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Location of the Transcription Defect in Group I Temperature-Sensitive Mutants of Vesicular Stomatitis Virus

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The ribonucleoprotein-dependent RNA transcriptase in vesicular stomatitis B virions of four temperature-sensitive (*ts*) mutants belonging to complementation group I was analyzed in vitro at permissive (31 C) and restrictive (39 C) temperatures. The RNA-synthesizing activity of all four *ts* mutants was more labile at 39 C than was the transcriptive activity of wild-type (*wt*) virions. In order to locate the temperature-sensitive transcription defect in the mutants, *wt* and *ts* mutant virions were fractionated by Triton X-100-high salt solubilizer into a sedimentable ribonucleoprotein template and a nonsedimentable enzyme fraction, each of which alone had little or no transcriptive activity. The template- and enzyme-containing fractions of *wt* virions were then tested for their capacity to restore transcriptive activity at 39 C to corresponding template and enzyme preparations of *ts* mutant virions. Recombination of *wt* template and *ts* enzymes resulted in no significant restoration of capacity to synthesize RNA at restrictive temperature. In contrast, transcriptive function at 39 C was reconstituted by recombining the *wt* enzyme with the template component of *ts* mutants. It appears, therefore, that the enzyme, rather than the template, is the temperature-sensitive component of the transcription complex of group I vesicular stomatitis virus mutants.

Vesicular stomatitis (VS) virions contain single-stranded RNA and RNA-dependent RNA polymerase (2). This transcriptase enzyme functions in vitro by catalyzing the synthesis of RNA complementary to virion RNA (2, 3, 5). Since the virus-specific mRNA transcribed in VS viral-infected cells is complementary to virion RNA (15, 18) and is made in the presence of cycloheximide (16), it is likely that the virion transcriptase functions early in infection to synthesize mRNA. The transcriptase has very stringent template requirements (4); the enzyme has been found to function only with nucleocapsids of infectious virions as template (9). Emerson and Wagner (9) have also shown that VS virions can be separated by exposure to Triton X-100 in a high salt environment into nucleocapsid template (high-speed pellet) and transcriptase (supernatant) fractions. Neither the pellet nor the supernatant fraction alone retains much or any capacity to direct the synthesis of viral RNA, but reconstitution of the two components results in restoration of most of the transcriptive function.

Temperature-sensitive (*ts*) mutants (11, 13, 19) of VS virus have been isolated in Glasgow (G group), Orsay (O group), and Winnipeg (W group) and provide a sensitive probe for delineating the physiological parameters of viral transcription. Mutants of complementation groups I and IV (7, 12, 19) are classified as RNA$^-$ owing to marked reduction of RNA synthesis in vivo at restrictive temperatures (17, 20, 26). Definitive evidence has also been provided for defective transcription at the restrictive temperature for certain group I mutants (6, 8, 21, 22). There appears to be some variability among the mutants in complementation group IV, the RNA$^-$ phenotype of which may or may not be related to defective transcription (6, 21, 22). If the in vitro transcriptase in either group is defective at restrictive temperature, the lesion could be in either the enzyme or the template.

The aims of the present study were: (i) to compare under carefully controlled conditions the degree to which transcriptase activity was defective in four different isolates of group I *ts* mutants, and (ii) to determine by reconstitution experiments whether the temperature-sensitive transcriptive function of these group I mutants is located in the enzyme or template of the VS virion. To this end transcriptase activity was measured after recombining the Triton-high salt nonsedimentable enzyme fraction of each mutant or wild-type virus with the sedimentable template fraction of the heterologous virus.

MATERIALS AND METHODS

Viruses and cell cultures. *ts* mutants of the Indiana serotype of VS virus designated *ts* G11, *ts* G13 and *ts* G16 were kindly supplied by C. R. Pringle, Institute of Virology, Glasgow; mutant *ts* O5 was obtained from Anne Flamand through P. Printz, Institut de Microbiologie, Orsay, France. The *wt* VS viral strain was one originally obtained from the U. S. Agricultural Research Center, Beltsville, Md. (24). Recently cloned stocks of virus were used for each experiment and were grown at 31 C in confluent monolayers of BHK-21, clone 13 cells, in 75-cm^2 Falcon flasks in the presence of 88% Eagle basal medium with glutamine, 10% tryptose phosphate broth, and 2% inactivated fetal calf serum. Cultures were inoculated at a multiplicity of ~1 PFU/cell and released virions in the medium were harvested after incubation at 31 C for 18 to 20 h (*wt*) or 22 to 24 h (mutants). Purified B virions were prepared by differential and rate-zonal centrifugation, as previously described (9), except that, if the virus transcriptase was to be assayed immediately, the final virion pellet was suspended in reticulocyte standard buffer (RSB; 10^{-2} M KCl, 1.5×10^{-3} M $MgSO_4$, 10^{-2} M Tris-hydrochloride, pH 8.0) to which was added 50 μliters of 6.5 $\times 10^{-3}$ M dithiothreitol (DTT) per ml. For reconstitution experiments in which virus was usually stored overnight at 4 C before dissociation, the final pellet was suspended in RSB containing DTT and 15% glycerol.

All preparations of virus were titrated by plaque assay on monolayers of L cells (24). The reversion frequency of the mutant stocks was tested for each preparation by plating at 31 and 39 C. No wild-type plaque revertants were detected among any of the four *ts* mutants ($<10^{-8}$ revertants). In some preparations of mutants *ts* O5 and *ts* G13 small plaques, quite distinct from large wild-type plaques, could be detected up to a level of 10^{-5} of the large plaques observed at 31 C. No plaques of any size were ever detected in preparations of *ts* G11 or *ts* G16 plated at 39 C.

Fractionation of VS virions. Virions were fractionated into pellet (template) and supernatant (transcriptase-containing) fractions by a modification of the method of Emerson and Wagner (9). At all stages the enzyme preparations were handled as gently as possible because the transcriptase is very labile (10). Virions suspended in RSB-15% glycerol were treated for 1 h at 0 C with an equal volume of 2X concentrated Triton-high salt solubilizer, which contained 18.7% glycerol, 3.74% Triton X-100, 1.44 M NaCl, 4.4×10^{-3} M KCl, 1.2×10^{-3} M DTT, 6.6×10^{-4} M $MgSO_4$, and 4.4×10^{-3} M Tris-hydrochloride, pH 8.0. The preparation was swirled gently every 15 min. Supernatant and pellet fractions were prepared from separate aliquots of the virion preparations exposed to Triton-high salt solubilizer. The supernatant fraction was prepared by centrifuging an undiluted sample at 150,000 $\times$ *g* for 110 min; the supernatant fluid was withdrawn and the pellet was discarded. Pelleted template fractions were obtained by diluting a solubilized virion preparation with six times its own volume of 1X Triton-high salt solubilizer (1 volume of 2X Triton-high salt solubilizer: 1 volume of RSB-15% glycerol) and then centrifuging at 150,000 $\times$ *g* for 110 min. The supernatant was discarded and the pellet was resuspended in an amount of 1X Triton-high salt solubilizer equal to ½ the volume of the solution which had been centrifuged. The pellet fraction was disaggregated by sonic treatment for 20 s in a Raytheon sonicator at an output voltage of 50 V. A third sample of the solubilized virions was diluted with an equal volume of 1X Triton-high salt solubilizer to provide an unfractionated preparation of solubilized virions at a protein concentration equal to that of a 1:1 mixture of the recombined supernatant and pellet fractions.

Transcriptase assay. The method of Emerson and Wagner (9) was modified in an attempt to increase incorporation at 39 C of ^{3}H-UTP by wild-type virus in order to make more feasible the detection of temperature-sensitive transcriptase activity. Several laboratories have reported marked thermolability of wild-type VS viral transcriptase (1, 6, 14, 22). In experiments in which the activity of solubilized virions was examined immediately after purification, virions were suspended in RSB and an equal volume of 2X Triton-high salt solubilizer was added. After 3 min at 0 C, 1.5 volumes of RSB-25% glycerol were added to 1 volume of the solubilized virion preparation, followed by 2.5 volumes of reaction mixture containing 1.3×10^{-3} M DTT, 8×10^{-3} M magnesium acetate, 5×10^{-2} M Tris-hydrochloride, pH 8.0, 1.4×10^{-3} M each of unlabeled ATP, CTP, and GTP, and 1.9×10^{-5} M ^{3}H-UTP (600 μCi/μmol). Samples (0.1 ml) were pipetted into test tubes which were capped and incubated at 31 or 39 C. All time points were assayed in duplicate. The amount of tritium incorporation was determined as previously described (9).

In reconstitution experiments one volume of sample (solubilized virion preparation, supernatant alone, pellet alone, or pellet plus supernatant fractions), which already contained solubilizer, was mixed with 1.5 volumes of RSB-20% glycerol and 2.5 volumes of reaction mixture. The preparation was then assayed as above. Thus, the glycerol concentration was the same in preparations subjected to the fractionation procedure or assayed directly upon purification.

Chemicals. Nucleoside triphosphates and dithiothreitol were purchased from Calbiochem, La Jolla, Calif. Triton X-100 was from Sigma Chemical Co., St. Louis, Mo. ^{3}H-uridine-5'-triphosphate (10 to 25 Ci/mmol) was obtained from Schwarz/Mann, Orangeburg, N.Y.

RESULTS

Comparative temperature-sensitivity of transcriptive activity of unfractionated VS virions. Before embarking on enzyme-template reconstitution studies, it was essential to characterize the relative temperature sensitivity of each of the group I *ts* mutants and *wt* virus. Therefore, a quantitative analysis was made of the RNA transcribed in vitro by purified virions of *ts* O5, *ts* G11, *ts* G13, and *ts* G16, as well as that of *wt* virions, at 31 and 39 C under carefully controlled conditions. In all these studies

RNA synthesis was assayed immediately after purification of virions without storage, and in each case *wt* virus was harvested and assayed simultaneously with each *ts* mutant. In this way any variability from one assay to another could be minimized and standardized.

Figure 1 shows the rate and extent of RNA synthesis by each *ts* mutant at 31 and 39 C compared directly with that of *wt* virus. As noted, incorporation at 31 C of ^{3}H-UTP was approximately the same for each preparation of *wt* virions when the infectivity of the sample varied little from 10^8 PFU. The fact that equivalent numbers of infectious virions of the four mutant viruses uniformly incorporated somewhat less ^{3}H-UTP at 31 C suggests reduced efficiency of the mutant transcriptase even at the permissive temperature. Nevertheless, linear kinetics at 31 C are demonstrable for both the *wt* and mutant enzymes for at least 3 h. Figure 1 also demonstrates that *wt* virus synthesized RNA far less efficiently at 39 C than at 31 C and the enzyme kinetics were not linear. However, the transcriptase of each *ts* mutant clearly functioned even less well at 39 C than did that of *wt* virus and enzyme activity of mutants *ts* G13 and *ts* G16 was almost completely inhibited at the restrictive temperature. Somewhat surprisingly, incorporation of ^{3}H-UTP by mutants *ts* O5 and *ts* G11 of the same

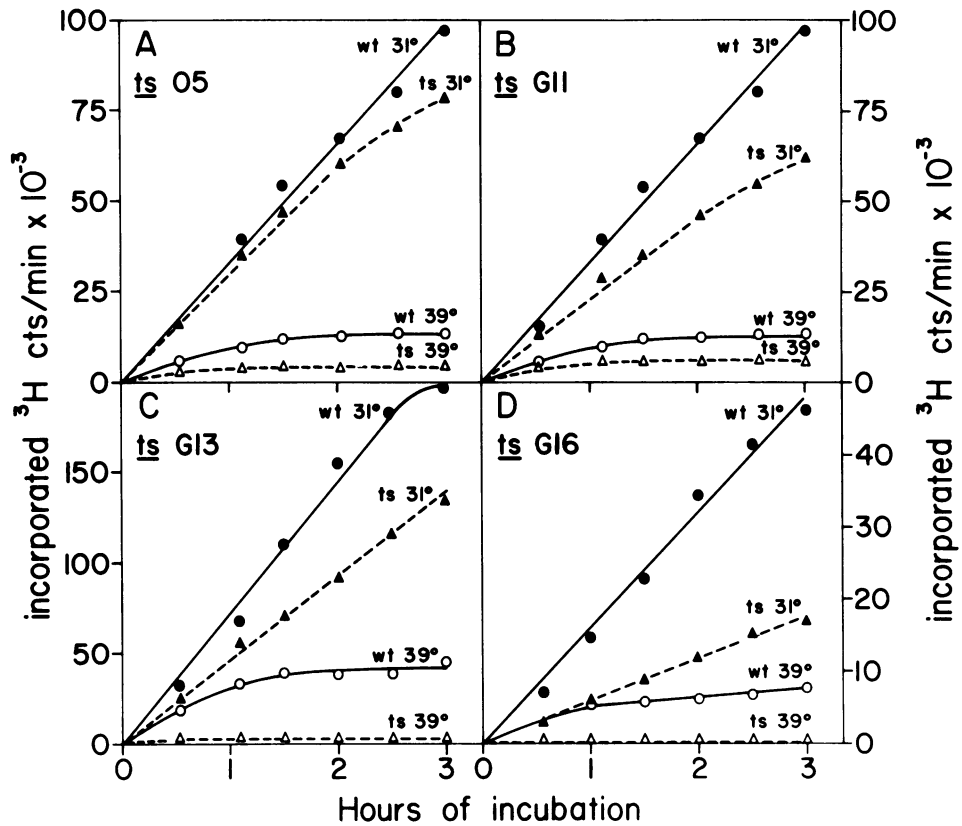

FIG. 1. *Temperature sensitivity of the transcriptive activity of solubilized, unfractionated virions of four group I ts mutants of VS virus compared to that of wt virions. All assays were performed immediately after harvesting and purifying a cloned preparation of virions grown at 31 C. Each mutant transcriptase was assayed in parallel with that of a wt preparation. The reaction mixtures for transcriptase assays contained DTT, magnesium acetate, Tris-hydrochloride, pH 8.0, and 1.4 x 10^{-3} M each of ATP, CTP, GTP, and 1.9 x 10^{-5} M ^{3}H-UTP (600 μCi/μmol). After incubation at 31 or 39 C for varying periods of time up to 3 h, the polymerase reaction was terminated by adding to each tube 0.6 ml of 0.067 M sodium pyrophosphate and 0.1 ml (200 μg) of yeast RNA. Newly synthesized viral RNA was precipitated by adding 0.5 ml of trichloroacetic acid and collected on 0.45-μm membrane filters. Radioactivity was counted in a scintillation spectrometer after eluting the precipitate with 0.3 N NaOH. The values plotted are the average of duplicate 0.1-ml samples. The infectivity titers, expressed as PFU present in 0.1 ml in each assay tube were: A, wt = 0.8 x 10^8, ts O5 = 1.6 x 10^8; B, wt = 0.8 x 10^8, ts G11 = 0.7 x 10^8; C, wt = 1.3 x 10^8, ts G13 = 1.7 x 10^8; D, wt = 0.4 x 10^8, ts G16 = 0.2 x 10^8.*

complementation group was not as temperature sensitive at 39 C as would be expected by their behavior in vivo at the same restrictive temperature (20, 21).

Table 1 shows quantitatively the comparative temperature sensitivity of the transcriptive activity of the four *ts* mutants. These calculations were made by applying a formula which relates the level of RNA synthesis of each mutant at 39 C to the simultaneously determined 31 C:39 C efficiency ratios of *wt* virus ^{3}H-UTP incorporation and to the relative transcriptive efficiency of each mutant at 31 C. The data are recorded as the observed ^{3}H-UTP incorporation of each mutant at 39 C as a percentage of the incorporation which would be expected at 39 C if the temperature sensitivity of mutant and *wt* RNA synthesis were identical. The expected value for incorporation by the mutant at 39 C can be calculated from the temperature sensitivity of *wt* virus and from the ^{3}H-uridine incorporation by the mutant virus at 31 C.

Clearly, the in vitro transcriptive activity of VS viral mutants *ts* O5 and *ts* G11 is only moderately temperature sensitive, compared to the extreme sensitivity of the other group I mutants *ts* G13 and *ts* G16, as previously noted by Szilágyi and Pringle (22).

Reconstitution of homologous VS viral template and enzyme fractions. The feasibility of comparing the temperature sensitivity of the transcriptase with that of the nucleocapsid template of *ts* mutants is greatly dependent on the ability to recover significant enzyme activity after recombination of homologous fractionated components of *wt* and mutant virions. Therefore, preliminary experiments were performed by mixing Triton-high salt-solubilized supernatant with pellet fractions of the same virus and testing the incorporation of ^{3}H-UTP at 31 and 39 C compared with that of solubilized but unfractionated virions. No experiments were done with mutant *ts* G11 because these virions were only slightly more temperature sensitive at 39 C than were *wt* virions. Preparations of *wt* or the three other *ts* mutant virions suspended in RSB-15% glycerol were exposed for 1 h at 0 C to an equal volume of the 2X Triton-high salt solubilizer described in Materials and Methods. One sample was fractionated into pellet and supernatant fractions by centrifugation at 150,000 × *g* and these fractions were tested individually for residual enzyme activity or recombined and then tested for restoration of ^{3}H-UTP incorporation in a complete transcription assay medium at 31 and 39 C. Solubilized but unfractionated virions served as controls.

TABLE 1. *Comparative temperature sensitivity of the in vitro intrinsic transcriptase activity of four ts mutants calculated as the percentage of expected ^{3}H-UTP incorporation at 39 C of wild-type VS virus corrected for decreased enzyme activity of wild type at 39 C and activity of the mutant at 31 C*

Mutant	Percent of control transcriptase activity[a]		
	40 min	90 min	180 min
ts O5	53	44	41
ts G11	85	77	67
ts G13	19	11	10
ts G16	22	11	11

[a] Percentage of control transcriptase activity = [(mutant activity at 39 C)/(mutant activity at 31 C)] × [(wild-type activity at 31 C)/(wild-type activity at 39 C)] × 100.

Table 2 compares the 39 C:31 C ratios of ^{3}H-UTP incorporation at 120 min of solubilized unfractionated and reconstituted virions of *wt* and mutant virus preparations. In each case incorporation of ^{3}H-UTP was negligible for the *wt* or mutant supernatant fractions alone at either 31 or 39 C (data not shown). The pellet fractions alone exhibited greater residual activity but in no instance did this represent more than 6% of the ^{3}H-UTP incorporation found for homologous *wt* or mutant reconstituted pellet plus supernatant. A considerable increase in activity was observed when pellet and supernatant fractions were reconstituted, compared to the activity of pellet and supernatant fractions assayed separately. As noted, temperature sensitivity of the reconstituted wild-type transcriptive activity was similar to that of unfractionated virions. The much greater degree of temperature sensitivity of the *ts* mutants was confirmed. A somewhat greater degree of temperature sensitivity after reconstitution of pellet and supernatant fractions was noted. This is illustrated by the fact that homologous recombination of the *wt* template and enzyme resulted in 51 to 67% recovery of transcriptive activity at 31 C but only 42 to 51% at 39 C. All recovery values are calculated from data for reconstituted fractions after correction for incorporation by pellet and supernatant fractions assayed separately. Recovery of transcriptive activity after homologous reconstitution of pellet and supernatant fractions of the three *ts* mutants was lower than that for *wt* VS virus. At 31 C, the restoration of ^{3}H-UTP incorporation for *ts* G13, *ts* O5, and *ts* G16 was 20 to 30%, 10 to 25%, and 40 to 50%, respectively. Restitution of

TABLE 2. *Temperature sensitivity of transcriptive activity of unfractionated, solubilized VS virions and reconstituted homologous template and supernatant of wt and mutant VS virions*

Virus	^{3}H-UTP incorporation after 120 min					
	Unfractionated, solubilized virions[a]			Reconstituted pellet + supernatant[b]		
	31 C (counts/min)	39 C (counts/min)	39 C:31 C (%)[c]	31 C (counts/min)	39 C (counts/min)	39 C:31 C (%)[c]
Wild type	34,402	7,012	20	22,918	3,028	13
*ts*O5	6,571	456	6.9	1,608	21	1.3
ts G13	16,872	448	2.7	4,317	6	0.1
ts G16	32,194	583	1.8	15,313	55	0.4

[a] Virions in RSB-15% glycerol were disrupted by treatment with Triton-high salt solubilizer and the transcriptase activity of the unfractionated preparation was assayed at 31 and 39 C.

[b] Virions in RSB-15% glycerol were disrupted by treatment with Triton-high salt solubilizer and separated into supernatant and pellet fractions by centrifugation at 150,000 × *g*. The two homologous fractions from each type of virion were recombined and assayed for ^{3}H-UTP incorporation at 31 and 39 C. All values are corrected for incorporation by pellet and supernatant fractions assayed separately.

[c] [(^{3}H-UTP incorporation for 120 min at 39 C)/(^{3}H-UTP incorporation for 120 min at 31 C)] × 100.

transcriptive activity of the *ts* mutants at 39 C was too low to calculate with any assurance of reliable comparison.

Restoration of ts mutant transcriptive activity at 39 C by fractionated components of wt VS virions. The preceding experiments with fractionated mutant virions showed that transcriptive function could be restored by recombining homologous template and enzyme fractions at 31 C but that the template-enzyme complex was still restricted at 39 C. Thus, it was possible to determine the site of the temperature-sensitive lesion of the mutant transcriptase-template complex by determining whether transcriptive activity at 39 C could be restored by *wt* virion template or enzyme. To this end virions of three *ts* mutants and wild type were fractionated by Triton-high salt solubilizer and centrifugation. The mutant supernatant fraction was combined with a *wt* virion pellet fraction and, conversely, the mutant pellet fraction was combined with a *wt* supernatant fraction. Samples of each heterologous mixture were assayed for incorporation of ^{3}H-UTP into an acid-precipitable fraction at 31 and 39 C.

Figure 2 shows that the supernatant fraction of *wt* virions restored transcriptase activity at 39 C to templates of mutants *ts* G13, *ts* G16, and *ts* O5. In marked contrast, extremely low ^{3}H-UTP incorporation was detected at restrictive temperature when enzyme in each mutant supernatant was recombined with the *wt* virion template. Clearly, the temperature-sensitive defect in all three group I mutants resides in the supernatant fraction, presumably the transcriptase. In each case the mutant enzyme was active at the permissive temperature as shown by ^{3}H-UTP incorporation at 31 C with *wt* pellet as template. These experiments also indicate that *ts* G13, *ts* G16, and *ts* O5 all possess a functional template at restrictive temperature as demonstrated by capacity of the mutant pellet fraction to support RNA synthesis at 39 C when recombined with *wt* supernatant enzyme.

In heterologous reconstitution experiments, the incorporation was usually fairly similar to that observed when the supernatant fraction used in the heterologous experiment was combined with its homologous pellet. The results in Table 2 (homologous reconstitution) and Fig. 2 (heterologous reconstitution) are taken from the same experiment for each *ts* mutant so that a comparison can be made.

DISCUSSION

In agreement with the results reported by other workers (6, 21, 22) all the complementation group I mutants examined in the present study had transcriptive activity which was more labile at the nonpermissive temperature of 39 C than was that of *wt* VS virus at 39 C. However, we observed, as did Szilágyi and Pringle (22), that there was considerable variation among group I mutants in the extent to which in vitro RNA synthesis was inhibited at 39 C. The fact that *ts* G11 and *ts* O5, especially the former, made considerable amounts of RNA in vitro at the nonpermissive temperature is not necessarily irreconcilable with the thesis that group I mutants all have a defect in transcriptive function such that infectious virions cannot develop at 39 C in vivo. It is possible that a group I *ts* mutant virion could possess a functional transcriptase but one which made at 39 C consider-

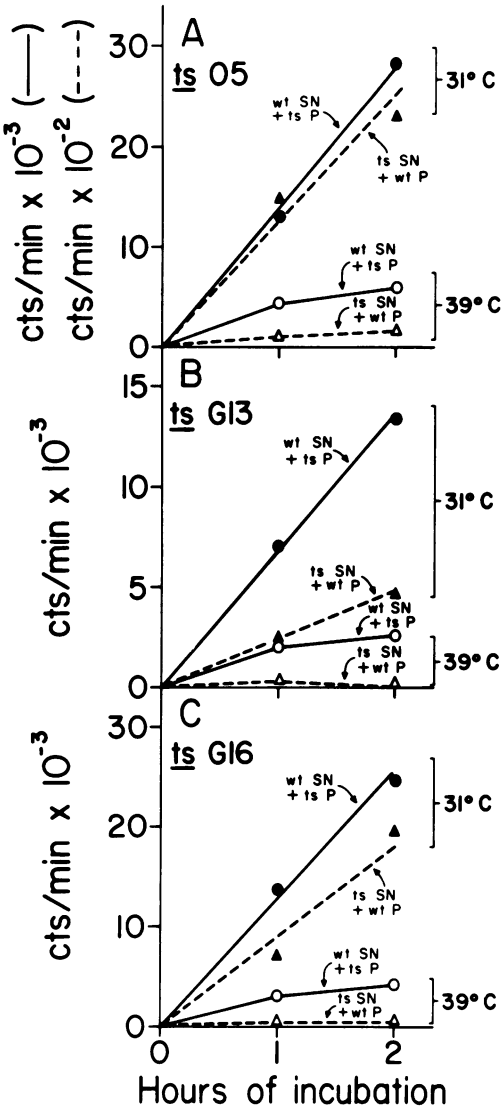

FIG. 2. *Transcriptive activity of the template pellet (P) and supernatant (SN) enzyme fractions of three VS viral ts mutants reconstituted with heterologous SN and P fractions of wt virons. In each experiment purified virions of mutants (A) ts O5, (B) ts G13, and (C) ts G16 were separated into P and SN fractions by treatment with Triton-high salt solubilizer and centrifugation at 150,000 × g, as were equivalent amounts of wt virions. The supernatant fraction of each ts mutant was mixed with wt pellet and the pellet fraction of each ts mutant was mixed with wt supernatant. Incorporation of ^{3}H-UTP into acid-insoluble material was determined at 31 or 39 C. All values are corrected for background radioactivity at 0 time (~200 counts/min) and for residual incorporation of ^{3}H-UTP into separately assayed supernatant fractions (negligible) and pellet fractions (never greater than 1,600 counts/min at 31 C and 210 counts/min at 39 C). All data represent the average of duplicate determinations.*

able amounts of faulty RNA that could not be faithfully translated into functional polypeptides, thus resulting in a deficiency of replication and in later secondary transcription.

Unfortunately, the in vitro transcription system is by no means ideal for delineating all the in vivo transcriptional events. For example, in vivo transcription in the presence of cycloheximide is far less temperature sensitive than is in vitro RNA synthesis (16). Furthermore, it seems reasonable to assume that in vivo transcription of the VS viral genome is complete or very nearly so at all temperatures at which *wt* VS virus is capable of undergoing a complete infective cycle. The requirement for total genome transcription seems implicit in the evidence that the five structural proteins of the VS virion account for most, if not all, of the viral genetic information (25). Unpublished results (D. M. Hunt) indicate that the plating efficiency of the *wt* VS virus used in the present experiments is quite similar at 31, 37, and 39 C. In contrast, it has been demonstrated that, although in vitro transcription is complete at 28 C (3), only one-half to one-quarter of the VS viral genome is transcribed at 37 C (5).

We have shown in these experiments that it is possible, as in the case of *wt* virions (9), to dissociate *ts* mutant virions into sedimentable and nonsedimentable fractions (i) which individually contain little or no transcriptional activity, (ii) homologous reconstitution of which results in restitution of much of their original capacity to incorporate ^{3}H-UTP at 31 C, and (iii) in which the recombined components exhibit transcriptional temperature sensitivity similar to that of solubilized but unfractionated virions. These data enabled us to perform heterologous reconstitution experiments in which pellet or supernatant fractions of *ts* O5, *ts* G13, or *ts* G16 were combined with the appropriate pellet or supernatant fraction of *wt* VS virions. When the reconstituted components consisted of *ts* pellets plus *wt* supernatant, the recombinant temperature sensitivity was always characteristic of *wt* virions. Conversely, when *ts* supernatants were recombined with *wt* pellet, transcriptional thermosensitivity was always that of the mutant from which the supernatant fraction was derived. Therefore, it seems evident that the supernatant fraction contains the temperature-sensitive component that is responsible for the transcriptive activity of group I *ts* mutants. From this and previous work (9, 10) it can be assumed that the Triton-high salt solubilizer dissociates the transcriptase enzyme from the nucleocapsid template. It also appears from these studies that the ribonucleoprotein template of group I *ts* mutants is similar

to and interchangeable with the ribonucleoprotein template of *wt* VS virions, as would be expected if the temperature-sensitive defect is in the mutant enzyme.

Further studies with these mutants will be undertaken to test the temperature-sensitivity of the purified L protein, which has been shown to be required for transcriptase activity (10). The supernatant fractions studied in these experiments are known to contain other virion proteins as well as L. It appears unlikely that envelope proteins G and M are required for accurate transcription in vivo because Szilágyi and Uryvayev (23) have shown that a ribonucleoprotein complex, derived from VSV, and containing only L, N, and NS proteins, could infect cells, producing normal virions. The NS protein is not essential for in vitro transcription but it does bind specifically to nucleocapsid template and could function as a cofactor responsible for fidelity of transcription (10). Reconstitution experiments with purified proteins of these and other mutants might elucidate those factors which regulate transcription.

It is also probable that there are VS viral *ts* mutants in which the template for transcription, which contains the major nucleocapsid N protein, does not function at nonpermissive temperatures. Possible candidates for such studies are in vivo RNA$^-$ mutants of complementation group IV. Of the two group IV mutants examined thus far, *ts* G44 makes more, and *ts* G41 makes less, RNA in vitro at 39 C than would be expected if mutant and *wt* transcriptive activities were identical (unpublished data). Studies with the group IV mutants are in progress but better characterization of their transcriptive activity and their temperature sensitivity is required before attempting to locate a possible lesion by enzyme-template reconstitution experiments.

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LITERATURE CITED

1. Aaslestad, H. G., H. F. Clark, D. H. L. Bishop, and H. Koprowski. 1971. Comparison of the ribonucleic acid polymerases of two rhabdoviruses, Kern Canyon virus and vesicular stomatitis virus. J. Virol. **7**:726-735.
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., and P. Roy. 1971a. Kinetics of RNA synthesis by vesicular stomatitis virus particles. J. Mol. Biol. **57**:513-527.
5. Bishop, D. H. L., and P. Roy. 1971b. Properties of the product synthesized by vesicular stomatitis virus particles. J. Mol. Biol. **58**:799-814.
6. Cairns, J. E., A. F. Holloway, and D. V. Cormack. 1972. Temperature-sensitive mutants of vesicular stomatitis virus: *in vitro* studies of virion-associated polymerase. J. Virol. **10**:1130-1135.
7. Cormack, D. V., A. F. Holloway, and C. R. Pringle. 1973. Temperature-sensitive mutants of vesicular stomatitis virus; homology and nomenclature. J. Gen. Virol. **19**:295-300.
8. Cormack, D. V., A. F. Holloway, P. K. Y. Wong, and J. E. Cairns. 1971. Temperature-sensitive mutants of vesicular stomatitis virus. II. Evidence of defective polymerase. Virology **45**:824-826.
9. Emerson, S. U., and R. R. Wagner. 1972. Dissociation and reconstitution of the transcriptase and template activities of vesicular stomatitis B and T virions. J. Virol. **10**:297-309.
10. Emerson, S. U., and R. R. Wagner. 1973. L protein requirement for *in vitro* RNA synthesis by vesicular stomatitis virus. J. Virol. **12**:1325-1335.
11. Flammand, A. 1969. Etudes des mutants thermosensibles du virus de la stomatite vésiculaire. Mise au point d'un test de complémentation. C. R. Acad. Sci. Paris **268D**:2305-2308.
12. Flammand, A., and C. R. Pringle. 1971. The homologies of spontaneous and induced temperature-sensitive mutants of vesicular stomatitis virus isolated in chick embryo and BHK-21 cells. J. Gen. Virol. **11**:81-85.
13. Holloway, A. F., P. K. Y. Wong, and D. V. Cormack. 1970. Isolation and characterization of temperature-sensitive mutants of vesicular stomatitis virus. Virology **42**:917-926.
14. Huang, A. S., D. Baltimore, and M. A. Bratt. 1971. Ribonucleic acid polymerase in virions of Newcastle disease virus: comparison with the vesicular stomatitis virus polymerase. J. Virol. **7**:389-394.
15. Huang, A. S., D. Baltimore, and M. Stampfer. 1970. Ribonucleic acid synthesis of vesicular stomatitis virus. III. Multiple complementary messenger RNA molecules. Virology **42**:946-957.
16. Huang, A. S., and E. K. Manders. 1972. Ribonucleic acid synthesis of vesicular stomatitis virus. IV. Transcription by standard virus in the presence of defective interfering particles. J. Virol. **9**:909-916.
17. Lafay, F. 1969. Etudes des mutants thermosensibles du virus de la stomatite vésiculaire (VSV). Classification de quelques mutants d'après des critères de functionnement. C. R. Acad. Sci. Paris **268D**:2385-2388.
18. Mudd, J. A., and D. F. Summers. 1970. Polysomal ribonucleic acid of vesicular stomatitis virus-infected HeLa cells. Virology **42**:958-968.
19. Pringle, C. R. 1970. Genetic characteristics of conditional lethal mutants of vesicular stomatitis virus induced by 5-fluorouracil, 5-azacytidine and ethyl methane sulfonate. J. Virol. **5**:559-567.
20. Pringle, C. R., and I. B. Duncan. 1971. Preliminary physiological characterization of temperature-sensitive mutants of vesicular stomatitis virus. J. Virol. **8**:56-61.
21. Printz-Ané, C., A. Combard, and C. Martinet. 1972. Study of the transcription and the replication of vesicular stomatitis virus by using temperature-sensitive mutants. J. Virol. **10**:889-895.
22. Szilágyi, J. F., and C. R. Pringle. 1972. Effect of temperature-sensitive mutations on the virion-associated RNA transcriptase of vesicular stomatitis virus. J. Mol. Biol. **71**:281-291.

23. Szilágyi, J. F., and L. Uryvayev. 1973. Isolation of an infectious ribonucleoprotein from vesicular stomatitis virus containing an active RNA transcriptase. J. Virol. **11**:279–286.
24. Wagner, R. R., A. H. Levy, R. M. Snyder, G. A. Ratcliff, Jr., and D. F. Hyatt. 1963. Biologic properties of two plaque variants of vesicular stomatitis virus (Indiana serotype). J. Immunol. **91**:112–122.
25. Wagner, R. R., L. Prevec, F. Brown, D. F. Summers, F. Sokol, and R. MacLeod. 1972. Classification of rhabdovirus proteins: a proposal. J. Virol. **10**:1228–1230.
26. Wong, P. K. Y., A. F. Holloway and D. V. Cormack. 1972. Characterization of three complementation groups of vesicular stomatitis virus. Virology **50**:829–840.