by addition of sodium dodecyl sulfate (SDS), phenol extraction, passage through Sephadex G-50 to remove triphosphates, and two alcohol precipitations to remove all traces of SDS. The RNA was finally suspended in 200 μliters of 0.01 M sodium phosphate buffer, 0.005 M ethylenediaminetetraacetic acid (EDTA), (*p*H 7.0) at a concentration of approximately 12 μg per ml. This concentration was determined from the ^{3}H label assuming that the viral RNA represents about 0.9% of the viral particle weight (6).

Melting and annealing conditions. RNA samples (10 μliter) were diluted 10-fold in the phosphate buffer described above, melted by heating at 100 C in a boiling water bath for 40 sec, and fast-cooled in a dry ice-methanol mixture. In particular experiments, the RNA was not diluted, prior to melting (*see below*). For annealing, the salt concentration of an RNA sample (20 μliter) was adjusted to 0.4 M LiCl, and the mixture was incubated at 65 C for 4 hr.

Test for ribonuclease resistance. RNA samples were diluted into 1 ml of 0.4 M LiCl, 0.01 M tris(hydroxymethyl)amminomethane (Tris)-hydrochloride buffer (*p*H 7.4) containing 10 μg of ribonuclease T_1 and 10 μg of ribonuclease A and incubated for 30 min at 37 C prior to determining the acid-insoluble radioactivity.

Gel electrophoresis. Gel electrophoresis was performed in swollen 3.2% polyacrylamide gels by using Tris-acetate buffer containing 0.1% SDS (4). Resolution of viral RNA species by gel electrophoresis was accomplished by combining 50 μliters of a ^{3}H-uridine-labeled influenza WSN virus preparation (3.5 mg of protein per ml) with 20 μliters of glycerol, 10 μliters of 10% SDS, and 2 μliters of ^{32}P-labeled BHK-21 cellular RNA (containing mostly 28 and 18*S* ribosomal RNA). After loading the mixture directly onto the gel surface, the RNA was subjected to electrophoresis for 3 hr at 50 v and 10 mamp. After electrophoresis, the gel was frozen, sliced in 0.5-mm sections (for the first 3 cm) or 1-mm sections (for the next 3 cm), and the slices were dissolved in 0.5 ml of 30% (v/v) H_2O_2 and counted in a liquid scintillation counter with Aquasol (New England Nuclear Corp., Boston, Mass.). The ^{32}P-labeled BHK-21 RNA was isolated by 1.5 M LiCl precipitation of the single-stranded RNA present in the aqueous phase of a phenol-cresol extract of BHK-21 cells grown in the presence of 100 μCi of ^{32}P-phosphoric acid per ml of medium (15).

Gel electrophoresis of reaction product RNA (^{3}H- and ^{32}P-labeled RNA species) was performed similarly except that the ^{32}P-BHK-21 cellular RNA was omitted.

RESULTS

Kinetics of product synthesis by influenza WSN viral polymerase. In examining the kinetics of in vitro product synthesis by a viral polymerase, it is informative to determine whether the viral (template) RNA is conserved intact or broken down into acid-soluble nucleotides during the course of the incubation. To answer this question, ^{3}H-uridine-labeled influenza WSN virus was used to prime a reaction in which α-^{32}P-UTP was employed to label the product synthesized. The linear incorporation of ^{32}P-uridine monophosphate (UMP) into product RNA during a 2-hr incubation period is shown in Fig. 1 where it can also be observed that the ^{3}H label was fully conserved in an acid-insoluble form. The preservation of acid-insoluble label justifies an earlier procedure we have employed to compensate for 5 to 15% sampling errors (viz., normalizing ^{32}P-product synthesis to a constant ^{3}H recovery; reference 5).

Gel electrophoresis of the RNA isolated from WSN virus. A ^{3}H-uridine-labeled preparation of influenza WSN virus was treated with SDS, mixed with ^{32}P-labeled BHK-21 cellular RNA, and subjected to electrophoresis in a swollen

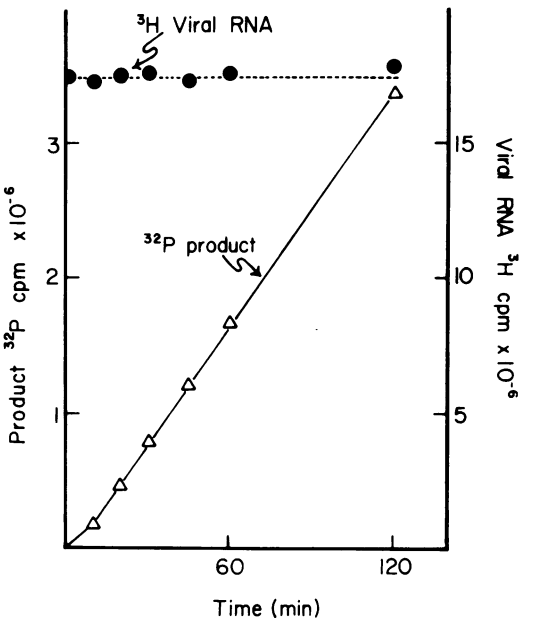

FIG. 1. *Kinetics of product synthesis by influenza (WSN) viral polymerase. A 100-fold standard influenza WSN polymerase reaction, 12.5 ml (5) containing α-^{32}P-UTP to label the product species and ^{3}H-uridine-labeled virus, was incubated at 31 C through 2 hr. Samples (50 μliter) were withdrawn at the indicated intervals to determine the acid-soluble radioactivity, expressed as the radioactivity per 12.5 ml of reaction mixture.*

3.2% polyacrylamide gel (*see above*). Five main peaks of the ^{3}H label were resolved (Fig. 2). Assuming that the ^{32}P-ribosomal RNA markers had molecular weights of 1.75×10^6 and 0.7×10^6 daltons (11), the molecular weights of the five ^{3}H peaks were determined: (from left to right) 1.05×10^6, 8.2×10^5, 7.0×10^5, 5.8×10^5, and 3.5×10^5 daltons, respectively. To determine the molar ratio of these RNA species, the sum of the ^{3}H label under each peak was computed and divided by their respective molecular weights. The ratio obtained was 2.0:0.9:1:2.2. Since the RNA was labeled with ^{3}H-uridine, one might expect some variance between the base-ratios of the various species; however, despite this, the molar ratio analysis suggests that there are probably seven species of RNA in the virus particle with a sum molecular weight of 4.9×10^6 daltons.

Gel electrophoresis of the RNA isolated from WSN polymerase reactions. A 100-fold standard polymerase reaction (12.5 ml), containing α-^{32}P-UTP to label the product species and ^{3}H-uridine-labeled virus to follow the fate of the viral RNA, was incubated through 2 hr at 31 C

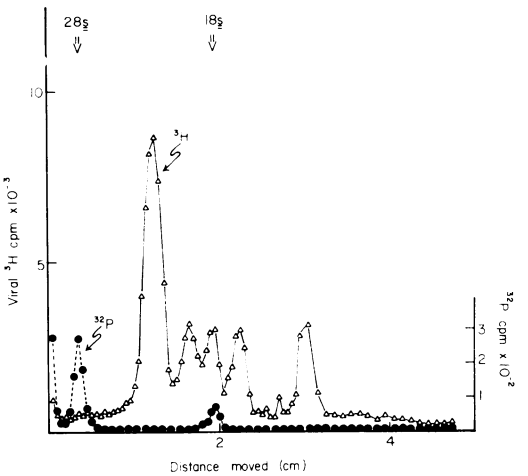

FIG. 2. *Gel electrophoresis of the RNA isolated from influenza (WSN) virus. The RNA-liberated by SDS treatment of complete influenza WSN virions, was subjected directly to gel electrophoresis in swollen 3.2% polyacrylamide gels (4) as described in the text. Cellular ^{32}P-labeled BHK-21 RNA was included as marker species.*

(*see above*). Portions were withdrawn at intervals and purified for RNA. A sample of each RNA was subjected to gel electrophoresis in swollen 3.2% polyacrylamide gels to monitor the distribution of both the ^{32}P and ^{3}H labels (Fig. 3).

Comparison of the profiles obtained indicated that the ^{3}H-viral RNA was not substantially degraded throughout the 2 hr of incubation, and all five RNA peaks could be clearly distinguished. The 0-min sample (not shown) which had been extracted with phenol, gave a profile of ^{3}H label similar to that shown in Fig. 2. Only the 120-min sample showed any significant evidence of ^{3}H label in small-molecular-weight RNA species. Throughout the time-course, there was no evidence of substantial involvement of ^{3}H label in slower moving complexes (*see below*) in contrast to results obtained previously with vesicular stomatitis virus (VSV) viral polymerase (3; Bishop and Roy, J. Mol. Biol., *in press*).

Comparison of the distribution of ^{32}P-product in the gels throughout the kinetic experiment indicated that product RNA was in the same area of the gels where most of the viral ^{3}H-RNA species were present. Almost all of the ^{32}P label was recovered in the 1- to 4-cm area of the gel for both the 10- and 20-min samples. Slower moving product species, by comparison to the main ^{3}H-viral species, were evident in the 30- to 120-min samples. Some fast moving (small-molecular-weight) ^{32}P-product species were evident in the 45- to 120-min samples (Fig. 3C).

Melting the products of an influenza WSN polymerase reaction. To determine whether the product RNA was hydrogen-bonded to other product strands, or hydrogen-bonded to viral RNA, or covalently joined to the viral RNA, a melting experiment was performed (Fig. 3D). A sample of the 120-min reaction product described in the previous section was diluted with phosphate buffer, melted (*see above*), and then immediately subjected to electrophoresis for 90 min (Fig. 3D). Comparison of the distribution of ^{3}H and ^{32}P labels shown in Fig. 3C with that of Fig. 3D indicated that the electrophoretic mobility of the ^{32}P-product RNA was increased after the heat treatment. However, the ^{3}H-labeled viral RNA peaks were conserved apparently intact despite the heating treatment, indicating that the melting process did not destroy covalently joined RNA. It was concluded by reference to the mobility of the ^{3}H-species that the majority of the product RNA recovered in the 2- to 4-cm region had a spectrum of molecular weights of between 5×10^4 and 3×10^5 daltons. Since the gel was only run for 90 min, the ^{3}H peaks were not fully resolved.

Ribonuclease resistance of the product and viral RNA isolated from influenza WSN polymerase reactions. It has been shown above that ^{32}P-product RNA from the polymerase reaction is recovered in some hydrogen-bonded form, possibly in association with the ^{3}H-viral RNA, from which it could be released by heat treatment. If the product RNA were hydrogen-bonded to the viral RNA then both species should be resistant to digestion by ribonuclease and the amount of ribonuclease resistance should indicate the degree of hydrogen bonding present. To determine this, samples (20 μliter) of the purified RNA isolated from the kinetic experiment described in Fig. 3 were mixed with 2 μliters of 4 M LiCl and a 5-μliter volume was precipitated with 10% trichloroacetic acid to determine the content of radioactivity. A second 5-μliter sample was diluted into a ribonuclease solution and incubated for 30 min at 37 C. After incubation, the residual acid-insoluble radioactivity was determined. The remaining 12 μliters of each sample were sealed in a capillary tube, and the RNA species were annealed by incubating at 65 C for 4 hr. After incubation, portions (5 μliter) were similarly taken to determine the acid-insoluble radioactivity with or without a ribonuclease treatment. Additionally, other 20-μliter portions of the original RNA samples were melted and then mixed with LiCl; the 22-μliter volumes were processed as described above. The results concerning the fate of the ^{3}H and ^{32}P labels are given in Fig. 4 and can be summarized as follows.

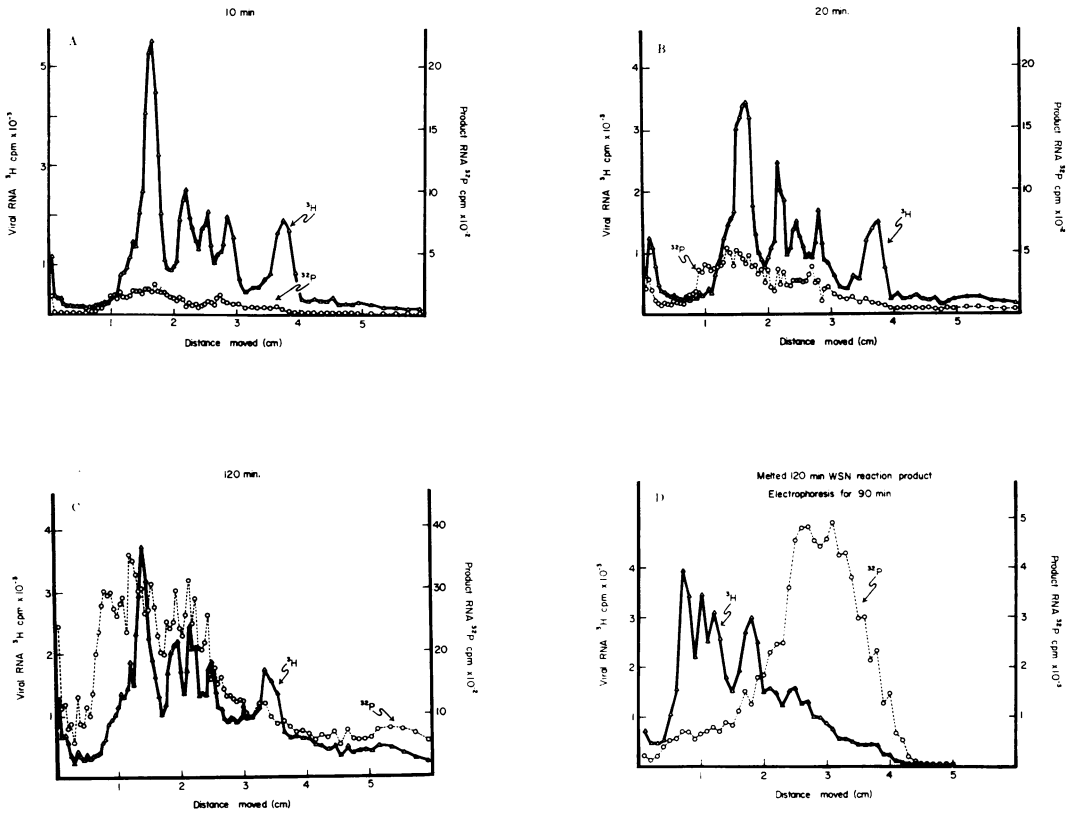

FIG. 3. *Gel electrophoresis of the RNA isolated from influenza (WSN) polymerase reactions. In the kinetic experiment described in Fig. 1, samples (1 ml at 0 min, 2 ml at 10, 20, 30, 45, and 60 min, and the remaining volume at 120 min) were extracted and purified for RNA as described by Bishop and Roy (J. Mol. Biol., in press). Each RNA sample was suspended in 200 μliters of 0.01 M sodium phosphate buffer, 0.005 M EDTA (pH 7.0), and a portion (10 to 20 μliters) was subjected to electrophoresis in swollen 3.2% polyacrylamide gels (4) to determine the distribution of ^3H and ^{32}P labels. A portion of the 120-min sample was diluted with phosphate buffer and then melted (see text), and the products were resolved by gel electrophoresis for 90 min. Figure 3A, 10 min; 3B, 20 min; 3C, 120 min; 3D, 120 min, melted.*

(i) **^3H-template RNA.** It was found that through the time-course of the experiment the ribonuclease resistance of the unmelted, unannealed ^3H-samples (viral RNA) increased to 7% (Fig. 4A). The melted, unannealed samples had a ribonuclease resistance of only 0.6% of the total ^3H label. For either the unmelted/annealed, or the melted/annealed samples, the ribonuclease resistance of the ^3H label increased to 14% in the 120-min samples (Fig. 4A).

(ii) **^{32}P-product RNA.** It was found that native, unmelted, unannealed ^{32}P-RNA (polymerase product) had ribonuclease resistances of 80% for the 10-min sample and around 60% for samples collected at 30 min through 120 min. After melting and without annealing, the ^{32}P-product RNA had a ribonuclease resistance of around 8%. When either the unmelted or melted samples were subsequently annealed, the ribonuclease resistance increased to 95 to 100% (Fig. 4B).

These findings support the conclusion that at least 60% of the product RNA isolated by phenol extraction from a WSN polymerase reaction mixture is in a hydrogen-bonded form possibly involving some of the viral RNA, since both the native ^{32}P-product as well as the ^3H-viral RNA show significant amounts of ribonuclease resistance. Moreover, the product RNA which, in the extract, is not hydrogen-bonded to viral RNA, can be almost totally annealed into a ribonuclease-resistant form. Since the ribonuclease resistance of the viral RNA alone (0-min sample) did not substantially increase with the annealing procedure, this indicates that the viral RNA preparation does not have complementary sequences. This suggests that the increase in

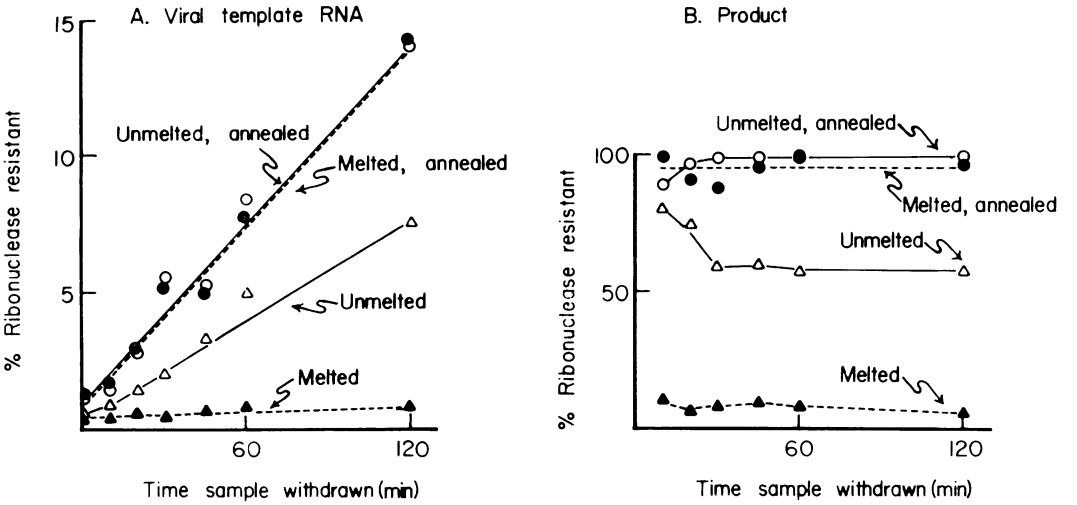

FIG. 4. *Ribonuclease resistance of the ^{32}P-product and ^{3}H-viral RNA isolated from influenza (WSN) polymerase reactions. Samples (20 μliter) of the RNA isolated from the kinetic experiment described in Fig. 1 and 3 and text were mixed with 4 M LiCl (2 μliters). A 5-μliter sample was precipitated with trichloroacetic acid to determine the content of radioactivity. A second 5-μliter sample was digested with ribonuclease (see text) to determine the native ribonuclease resistance of either the ^{3}H-viral RNA (A: continuous line, unmelted) or the ^{32}P-product RNA (B: continuous line, unmelted). The ribonuclease resistance is expressed as the per cent of acid-insoluble undigested material by reference to the initial acid-insoluble radioactivity. The remaining mixture was sealed in a capillary and incubated at 65 C for 4 hr. A 5-μliter portion of this annealed material was precipitated with trichloroacetic acid to determine the acid-insoluble radioactivity. A final 5-μliter sample was digested with ribonuclease to determine the per cent ribonuclease resistance of either the annealed ^{3}H-viral RNA (A: continuous line, unmelted, unannealed) or the annealed ^{32}P-product RNA (B: continuous line, unmelted, annealed). Alternatively, the initial reaction product was melted prior to addition of LiCl then similarly processed (broken lines, melted or melted, annealed). The ^{32}P:^{3}H counts/minute ratios of ribonuclease-resistant RNA for the native/unmelted samples were 23, 23, 19, and 26 for the 30-min, 45-min, 60-min and 120-min samples, respectively. The ratios for the native/annealed samples were 16, 25, 24, and 23, respectively (see text). The counts/minute at other times were insufficient to obtain accurate ratios.*

ribonuclease resistance of viral RNA after annealing the 120-min sample was due to hydrogen bonding with some of the product molecules.

Complementarity of the product RNA to the viral RNA. The results presented in the previous section suggest that the influenza virion polymerase transcribes the viral RNA into product species. To determine the amount of product RNA which was identical, complementary, or irrelevant to the WSN genome, an annealing experiment was performed (Table 1). Melted reaction-product RNA (^{3}H and ^{32}P species) was diluted to a concentration at which it could not anneal (since RNA annealing is concentration-dependent, reference 15), and then subjected to an annealing treatment in the presence of an excess of added, unlabeled WSN viral RNA. The results summarized in Table 1 indicate that 95% of the diluted product RNA was complementary to the viral WSN genome. Less than 5% of the product species was identical or irrelevant to the viral genome.

We have demonstrated that the product RNA as isolated from a reaction mixture is involved in hydrogen-bonded complexes from which it can be freed by a melting treatment. Since the product in these complexes is mostly in a ribonuclease-resistant form and since 95% of the product is complementary in composition to the viral RNA, these results can only be interpreted to mean that the product RNA is transcribed from the viral genome and is present in complexes involving the viral RNA.

DISCUSSION

RNA of WSN virus. The profile of RNA species obtained after electrophoresis of ^{3}H-uridine-labeled WSN virus agrees well with the results obtained by Duesberg (9) and Pons and Hirst (14). It is apparent that the influenza genome is in the form of separate polynucleotides since SDS treatment or phenol extraction give the same result. We suggest from the molar ratios of the RNA species detected in these gels that there are a minimum of seven RNA species with a sum molecular weight of about 5×10^{6} daltons or

TABLE 1. *Complementarity of the ^{32}P-product RNA to the influenza (WSN) genome*[a]

Time (min)	Per cent ribonuclease-resistant RNA					
	Melted		Melted/annealed		Melted/annealed with WSN RNA	
	^{3}H	^{32}P	^{3}H	^{32}P	^{3}H	^{32}P
10	0.1	2	1	0	1	94
20	0.2	4	1	5	1	95
30	0.2	3	1	9	1	98
45	0.2	3	1	9	1	100
60	0.2	4	1	6	1	96
120	0.2	3	1	7	2	99

[a] Portions of the RNA isolated in the kinetic experiment described in Fig. 1 and 3 and in the text were diluted 5,000-fold (= 0.002 μg/ml) and melted by heat treatment (*see text*). Samples were precipitated with trichloroacetic acid to determine the acid-insoluble radioactivity or treated with ribonuclease to determine the per cent of ribonuclease-resistant ^{3}H-viral or ^{32}P-product RNA. Other samples were mixed with unlabeled WSN viral RNA (final concentration, 10 μg per ml) and annealed in 0.4 M LiCl at 65 C for 4 hr before determining the content of acid-insoluble label and per cent ribonuclease-resistant ^{3}H-viral and ^{32}P-product RNA.

about twice the size previously reported (9, 14). In this connection, seven species of RNA have been detected in the PR8 strain of influenza virus (12) and, based on the sum molecular weights of viral-coded polypeptides, it has recently been suggested that the true size of the influenza genome had been previously underestimated (8, 16). The viral RNA does not contain significant amounts of complementary RNA molecules.

Product synthesized by WSN RNA polymerase. The results described in this report concern the nature and time-course of product synthesis in WSN-primed reactions. In determining the fate of the viral RNA, we have addressed our study to the question of how the product RNA is related to the viral genome. Consequently, we employed ^{3}H-uridine-labeled virus preparations to follow the fate of the viral RNA and ^{32}P-labeled triphosphates to monitor product synthesis. Although we have run template-product analyses under the most optimal reaction conditions that we can delineate (5), it is still possible that these conditions are insufficient for accurate and complete enzyme activity.

Our results lead us to suggest that the WSN virion possesses a transcriptase enzyme activity which is responsible for transcribing some or all of the viral genome into complementary product RNA species. We do not know whether all components of the viral RNA are transcribed although we have observed that part of the product RNA is recovered in a hydrogen-bonded state in the same areas of the gel where the viral RNA is recovered (Fig. 3). We have also presented evidence that the transcriptase activity of influenza WSN is much less active than a similar enzyme activity present in VSV particles (5), although this property may be strain-dependent within the myxovirus group (7). Recently it has been shown that the VSV transcriptase compeletely transcribes the VSV genome (3). Since the VSV polymerase takes 50 to 60 min for complete transcription under ideal in vitro conditions, we are currently involved in determining the completeness of the WSN transcription process by using product isolated from longer incubations.

It has been assumed in this discussion that involvement of ^{3}H-template RNA in ribonuclease-resistant, hydrogen-bonded structures is indicative of enzyme activity. When melted reaction product RNA (60-min sample) was subsequently extracted with phenol and then alcohol-precipitated prior to electrophoresis, the distribution of labels in the gel showed that product RNA (^{32}P) and template RNA (^{3}H) were not associated (Fig. 3D). This suggests that the complexes obtained from reaction mixtures were not artifacts generated by the extraction procedure but were derived from preexisting RNA associations.

No ^{3}H-viral RNA was visibly associated with the slowest moving product species of the 120-min sample (Fig. 3C) resolved by gel electrophoresis. Two possible explanations of this observation can be proposed. Either the slow-moving complexes were composed solely of product RNA or the amount of viral RNA present was too little to detect. The possibility that the complexes are derived from product species alone can be excluded since at least 95% of the product was complementary to the viral RNA and none of the viral RNA contains complementary ^{3}H species. Secondly, it was observed that at 120 min only 7% of the viral genome was ribonuclease-resistant so that the amount of viral RNA involved in product synthesis was not very substantial. Further, the specific activity of the product RNA was ca. 1.3×10^{7} per μg, as calculated from the specific activity of the precursor triphosphate and assuming a 25 mole % of UMP in the product. The specific activity of the template RNA, based on the radioactivity of the viral preparation and an assumed 0.9% RNA in the virions (6), was around 4×10^{5}. Consequently, a 30 to 1 ratio of ^{32}P and ^{3}H labels would

be expected for a mass parity of product and viral RNA species (see Fig. 4, where the $^{32}P:^{3}H$ counts/minute ratio for the ribonuclease-resistant RNA was found to be approximately 24, in good agreement with this calculation). In Fig. 3, the ratio of labels in the slow-moving complexes was around 3:1 for the ^{32}P to ^{3}H radioactivity in the 0.8- to 1.2-cm gel region of the 120-min sample. From this ratio it can be suggested that the mass of product present was equivalent to a maximum of 10% of the amount of viral RNA there. However, because the 0-min sample had a significant amount of ^{3}H label in the same gel region (*see* Fig. 2), these figures only give an approximate idea of the mass equivalence of product and viral RNA in these complexes. If it can be assumed that the product species with a single-stranded molecular weight of 10^5 daltons were derived by transcription of the largest species of viral RNA, then this product mass would also be equivalent to 10% of those viral species.

One final point should be mentioned regarding the relationship of product to viral RNA. For the 30-min sample, approximately 60% of the product RNA was ribonuclease-resistant although almost all of the product was associated with viral RNA species (Fig. 3B). This suggests, therefore, that the product-template RNA complexes are multistranded with respect to the product strands. If so, then it is tempting to speculate that there may be more than one enzyme molecule per viral RNA in the virions or, alternatively, that the polymerase can reinitiate transcription of the same template RNA molecule.

We do not know how many of the virions in the reaction mixture have active enzyme or competent template RNA. The 7% figure obtained for the ribonuclease resistance of the ^{3}H-template RNA (120-min sample) suggests that at least 7% of the available virion template is involved in enzyme activity. Since the melted single-stranded product size is smaller than any of the viral template species, it is probable that none of the template molecules were completely transcribed under the reaction conditions reported here, so that this 7% value is a minimal estimate.

ACKNOWLEDGMENTS

We are indebted to Ulrike Stadler and Rebecca M. Shamy for their expert technical assistance.

This study was supported by funds from Public Health Service research grants CA-02332 from the National Cancer Institute and AI-09124 from the National Institute of Allergy and Infectious Diseases. J. F. O. is a predoctoral fellow in virology, the recipient of training grant GM-507-11 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Baltimore, D. 1970. Viral RNA-dependent DNA polymerase. Nature (London) 226:1209–1211.
2. Baltimore, D., A. S. Huang, and M. Stampfer. 1970. Ribonucleic acid synthesis of vesicular stomatitis virus. II. An RNA polymerase in the virion. Proc. Nat. Acad. Sci. U.S.A. 66:572–576.
3. Bishop, D. H. L. 1971. Complete transcription by the transcriptase of vesicular stomatitis virus. J. Virol. 7:486–490.
4. Bishop, D. H. L., J. R. Claybrook, and S. Spiegelman. 1967. Electrophoretic separation of viral nucleic acids on polyacrylamide gels. J. Mol. Biol. 26:373–387.
5. Bishop, D. H. L., J. F. Obijeski, and R. W. Simpson. 1971. Transcription of the influenza ribonucleic acid genome by a virion polymerase. I. Optimal conditions for in vitro activity of the ribonucleic acid-dependent ribonucleic acid polymerase. J. Virol. 8:66–73.
6. Blair, C. D., and P. H. Duesberg. 1970. Myxovirus ribonucleic acids. Annu. Rev. Microbiol. 24:539–574.
7. Chow, N., and R. W. Simpson. 1971. RNA-dependent RNA polymerase activity associated with virions and subviral components of myxoviruses. Proc. Nat. Acad. Sci. U.S.A. 68:752–756.
8. Compans, R. W., H. Klenk, L. A. Caliguri, and P. W. Choppin. 1970. Influenza virus proteins. I. Analysis of polypeptides of the virion and identification of spike glycoproteins. Virology 42:880–889.
9. Duesberg, P. H. 1968. The RNA's of influenza virus. Proc. Nat. Acad. Sci. U.S.A. 59:930–937.
10. Huang, A. S., D. Baltimore, and M. A. Bratt. 1971. An RNA polymerase in virions of Newcastle disease virus: comparison with vesicular stomatitis virus polymerase. J. Virol. 7:389–394.
11. Loening, U. E. 1968. Molecular weights of ribosomal RNA in relation to evolution. J. Mol. Biol. 38:355–365.
12. Lerner, R. A., and L. D. Hodge. 1969. Nonpermissive infections of mammalian cells: synthesis of influenza virus genome in Hela cells. Proc. Nat. Acad. Sci. U.S.A. 64:544–551.
13. Lin, F. H., and H. Thormar. 1970. Ribonucleic acid-dependent deoxyribonucleic acid polymerase in visna virus. J. Virol. 6:702–704.
14. Pons, M. W., and G. K. Hirst. 1968. Polyacrylamide gel electrophoresis of influenza virus RNA. Virology 34:386–388.
15. Roy, P., and D. H. L. Bishop. 1970. Isolation and properties of poliovirus minus strand ribonucleic acid. J. Virol. 6:604–609.
16. Schulze, I. T. 1970. The structure of influenza virus. I. The polypeptides of the virion. Virology 48:890–904.
17. Shatkin, A. J., and J. D. Sipe. 1968. RNA polymerase activity in purified reoviruses. Proc. Nat. Acad. Sci. U.S.A. 61:1462–1469.
18. Temin, H. M., and S. Mitzutani. 1970. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature (London) 226:1211–1213.