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Genome Homology of Vesicular Stomatitis Virus and Defective T Particles and Evidence for the Sequential Transcription of the Virion Ribonucleic Acid

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The small defective T particle of vesicular stomatitis virus (VSV-111) has no detectable enzyme activity when assayed at 31 C, although qualitatively it possesses all the virion proteins found in complete VSV-1 virions. With VSV-1 transcription product ribonucleic acid (RNA), it is shown that the VSV-111 RNA is identical to part of the VSV-1 genome. Evidence is also presented to support the idea that in vitro VSV-1 transcription is sequential.

The virion-associated ribonucleic acid (RNA)-dependent RNA polymerase of complete infectious vesicular stomatitis virus (VSV-1) is responsible for transcribing the virion genome and making complementary messenger RNA species (2, 10, 15). In vitro this transcription process is repetitive, optimal around 31 C, and continues for many hours, although it takes about 60 to 90 min to yield a complete complement of product RNA molecules (1, 3). At 37 C, the in vitro transcription is slower, incomplete (terminating between 60 and 90 min of incubation), and, although it is repetitive, only about 30% of the genome is transcribed (6, 7).

We have previously shown that in incubations at 37 C the larger defective particles (17) found in some vesicular stomatitis virus (VSV) preparations (called LT or VSV-11 particles) also demonstrate enzyme activity (7). However, when assayed for 60 min at 37 C, little enzyme activity was detected (7) with the smaller defective particle (called T or VSV-111 particles) (8, 9, 11). These results suggest that the VSV-111 particle either possesses no enzyme or that its enzyme is inactive at 37 C. In this communication, we present evidence which indicates that VSV-111 particles do not have active enzyme when assayed in vitro at 31 C, although qualitatively they have all the proteins identifiable in VSV-1 preparations (14, 16, 19, 20).

It is conceivable that the defective VSV-111, although requiring VSV-1 for growth in an infected cell, does not have an identical sequence to any part of the VSV-1 genome. Conversely, it is conceivable that a population of VSV-111 could have a spectrum of sequences which is overall identical to the entire VSV-1 genome. In between these two extremes is the possibility that VSV-111 is identical to a specific part of the VSV-1 genome. Evidence will be presented to support this latter hypothesis and also to suggest that transcription of VSV-1 is sequential in that not all regions of VSV-1 are transcribed at the same time.

MATERIALS AND METHODS

Preparation of virus. VSV was grown at 37 C in monolayer cultures of BHK-21 cells as described previously (1, 6). The purification procedure of virus from tissue culture supernatant fluids was as follows. The fluids were clarified from cellular debris by centrifugation at 4 C for 30 min at 7000 × *g*. Per liter of supernatant fluid, 25 g of NaCl and 75 g of polyethylene glycol 6000 (Carbowax 6000) were added, and, after dissolving, the mixture was stored in centrifuge bottles at 4 C for 2 hr. The precipitated material was then collected by centrifugation at 4 C and 7000 × *g* for 30 min, and the supernatant fluid discarded. After draining, the precipitate was suspended in Eagle medium (10 ml per original liter of tissue culture supernatant fluid), homogenized in a Dounce homogenizer, and 5-ml samples were layered over 7-ml linear gradients of 70 to 20% (w/v) sucrose in 0.15 M NaCl–0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (*p*H 7.5) prepared in a Spinco SW41 centrifuge tube. The tubes were then centrifuged at 35,000 rev/min for 5 hr at 3 C, and the virus band was removed with a pipette. Under

these conditions, the virus particles had been centrifuged to equilibrium. Sucrose was removed by passing up to 5 ml of the virus suspension through a 13-ml column of G-25 Sephadex (coarse grade) equilibrated and subsequently eluted with 0.15 M NaCl-0.01 M Tris-hydrochloride buffer, *p*H 7.5. To separate VSV-1 from the defective particles, the virus suspension (3 ml per gradient) was loaded over an 8.5-ml linear gradient of 30 to 15% (w/v) sucrose in 0.15 M NaCl-0.01 M Tris-hydrochoride buffer (*p*H 7.5) prepared over a 0.5-ml cushion of 70% sucrose in a Spinco SW41 centrifuge tube. The tubes were centrifuged at 35,000 rev/min for 35 min at 3 C, and the virus bands were collected as they dripped from the bottom of the tube. Sucrose was removed from the virus preparation as described above. The advantages of this purification procedure are with regard to the speed of purification—the whole process can be completed in one day—and result in the removal of both cellular debris (of all sorts of density and size) and aggregated virus. Judged by the ^{3}H-RNA (6), about 70% of the postequilibrium virus preparation was recovered on the 70% sucrose pad of the velocity gradient and presumably represented virus aggregated together or with cellular material. As defined operationally by the sustained kinetics as well as the per cent virus demonstrating virion transcriptase activity (*see below*), the best virus preparations we have obtained are those purified by both equilibrium and velocity centrifugations as described above.

Where necessary, further purification of VSV-111 particles from contaminating VSV-1 virions was achieved by successive velocity centrifugations as described above. Virions labeled with ^{3}H-uridine were prepared similarly by including ^{3}H-uridine (10 μCi per ml) in the tissue culture medium (1).

Reaction conditions: preparation of product RNA. The reaction conditions and constituents are described elsewhere (5–7). In some experiments, the specific activity of the ^{32}P-α-uridine triphosphate (UTP) used to label the product RNA was varied by including more unlabeled UTP in the reaction mixture (see text and figure legends). The purification of reaction product RNA (^{3}H and ^{32}P species) from proteins and labeled triphosphates has also been described (6).

Melting and annealing conditions, treatment with ribonuclease, and RNA gel electrophoresis. Procedures for gel electrophoresis, ribonuclease treatment, and melting and annealing conditions have been described elsewhere (4, 6, 7, 18). Unless otherwise stated, RNA samples were subjected to electrophoresis in 2% polyacrylamide gels for 90 min at 10 mamp per gel (4, 6). After electrophoresis, the gels were sliced, dissolved in H_2O_2, and counted in Aquasol (New England Nuclear Corp.) (6).

RESULTS

The relative polymerase activities of VSV-1 and VSV-111 virions. A preparation of ^{3}H-uridine-labeled VSV was purified as described above, and, after velocity gradient centrifugation, the two visible bands of virus ("VSV-1" and "VSV-111") were collected. Each virus band contained a population of particles. The lower "VSV-1" band contained principally VSV-1 particles but also VSV-111 species, whereas the upper "VSV-111" band contained principally VSV-111 particles with some VSV-1 virions (Fig. 1). Since all the particles were derived from the same batch of infected cells and purified together, it can be assumed that both species have the same ^{3}H specific activity for their RNA. It has been shown (6) that VSV-111 RNA has about 25% of the molecular weight of VSV-1 RNA, so that from the relative amounts of label in the two peaks (Fig. 1) it was calculated that the relative number of VSV-1 to VSV-111 viral genomes in the lower "VSV-1" preparation was about 1.5:1, whereas for the upper "VSV-111" preparation it was about 1:14.

Without further purification, each virus band was used separately to prime reactions containing ^{32}P-α-UTP to label the product RNA. The specific activity of the product RNA was designed such that under the Aquasol counting conditions the specific activity of the ^{32}P product RNA of the "VSV-111" reaction was approximately equivalent to that of the ^{3}H-RNA (*see below*). In the "VSV-1" reaction however, the ^{32}P product RNA was one-fifth the specific activity of the ^{3}H-RNA. The relative enzyme activity at 31 C of the two virus preparations is shown in Fig. 2. For the first 4 hr of reaction, the "VSV-1" reaction rate was four times greater than the "VSV-111" reaction (as related to the ^{3}H label in each preparation). Since about one-fifth to one-quarter of the ^{3}H label in the "VSV-111" preparation was present in VSV-1 RNA (Fig. 1), this result suggested that there was probably very little enzyme activity ascribable to VSV-111 particles.

Association of product and template RNA species in a VSV-111-primed reaction. To determine whether any product could be observed in association with VSV-111 RNA (i.e., ascribable to VSV-111 transcriptase activity), samples of the "VSV-111" and "VSV-1" reactions were extracted for RNA at various time intervals. The reaction product RNA species, containing both ^{3}H- and ^{32}P-labeled species, were resolved by gel electrophoresis with 2% polyacrylamide gels. This concentration of polyacrylamide was chosen to allow observation of the total size spectrum of product species (20 to 1*S*) as well as free VSV-1 and VSV-111 RNA species and complexes of either with product RNA. It has been shown in a previous communication that during the time-course of RNA synthesis by VSV-1 transcriptase, product RNA is initially recovered in association with the viral RNA in complexes

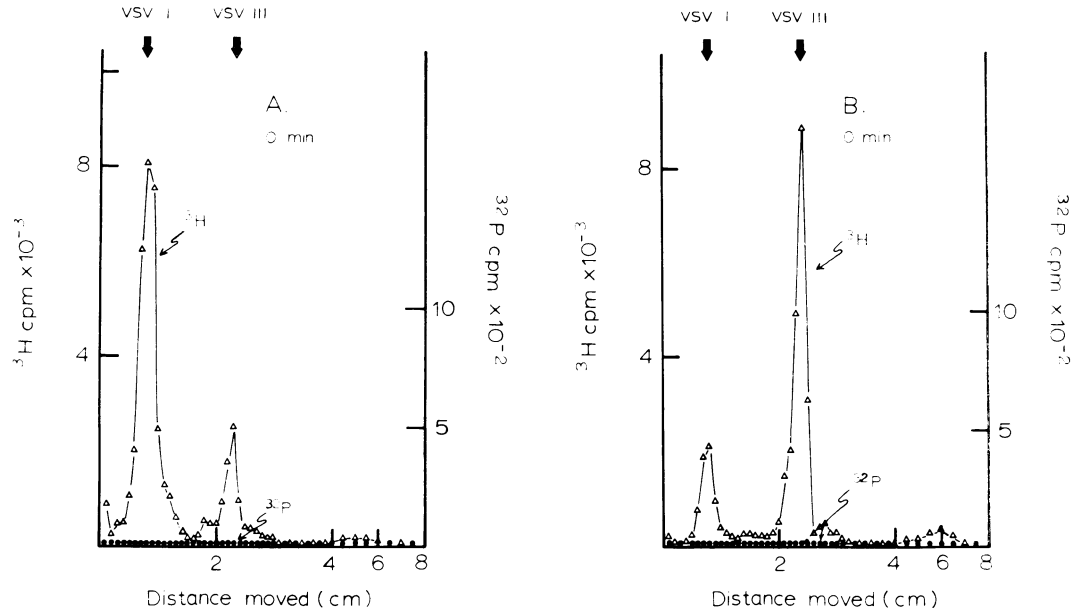

FIG. 1. *RNA gel electrophoresis of populations of VSV-1 and VSV-111 particles. A preparation of ^{3}H-uridine-labeled VSV was centrifuged on a linear gradient of sucrose (see Materials and Methods), and the two bands of virus were collected. After removal of sucrose, the lower "VSV-1" band and the upper "VSV-111" band were used separately to prime 100-fold reactions containing ^{32}P-α-UTP to label the product RNA (reference 5; Materials and Methods). At intervals, samples of each reaction were extracted for RNA, separated from triphosphates by Sephadex G-50 column chromatography (6), and the labeled RNA was recovered by alcohol precipitation. Gel electrophoresis of the RNA in 2% polyacrylamide gels (4, 6, 7) was conducted at 10 mamp per gel for 90 min, and the distribution of labels was determined by slicing the gels, dissolving in H_2O_2, and counting in Aquasol. In the "VSV-1" reaction, the specific activity of the ^{32}P-α-UTP was one-fifth that in the "VSV-111" reaction. The zero-time samples of the "VSV-1" reaction (A) and the "VSV-111" reactions (B) are given.*

which move slower upon electrophoresis than free VSV-1 RNA (7). Since these complexes can be pulse-labeled and the pulsed RNA can be chased into free product species (6), it is apparent that the complexes represent the active sites of product RNA synthesis and relate to the possession of active enzyme by those virions (7).

Hence, by examining the RNA profiles of the total reaction product nucleic acids in the "VSV-111" reaction, the fate of both ^{3}H-VSV-1 and -VSV-111 RNA species was followed (Fig. 3). The zero-time sample of the reaction is given in Fig. 1 and allowed identification of the relative electrophoretic mobility of free VSV-1 and free VSV-111 RNA.

It is evident, from visual examination of the 30-min through 180-min samples of the "VSV-111" reaction, that most of the VSV-111 RNA was recovered free throughout the reaction time course and that little, if any, ^{32}P was associated with the VSV-111 RNA. However, many of the VSV-1 RNA species were involved in complexes with ^{32}P product RNA which moved slower than free VSV-1 RNA (7). The relative ^{32}P to ^{3}H label in the first 1 cm of each gel (including both free and complexed ^{3}H-VSV-1) was much greater than for the next 1.5 cm (i.e., the gel area encompassing VSV-111 RNA species).

To determine whether any of the ^{3}H-RNA present in the first 1 cm of the gel contained VSV-111 RNA, a melting experiment was performed on the 180-min sample (Fig. 4). The percentages of ^{3}H label recovered after melting in free VSV-1 RNA and free VSV-111 RNA species were indistinguishable, from the percentages in the 1st cm and the 2- to 2.5-cm region, respectively, of the 180-min unmelted sample (Fig. 3). Moreover, although in the melted sample there were no complexes containing ^{32}P in the first centimeter of the gel, the ^{32}P profile from the second to eighth centimeter was essentially identical to that of the unmelted sample, indicating that the ^{32}P in the 2- to 2.5-cm region of the unmelted sample represents free product species and not ^{3}H-VSV-111 template-^{32}P product complexes.

Annealing of "VSV-111" reaction product RNA to VSV-1 RNA. When purified, unlabeled VSV-1 RNA [a 100-fold (w/w) excess to the ^{3}H-RNA present] was annealed to the "VSV-111"

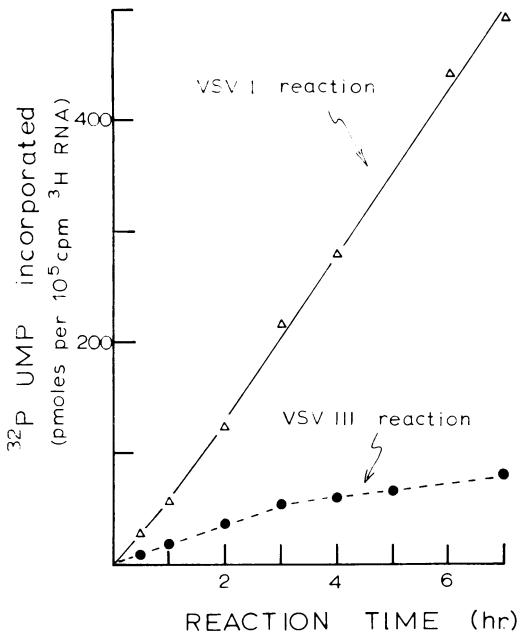

FIG. 2. *Kinetics of RNA synthesis of the "VSV-I"- and "VSV-III"-primed reactions. Samples (50 μliter) of the two reactions described in Fig. 1 were precipitated at intervals with 5% trichloroacetic acid, and the acid-insoluble radioactivity was determined. From the specific activity of the ^{32}P-α-UTP in the two reactions, the incorporation of label in pmoles was calculated and is expressed as the incorporation per 10^5 counts/min ^{3}H-RNA in each sample. This manner of expressing the synthesis of RNA relates to the mass of ^{3}H-RNA and not to the molar amounts of the various ^{3}H-RNA species (see Discussion).*

180-min reaction product nucleic acids, almost all the ^{32}P was recovered in complexes at the front of the gel (Fig. 4). This result indicated that the majority of the ^{32}P product present in the 180-min sample was complementary to VSV-1 RNA.

This observation could be interpreted two ways. Either the product present was synthesized on the VSV-1 template or it was synthesized on the VSV-111 template and VSV-111 RNA is homologous to the VSV-1 genome. Although no VSV-111 enzyme activity had been detected (as judged by the presence of template-product complexes), to obtain an answer to these proposals by an alternate approach, a self-annealing experiment was performed on the "VSV-111" reaction product species.

Self-annealing of "VSV-111" reaction product RNA. The reaction products from the "VSV-111"-primed experiment described above were self-annealed, and the annealed RNA was resolved by gel electrophoresis (Fig. 3). It was observed that, as judged by their relative electrophoretic mobility, free ^{32}P product and free ^{3}H-VSV-111 RNA persisted after the annealing even though some of the product annealed to VSV-1 RNA, as evidenced by the increased ^{32}P to ^{3}H ratio in the first centimeter of the gel (by comparison to the unannealed samples) as well as the increased ^{32}P to ^{3}H ratio in these annealed VSV-1 complexes throughout the reaction time course (approaching parity by the 180-min annealed sample). These results suggest then that the majority of the "VSV-111" reaction product ^{32}P-RNA was not homologous to the VSV-111 genome and hence derived from VSV-1 transcription.

It was also observed that some annealing between ^{32}P product RNA and ^{3}H-VSV-111 RNA species did occur only at the later time points, as evidenced by a broadening of the ^{3}H-VSV-111 RNA profile (recovered over a 1.2-cm span for the annealed 180-min sample, by comparison with 0.6 cm for the annealed 30-min sample, or 0.4 cm for the unannealed 180-min sample). As the broadening was principally on the slower-moving edge of the peak and there was ^{32}P product RNA in the same region, these results can be interpreted to indicate that VSV-111 RNA had annealed to a fraction of the ^{32}P product species present. The relative ^{32}P to ^{3}H ratio varied through the whole region (1–2.5 cm), indicating that not all of the ^{3}H-VSV-111 RNA had completely annealed to ^{32}P product.

Whether the product which annealed to the VSV-111 RNA was derived from VSV-1 transcription or from a very slow VSV-111 transcription could not be determined by these techniques.

Sequence homology of VSV-1 and VSV-111 RNA. It has previously been shown that VSV-1 and VSV-111 RNA species do not have complementary sequences (7) and that at least 87% of the product synthesized by VSV-1 virion preparations is complementary to the VSV-1 viral RNA (1). It is possible that VSV-111 is genetically unrelated to VSV-1; consequently, annealing experiments were devised to determine whether VSV-111 RNA possesses sequence homology to the VSV-1 genome.

Three questions were asked relevant to determining VSV-111 and VSV-1 RNA sequence homology. (i) Is the population of VSV-111 RNA species homologous to all of the VSV-1 RNA? (ii) Is only part of the VSV-111 RNA homologous to part of the VSV-1 genome? (iii) Is all of the VSV-111 RNA homologous to part of the VSV-1 genome?

It has been shown in a previous section that a population of VSV-111 RNA molecules would not anneal to product RNA derived at early

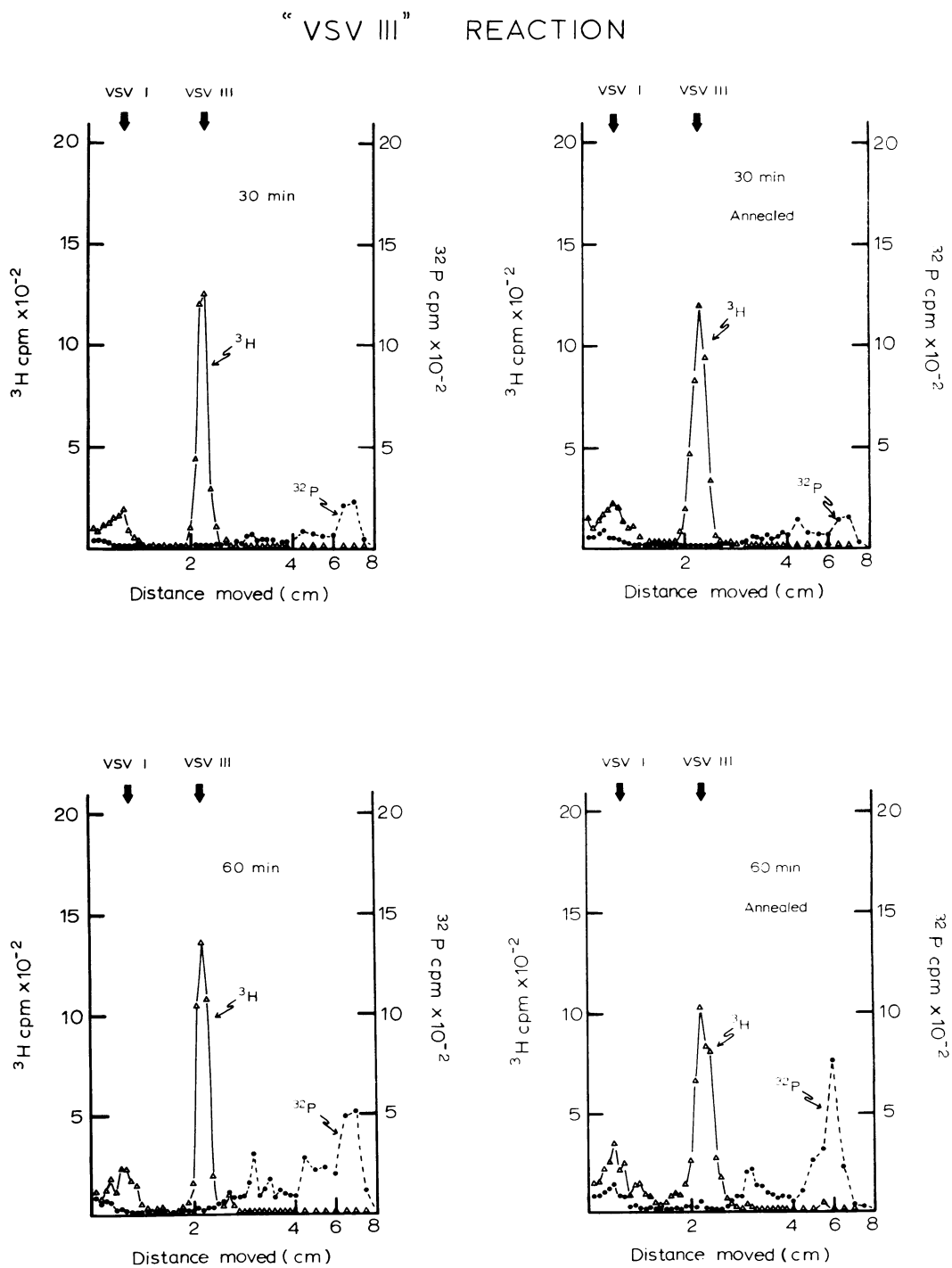

FIG. 3. *Gel electrophoresis of the RNA extracted from the "VSV-111"-primed kinetic experiment, with or without a self-annealing pretreatment. The RNA extracted at intervals from the "VSV-111"-primed experiment (Fig. 1) was subjected to gel electrophoresis (Fig. 1) with or without an annealing pretreatment (7). The zero-time sample is given in Fig. 1B. The 30-, 60-, 120-, and 180-min samples are shown. The positions of free VSV-1 and free VSV-111 RNA are shown.*

times from VSV-1 transcription (Fig. 3). This suggests therefore that the majority of VSV-111 molecules are not identical to the whole VSV-1 genome and probably are either partially or completely identical to part of the VSV-1 genome or not identical at all.

To determine whether all of the VSV-111 RNA is identical to any part of the VSV-1 RNA, a

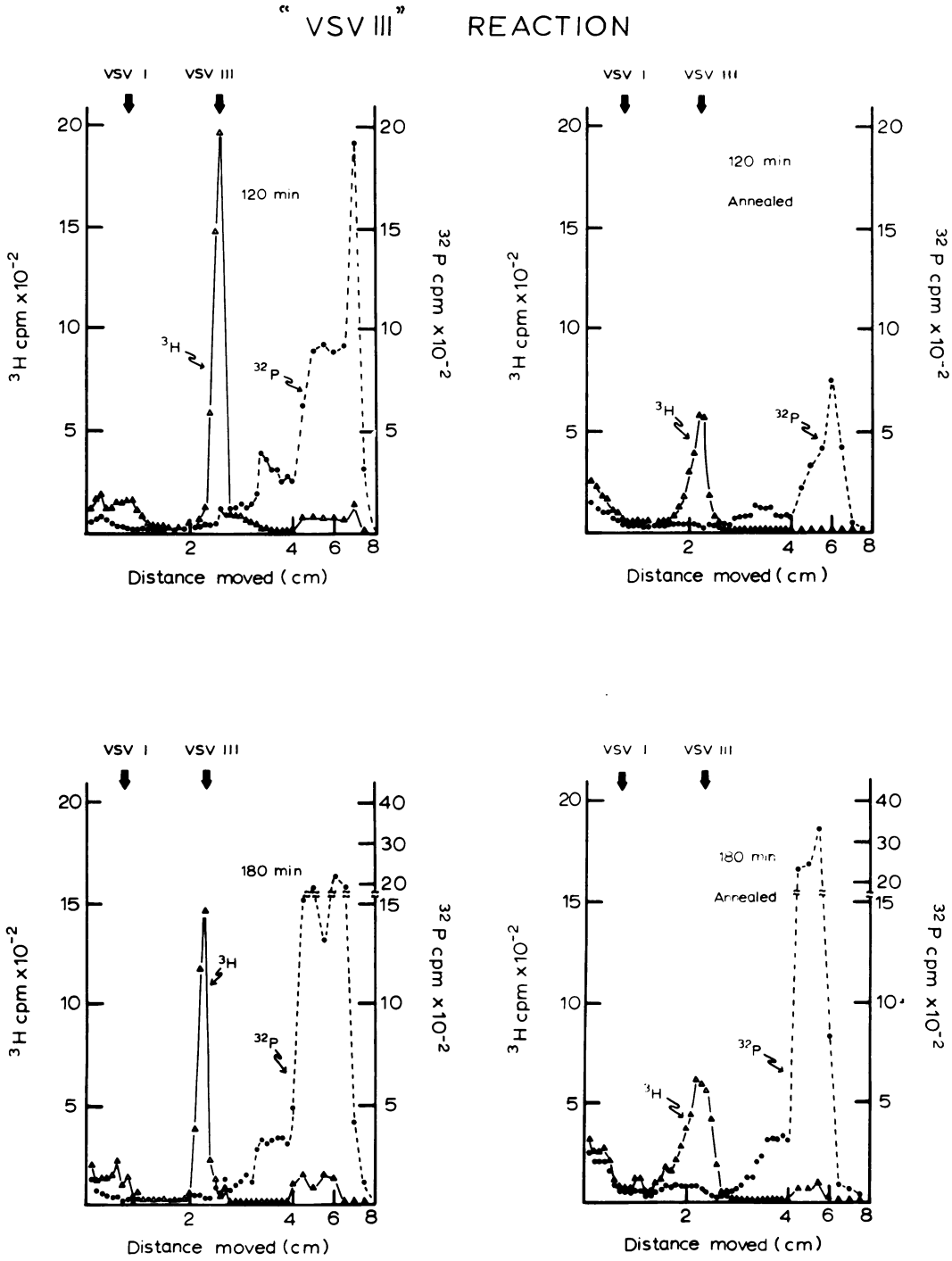

FIG. 3b,

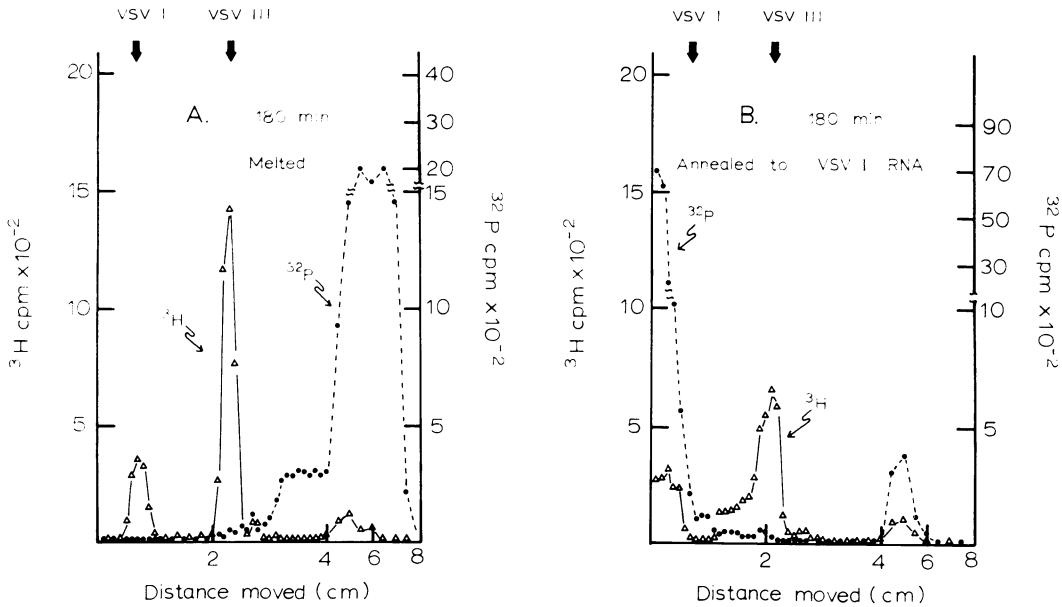

FIG. 4. *Properties of the 180-min "VSV-111" reaction product RNA: melting or annealing to unlabeled VSV-1 RNA. A sample of the 180-min "VSV-111" reaction product was melted (7) or annealed with a 100-fold excess of unlabeled, purified VSV-1 RNA (7) prior to electrophoresis. The VSV-1 RNA was obtained from a culture of VSV grown at 32 C in which there were no detectable VSV-111 particles when the crude VSV was centrifuged on a sucrose gradient (reference 1; Materials and Methods). The amount of unlabeled RNA added was calculated after assuming that the specific activity of the ^{3}H-RNA was 3 × 10^{5} counts per min per μg of RNA (3). The positions of free VSV-1 and free VSV-111 RNA are indicated.*

reaction primed by pure VSV-1 was run and the reaction product nucleic acids were purified. The VSV-1 contained less than 0.5% (w/w) VSV-111, as judged by its RNA gel profile (6). An annealing experiment was performed on the total reaction product nucleic acids, purified ^{3}H-VSV-111 RNA was added to duplicate samples, and the ribonuclease resistance of the total ^{3}H-RNA was monitored. The amount of ^{3}H-VSV-111 RNA added was equal to 20% of the final total ^{3}H label, i.e., on a molar basis almost equal to the amount of VSV-1 RNA present in the reaction product nucleic acids. This amount was chosen so that it would not, on a molar basis, be disproportionately large by comparison to the ^{3}H-VSV-1 RNA, nor on a mass basis be too small for monitoring its fate during annealing.

The amount of ^{3}H-ribonuclease resistance of the annealed VSV-1 reaction product nucleic acids in the presence or absence of 20% (w/w) ^{3}H-VSV-111 RNA is shown in Fig. 5. The difference in ^{3}H-ribonuclease resistance between the two experiments is also plotted.

It was found that with the 30- and 60-min samples there was a 21 to 19% difference in ^{3}H-ribonuclease resistance. Note, though, that the ^{32}P product was only 40% ribonuclease-resistant with or without the added VSV-111 RNA. The difference in ^{3}H-ribonuclease resistance dropped from 2 to 1% for the 6- and 7-hr samples. It can be concluded therefore that almost all of the VSV-111 genome is complementary to the reaction product nucleic acids generated in the VSV-1 reaction.

In confirmation of this result, it was found that the ribonuclease resistance of the ^{32}P product species increased proportionately in the later samples containing added VSV-111 RNA (Fig. 5).

These results are consistent then with the suggestion that all of the VSV-111 RNA is complementary and hybridizable to some of the product transcribed from VSV-1 RNA and therefore homologous to part of the VSV-1 genome.

An examination of the proteins of VSV-1 and VSV-111 virions. The simplest explanation of the lack of enzyme activity in VSV-111 particles is that they do not have the transcriptase enzyme VSV-1 particles possess. At present, the protein(s) which constitutes the VSV-1 virion transcriptase has not been identified. However, with VSV-1 and VSV-111 particles purified by three successive velocity gradient centrifugations [whereby the contamination of either by the other was less than 5% (w/w) as judged by

the ^{3}H-RNA profiles; Fig. 1], no qualitative difference in the protein profiles was observed, in confirmation of previously reported observations (14–16, 19, 20).

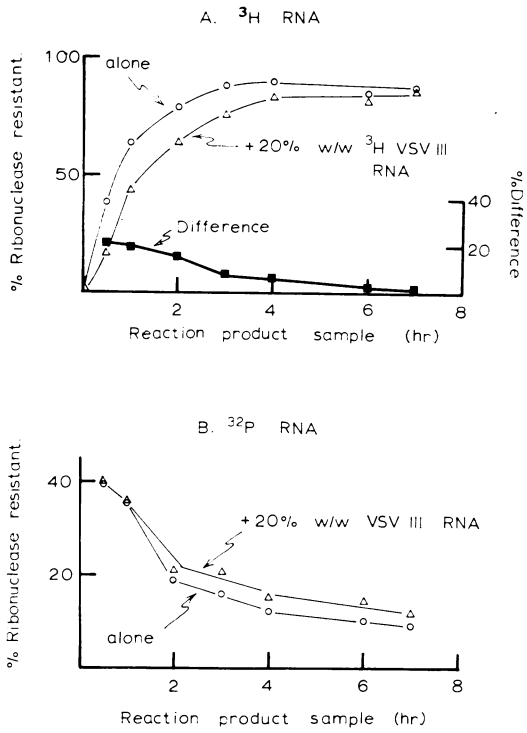

FIG. 5. *Ribonuclease-resistant RNA of VSV-1 reaction product nucleic acids determined after annealing in the presence or absence of 20% (w/w) added ^{3}H-VSV-111 RNA. A preparation of ^{3}H-VSV-1 virions was obtained from the virus preparation described in Fig. 1 and purified by three successive velocity gradient centrifugations. The preparation contained less than 0.5% (w/w) ^{3}H-VSV-111 RNA. A reaction mixture containing ^{32}P-α-UTP was primed with this virus preparation (Fig. 1), and the reaction product nucleic acids were extracted at intervals. After purification, the nucleic acid samples were annealed at 60 C for 14 hr in 0.4 M NaCl-0.01 M Tris-hydrochloride buffer, pH 7.5 (3, 7, 18), in the presence or absence of 20% (w/w) ^{3}H-VSV-111 RNA. The ^{3}H-VSV-111 RNA was obtained by phenol extraction of VSV-111 virions, purified by three successive sucrose gradient centrifugations. The ^{3}H-VSV-111 RNA contained less than 5% (w/w) ^{3}H-VSV-1 RNA (Fig. 1). After annealing, the samples were diluted to 2 ml with 0.4 M NaCl-0.01 M Tris-hydrochloride buffer (pH 7.5), and 1 ml was subtracted to determine the acid-insoluble radioactivity. Ribonuclease T_1 (10 μg) and ribonuclease A (10 μg) were then added to the remaining 1 ml. After incubation for 30 min at 37 C, the residual acid-insoluble, ribonuclease-resistant RNA was determined. The per cent ribonuclease-resistant total ^{3}H-RNA (A) or ^{32}P-RNA (B) is given. In part A, the difference in per cent ^{3}H-ribonuclease-resistant between the samples with or without added ^{3}H-VSV-111 RNA is also given.*

DISCUSSION

Lack of transcriptase activity of VSV-111 particles. In the experiments described above, populations of VSV-111 and VSV-1 particles, as isolated from a velocity gradient of ^{3}H-uridine-labeled virus, were used separately to prime reactions with ^{32}P-α-UTP to monitor product RNA synthesis. The purpose of using mixtures of particles in this manner was to determine the relative enzyme activity of both particles treated similarly throughout the purification procedure. Notice that, although in Fig. 2 the rate of product synthesis in the "VSV-111" reaction was one-quarter that of the "VSV-1" reaction and since the comparison was made relevant to the ^{3}H present in each reaction and VSV-111 RNA is only 25% the mass of VSV-1 RNA, on a molar basis the relative rate of the two reactions was much less. Moreover, as indicated, the majority of the enzyme activity observed was probably due to the contaminating VSV-1 virions.

We have done similar transcriptase assays with VSV-111 preparations containing no detectable VSV-1 contamination and found less than 1% of the activity of comparably purified VSV-1 virions (at 37 or 31 C). However, since in that case the lack of activity could be ascribable to inactivation of an unstable transcriptase by the extensive purification procedure involved, the experiments reported here were performed with a mixture of identically grown and purified virions, and this preparation contained demonstrably active VSV-1 transcriptase.

It could be argued that VSV-111 virions possess a very slow, unstable transcriptase undetected in the experiments reported here and inactivated by extensive virion purification and that it is VSV-111 transcribed product which hybridizes to the VSV-111 RNA (Fig. 3, 180-min annealed sample). However even if VSV-111 contains an undetectable amount of a slow transcriptase, VSV-111 RNA anneals to the product made by VSV-1 transcriptase (Fig. 5). Moreover, we cannot necessarily ascribe the ^{3}H-VSV-111 template-^{32}P product complexes obtained by annealing the 180-min "VSV-111" reaction product samples to VSV-111 transcribed RNA, and, in view of the results presented in Fig. 5, we would suggest that they originate by hybridization of ^{3}H-VSV-111 RNA to VSV-1 transcribed product.

It should be recognized however that these are in vitro assays and not necessarily similar to the in vivo situation. To determine whether VSV-111 is transcribed in vivo, it will be necessary to follow the fate of ^{3}H-VSV-111 RNA in single or mixed infections with unlabeled VSV-1 virions. Such experiments are currently being conducted.

Identity of VSV-111 to the VSV-1 genome.

From the results presented, it can be concluded that VSV-111 RNA is identical in sequence to part of the VSV-1 genome. Whether it is identical to one or other end of VSV-1 RNA will have to await an accurate chemical analysis of the genomes of both virions and an examination of the nucleotide fingerprints obtained by specific ribonuclease digestion of their RNA species.

Evidence to suggest the sequential transcription of VSV-1. In the experiments described above, it was found that there was very little annealing of VSV-111 RNA to VSV-1 product species isolated at early times (30 and 60 min) from a reaction time course (Fig. 3, 5). As can be seen in Fig. 5, the degree of completeness of transcription, as defined by the per cent ribonuclease-resistant ^{3}H-RNA obtained after annealing VSV-1 reaction product nucleic acids, was about 65% by 1 hr and 90% complete by 3 hr of reaction incubation. When ^{3}H-VSV-111 RNA was added to these samples, it annealed only to product species of the 120-, 180-min, and later samples, as shown by the difference plot in Fig. 5. Consequently, these results suggest that there is some sort of sequential transcription of the VSV-1 RNA whereby the free product obtained in early reactions is not complementary to VSV-111 RNA, whereas in late reactions free product is present which is hybridizable to VSV-111 RNA.

Model for the mechanism of interference of VSV-1 infections by VSV-111. In mixed infections where both particles are used to infect a cell at high VSV-111 input, little VSV-1 is produced although much VSV-111 is obtained [thus "interfering" with the VSV-1 growth cycle (12, 13)]. If VSV-111 lacks an active transcriptase in vivo as well as in vitro, then the question arises as to how interference is mediated. Evidence has been presented from other laboratories to suggest that VSV-1 transcriptase products are the messenger RNA species which are responsible for synthesizing viral proteins in an infected cell (10, 15). If VSV-111 does not have an active transcriptase, this suggests either that it borrows the transcriptase of VSV-1 or that VSV-111 transcription into messenger RNA is not necessary for its life cycle.

One might ask why should VSV-111 RNA be transcribed since all the viral proteins are being synthesized by the VSV-1 growth cycle? Two possible answers present themselves: either it is transcribed to make messenger RNA and protein(s) which it needs to give it a selective growth advantage, or it is not transcribed at all. In the latter case, one could predict that in infected cells the VSV-111 genome waits and competes for a replication function (such as a VSV-1-determined protein to change an inactive transcriptase into an active replicase). Such predictions are amenable to experimental analysis and are currently being investigated.

ACKNOWLEDGMENTS

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