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Effect of Temperature-Sensitive Mutation on Activity of the RNA Transcriptase of Vesicular Stomatitis Virus New Jersey

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The virion-associated RNA transcriptase activity of vesicular stomatitis virus New Jersey temperature-sensitive (*ts*) mutants was assayed in vitro at the permissive (31°C) and restrictive (39°C) temperatures. RNA synthesis at 39°C by the RNA-negative *ts* A1 and the RNA-positive *ts* C1 and *ts* D1 mutants was similar to that of wild-type virus. The RNA-negative *ts* B1 synthesized only small amounts of RNA in vitro at 39°C. The three mutants of complementation group E were dissimilar in the amounts of RNA they synthesized at 39°C: *ts* E1 synthesized very little RNA, *ts* E2 synthesized moderate amounts, and RNA synthesis by *ts* E3 was not inhibited. The two mutants of group F were also dissimilar, since *ts* F1 synthesized very little RNA at 39°C, whereas *ts* F2 synthesized as much RNA as wild-type virus. The revertant clones *ts* B1/R1, *ts* E1/R1, and *ts* F1/R1 synthesized RNA at 39°C in amounts comparable to wild-type virus, indicating that the heat sensitivity of the transcriptase activity of the mutants *ts* B1, *ts* E1, and *ts* F1 was associated with temperature sensitivity. Similar heat sensitivities were observed when transcribing nucleoprotein complexes were used in the assays, showing that the mutated polypeptides were part of the viral core. The heat stability of the mutant *ts* B1 was similar to that of wild-type virus, and in vitro RNA synthesis was fully restored when the temperature was lowered to 31°C after 30 min of preincubation at 39°C, showing that the inhibition was due to reversible configurational change of the mutated polypeptide. When virions of the mutant *ts* E1 were heated for 5 h at 39°C, their infectivity and transcriptase activity were as stable as those of the wild-type virus, whereas transcriptase activity became very heat labile after disruption of the viral coat with a neutral detergent. This suggests an interaction between the mutated polypeptide and a coat polypeptide which stabilizes the activity of the transcriptase. The RNA transcriptase activity of the mutant *ts* F1 was also heat labile, although to a lesser extent than that of *ts* E1. Thus, the defects in transcriptase activity of groups B, E, and F suggest that all three polypeptides of the virus core, polypeptides L, N, and NS, are involved in the transcription. In addition, we postulate that the mutated gene products of groups E and F are multifunctional, being required both in transcription and replication, and that the gene product of group E may also be involved in some late stage of virus development.

Temperature-sensitive (*ts*) mutants of vesicular stomatitis virus (VSV) Indiana have been classified into six non-overlapping complementation groups (3, 4, 13, 19). At the restrictive temperature (39°C), mutants of complementation groups I and IV do not synthesize virion RNA, whereas mutants of groups III and V do (15). Group II is unique in that it contains mutants of both RNA-negative and RNA-positive phenotypes (15). The virion transcriptase activity of several *ts* mutants was compared in vitro at 31 and 39°C by Szilágyi and Pringle, who found that mutants of groups II, III, and IV all synthesized RNA at 39°C, indicating that in these groups the RNA transcriptase was not affected by the mutation (20). However, several mutants in group I did not synthesize RNA in vitro at 39°C because of the heat lability of their transcriptase (20). Studies of primary RNA transcription in infected cells confirmed that in complementation group I the mutation altered the activity of the RNA transcriptase (18). Dissociation and reconstitution experiments indicated that polypeptide L was the site of the mutational lesion (2, 5, 6). Recently we examined mutant *ts* O45 from the RNA-positive complementation group V (17, 24) and mutant *ts* M602 from the RNA-negative group VI (7, 8, 19) and found that

both mutants synthesized RNA in vitro at 39°C, indicating that the transcriptase activity in these groups is not affected by the mutation (unpublished data).

ts mutants isolated from VSV New Jersey have also been classified into six non-overlapping complementation groups by Pringle et al. (16). Complementation groups A, B, and F did not synthesize virion RNA at the restrictive temperature (39°C), whereas mutants of groups C and D did (16). Complementation group E contained both RNA-negative and RNA-positive mutants, since two of the mutants (*ts* E1 and *ts* E3) did not synthesize virion RNA at 39°C whereas the third mutant (*ts* E2) did (16). Lesnaw and Reichmann used one mutant from each of the six complementation groups to show that in infected cells two of the RNA-negative mutants, *ts* A1 and *ts* E1, were able to carry out primary transcription but not replication of virion RNA at 39.5°C (10). The RNA-positive mutants *ts* C1 and *ts* D1 synthesized virion RNA at the restrictive temperature, whereas mutants from the other two RNA-negative complementation groups (*ts* B1 and *ts* F1) exhibited neither transcription nor replication (10). Recently Lesnaw and Dickson have carried out in vitro dissociation and reconstitution experiments which indicate that the mutational lesion in *ts* B1 involves the L or NS polypeptides (9).

Conditional temperature-dependent (*td* CE) mutants have been isolated which grow both at 31 and 39°C in BHK cells but only at 31°C in secondary chicken embryo cells (14, 21). Some of the *td* CE mutants of VSV New Jersey synthesize RNA in vitro at 39°C similarly to wild-type virus, while others do not (21). One mutant of the latter category, *td* CE3, was studied in some detail, and it was shown that the activity of its transcriptase was fully and instantaneously reversible by lowering the temperature from 39 to 31°C (21). Thus, the mode of inhibition of the transcriptase of this mutant differs from that observed in some of the group I mutants of VSV Indiana, where thermal inactivation was irreversible (20). Dissociation and reconstitution experiments showed that the *td* CE3 mutation affected polypeptide L (22).

In this communication we examine the kinetics of in vitro RNA synthesis by purified virions of the conventional *ts* mutants of VSV New Jersey to determine whether virion transcriptase activity was defective in any of the six complementation groups.

MATERIALS AND METHODS

Mutants. The *ts* mutants of VSV New Jersey described by Pringle et al. (16) were used. We chose one mutant from groups A, B, and C (*ts* A1, *ts* B1, and *ts* C1), the only mutant of group D (*ts* D1), all three mutants of group E (*ts* E1, *ts* E2, and *ts* E3), and both mutants of group F (*ts* F1 and *ts* F2). We also isolated revertant clones from mutants *ts* B1, *ts* E1, and *ts* F1. These revertants (*ts* B1/R1, *ts* E1/R1, and *ts* F1/R1) were isolated from BS-C-1 monolayers incubated at 39°C.

Growth and purification of virus. BHK-21 clone 13 monolayers in 6 to 10 Burrler bottles were infected with low multiplicity of the mutants and incubated at 31°C. Growth and purification followed the method described by Szilágyi and Pringle (20). The purified virus was suspended in 20 mM Tris-hydrochloric acid buffer (pH 8.0), the amount of the protein in this purified preparation was determined by the method of Lowry et al. (11), and the concentration of protein in each suspension was adjusted to 1.3 mg/ml. Infectivity assays were carried out under agar overlay using BS-C-1 cell monolayers.

Thermal inactivation of infectivity. Purified virus preparations (0.005 ml) were suspended in 1 ml of Eagle medium containing 10% fetal calf serum and were incubated at 39°C for 5 h. The residual infectivity of these suspensions was titrated at 31°C on BS-C-1 cell monolayers, and results are expressed as a percentage of the infectivity of identical samples which were kept at 0°C for the same length of time.

TNP preparations. Transcribing nucleoprotein (TNP) complexes were prepared from purified virions by the method of Szilágyi and Uryvayev (23) using the modifications described by Szilágyi and Pringle (21).

Assay of the RNA transcriptase. The previously described RNA transcriptase assays (1, 12, 20, 21, 23) were modified. Incubation mixtures (0.3 ml) were made up at 0°C by adding the following ingredients: calculated volume of 20 mM Tris-hydrochloride buffer (pH 8.0) to make the final volume 0.3 ml, 0.03 ml of TNP or purified virus suspension, 0.015 ml of 0.8% Triton-N 101 (added when virus was used but not when TNP was used), 0.015 ml of 70 mM dithiothreitol, 0.006 ml of Searle "ribonuclease inhibitor" preparation (50 units/ml), 0.018 ml of 32 mM ATP, 0.0075 ml of 1 mM *S*-adenosyl methionine, and 0.075 ml of "reagent mixture" (containing Tris-hydrochloride buffer, pH 8.0, NaCl, actinomycin D, ATP, CTP, GTP, and ^{3}H-labeled UTP) (22). The incubation mixtures were then placed in 31 or 39°C water baths for 1 min, and RNA synthesis was started by the addition of 0.0075 ml of 220 mM $MgCl_2$. Samples (0.02 ml) were taken either before the addition of $MgCl_2$ (zero-time sample) or at intervals during incubation and were placed on Whatman DE-81 paper disks. The disks were washed at room temperature five times with 5% sodium pyrophosphate, twice with water, twice with ethanol, and twice with ether, and radioactivity was measured by liquid scintillation spectrophotometry.

Thermal inactivation of transcriptase. Two identical incubation mixtures containing all the ingredients except $MgCl_2$ were prepared for each mutant. One incubation mixture of each pair was incubated at 39°C for 30 min while the other was kept in an ice-water bath. The transcriptase activity of both mixtures was assayed at 31°C after the addition of $MgCl_2$, and the amount of RNA synthesized by the heat-treated

incubation mixture during 3 h was expressed as a percentage of the amount synthesized by its control.

RESULTS

Temperature sensitivity of the mutants of VSV New Jersey. Throughout the experiments described in this communication, we used purified preparations of the various *ts* mutants of VSV New Jersey. In preliminary experiments we noticed that some preparations of mutant *ts* E1, and to a lesser extent *ts* D1, appeared to have a high frequency of revertant virus. This difficulty was overcome by recloning these mutants. The purified preparations of the *ts* mutants used in the experiments exhibited a *ts* phenotype, and the revertant clones showed efficiencies of plating at 39°C similar to wild-type virus.

In vitro RNA synthesis by the *ts* mutants. To determine the temperature sensitivity of the transcriptase activity of the various *ts* mutants of VSV New Jersey, we assayed the virion-associated RNA transcriptase in vitro at 31 and 39°C and compared the results to those obtained with wild-type virus. These experiments were repeated three times, and the results of a single experiment are shown in Fig. 1. The results obtained in the other two experiments were in very good agreement with those given in Fig. 1.

Wild-type virus synthesized RNA at 31°C linearly for 1 h, and RNA synthesis continued at a reduced rate throughout the 3 h of the experiment. At 39°C the initial rate of RNA synthesis was about half of the rate at 31°C, and a plateau was reached by about 60 min, the total amount of RNA produced being about 13 to 16% of that synthesized at 31°C.

Mutant *ts* A1 consistently synthesized more RNA at 39°C than the wild-type virus. Mutants *ts* C1 and *ts* D1 synthesized RNA at 39°C similarly or slightly better than wild-type virus.

In the case of *ts* B1, the mutation almost certainly affected a polypeptide involved in the transcription process, since this mutant synthesized very little RNA at 39°C (only 2 to 4% of that synthesized at 31°C), whereas its revertant, *ts* B1/R1, synthesized RNA at 39°C at least as well as wild-type virus.

Likewise, mutant *ts* E1 synthesized very small amounts of RNA at 39°C, whereas the amount of RNA synthesized at 39°C by its revertant, *ts* E1/R1, was comparable to that produced by wild-type virus. This indicates that the mutated polypeptide of *ts* E1 is involved in transcription. However, mutant *ts* E3 synthesized RNA normally at 39°C, and *ts* E2 synthesized RNA at a somewhat reduced rate.

There was limited RNA synthesis at 39°C by mutant *ts* F1, about 5% of the amount synthesized at 31°C. Since the revertant clone (*ts* F1/R1) of this mutant synthesized RNA as well as or better than the wild-type virus, it is likely that the mutated polypeptide of *ts* F1 is also involved in the transcription process. However, mutant *ts* F2 synthesized RNA at 39°C similarly to wild-type virus.

These experiments were repeated using TNP complexes of the *ts* mutants. These TNP preparations are ribonucleoprotein complexes isolated from purified virions (23). They contain only three polypeptides (L, N, and NS) in close association with the virion RNA and retain the transcriptase activity of the virion (23). Results with the TNPs of the *ts* mutants were very similar to those given in Fig. 1, indicating that the temperature-sensitive polypeptides of *ts* B1, *ts* E1, and *ts* F1 are part of the viral core (Table 1).

Thermal stability of the transcriptase activity of the *ts* mutants. The mode of inhibition of RNA synthesis at 39°C was investigated by examining the heat stability of the transcriptase activity of the *ts* mutants. Identical reaction mixtures, containing all ingredients except $MgCl_2$, were incubated for 30 min at either 39 or 0°C, and their residual transcriptase activities were assayed after the addition of $MgCl_2$ at 31°C.

Figure 2 shows that wild-type virus retained approximately 40% of its transcriptase activity after heat treatment at 39°C. Similar heat stabilities were observed in the case of mutants *ts* A1, *ts* C1, and *ts* D1 (Fig. 2).

Since preliminary experiments indicated that the transcriptase activity of *ts* B1 was heat stable, we assayed the heat stability of two additional preparations of this mutant. In all three preparations, the heat stabilities of the transcriptase activity were similar to or only slightly less than that of wild-type virus or the revertant clone *ts* B1/R1 (Fig. 2). Thus, the inhibition of RNA synthesis at 39°C by the mutant *ts* B1 was probably the result of a reversible configurational change of the mutated polypeptide.

The transcriptase activity of mutant *ts* E1 was very heat labile at 39°C. However, the revertant clone *ts* E1/R1, as well as the mutants *ts* E2 and *ts* E3, had more stable transcriptase activities than wild-type virus (Fig. 2). Thus, the inability of *ts* E1 to synthesize RNA in vitro at 39°C is due to thermal inactivation of the transcription process.

Mutant *ts* F1 retained only 10% of its transcriptase activity after heat treatment, showing that the transcriptase activity of this mutant is slightly less heat labile than that of *ts* E1. The

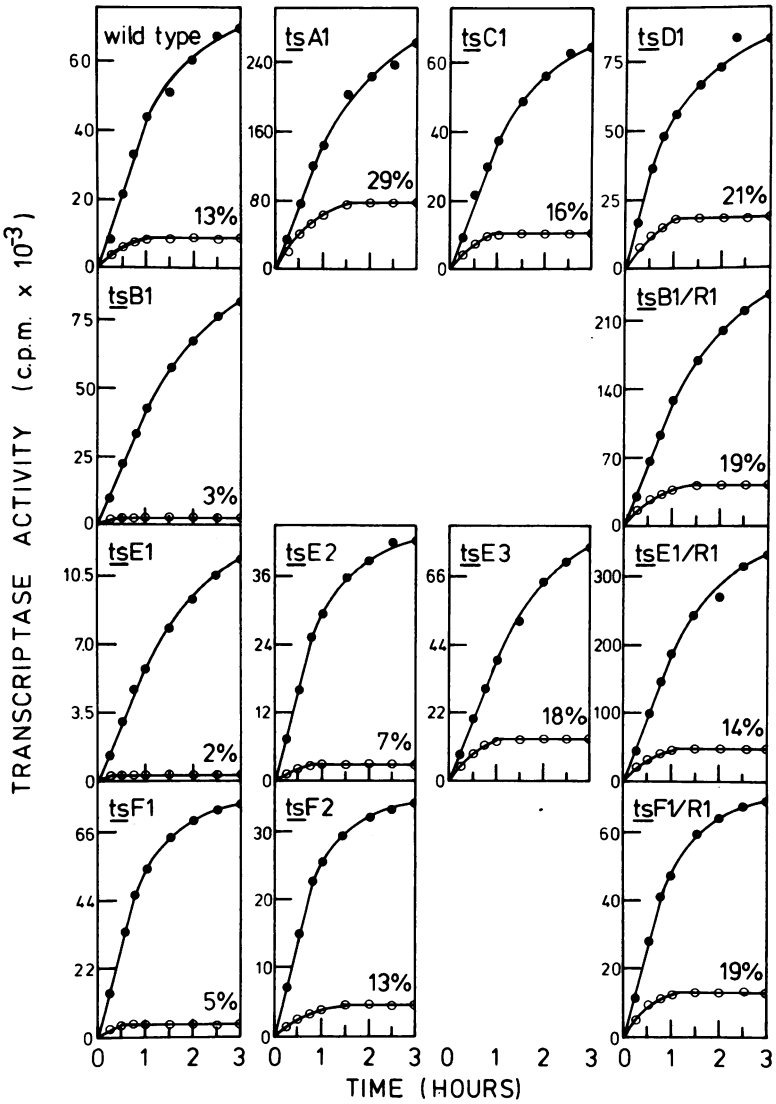

FIG. 1. *Assay of the virion-associated RNA transcriptase of the ts mutants of VSV New Jersey at 31 and 39°C. The virion-associated RNA transcriptase in purified preparations of the ts mutants was assayed either at 31°C (●) or at 39°C (○) as described in the text.*

transcriptase activity of *ts* F2 was consistently more heat stable than that of wild-type virus, and the transcriptase activity of the revertant *ts* F1/R1 was the most heat stable of all the clones tested (Fig. 2). Thus, heat lability of the transcriptase activity of the mutant *ts* F1 probably accounts for its inability to synthesize RNA in vitro at 39°C.

In vitro temperature-shift experiments with *ts* B1. The mode of inhibition of the transcriptase activity of the mutant *ts* B1 was further studied by temperature-shift experiments, where a reaction mixture was incubated at 39°C for 30 min before transfer to 31°C (Fig. 3). The amount of RNA synthesized after the temperature was lowered to 31°C was approximately 25% of that synthesized by a reaction mixture that was incubated at 31°C throughout the experiment. Another reaction mixture containing all the ingredients except $MgCl_2$ was incubated for 30 min at 39°C, and the residual enzyme activity was assayed at 31°C after the addition of $MgCl_2$. The amounts of RNA synthesized by the two preparations were very similar, showing that enzyme activity was fully restored after temperature shift from 39 to 31°C. These results suggest that a reversible configurational change of the mutated polypeptide is responsible for the

TABLE 1. *RNA synthesized by TNP preparations of the ts mutants of VSV New Jersey*[a]

Mutant	% RNA synthesized at 39°C
Wild type	16
ts A1	26
ts C1	20
ts D1	12
ts B1	4
ts B1/R1	22
ts E1	3
ts E2	8
ts E3	15
ts E1/R1	19
ts F1	3
ts F2	12
ts F1/R1	42

[a] Using TNP preparations of the *ts* mutants of VSV New Jersey, complementary RNAs were synthesized at 31 and 39°C under conditions otherwise identical to those described in Fig. 1. The amounts of RNA synthesized at 39°C after 3 h of incubation are expressed as the percentage of the amounts of RNA synthesized at 31°C after the same length of incubation by the same TNP preparation, as follows: percent RNA synthesized at 39°C = (counts per minute after 3 h at 39°C/counts per minute after 3 h at 31°C) × 100.

inhibition of the transcriptase activity of the mutant *ts* B1.

Heat sensitivity of the infectivity of the *ts* mutants. Purified preparations of the *ts* mutants were incubated for 5 h at 39°C, and their residual infectivity was compared with the infectivity of identical suspensions kept at 0°C for the same length of time. In the case of wild-type New Jersey, around 5% of the infectivity survived this heat treatment. Considering the variations inherent in such experiments, the results indicate that the mutants and the revertant clones under investigation showed similar heat stabilities to the wild-type virus. The only possible exception is *ts* F1, where only 0.7% of the infectivity was recovered, suggesting that this mutant may be heat labile.

Since it has been shown that mutant *ts* E1 has a very heat-labile transcriptase activity (Fig. 1 and 2), it was surprising that the proportion of infectious virus surviving the heat treatment at 39°C for 5 h was similar to that of wild-type virus. For this reason we retested the heat stability of the infectivity of this mutant using two further independently grown and purified preparations (Table 2). The results confirm that the heat stability of the mutant *ts* E1 is similar to that of the wild-type virus, since in both cases 10% of the infectivity was recovered after treatment for 5 h at 39°C. We believe that the low survival in the case of *ts* E1/R1 is erroneously low, since in another experiment the heat stability of this virus was significantly higher (13.5%).

Comparison of the heat lability of the transcriptase activity of disrupted and intact virions of mutant *ts* E1. Two further experiments were conducted to determine how mutant *ts* E1 retains its infectivity after a relatively long incubation at 39°C, even though the activity of its transcriptase is heat labile.

In the first experiment, a purified preparation of the mutant *ts* E1 was incubated for 5 h at 39°C, and then its residual transcriptase activity was assayed at 31°C. The results showed that, like wild-type virus, mutant *ts* E1 retained approximately 8 to 10% of its RNA transcriptase activity. Therefore it appears that, when the undisrupted virions of *ts* E1 are heated, both the mutant's infectivity and transcriptase activity are as stable as those of wild-type virus.

Next, the heat stability of the in vitro transcriptase activity of *ts* E1 was reexamined using a TNP preparation (Fig. 4). There was very little 1 173RNA synthesis at 31°C after an initial incubaRNA synthesis at 31°C after an initial incubation of the reaction mixture at 39°C for 30 min. Similarly, almost all transcriptase activity was lost when a control reaction mixture containing all the ingredients except $MgCl_2$ was incubated at 39°C for 30 min and then the residual transcriptase activity was assayed at 31°C. These results show that transcriptase activity of the viral core, unlike the activity of the undisrupted virion, is heat labile, presumably due to the irreversible denaturation of the mutated polypeptide.

DISCUSSION

In this paper we attempt to identify the number of viral polypeptides involved in the transcription of the genome of VSV New Jersey by comparing the in vitro activity of the virion-associated RNA transcriptase of the temperature-sensitive (*ts*) mutants of all six complementation groups. Similar experiments made with the *ts* mutants of VSV Indiana (20) and the temperature-dependent host range (*td* CE) mutants (21) provided the first evidence that some mutants possess a temperature-sensitive transcriptase.

The RNA transcriptase of the mutants of complementation groups C and D is unlikely to be affected by the mutation since mutants *ts* C1 and *ts* D1 synthesized RNA in vitro at 39°C similarly to wild-type virus. These results agree with the finding that both primary transcription and replication of the viral genome occur in cells infected with either of these mutants (10, 16). Since the mutations did not appear to affect any

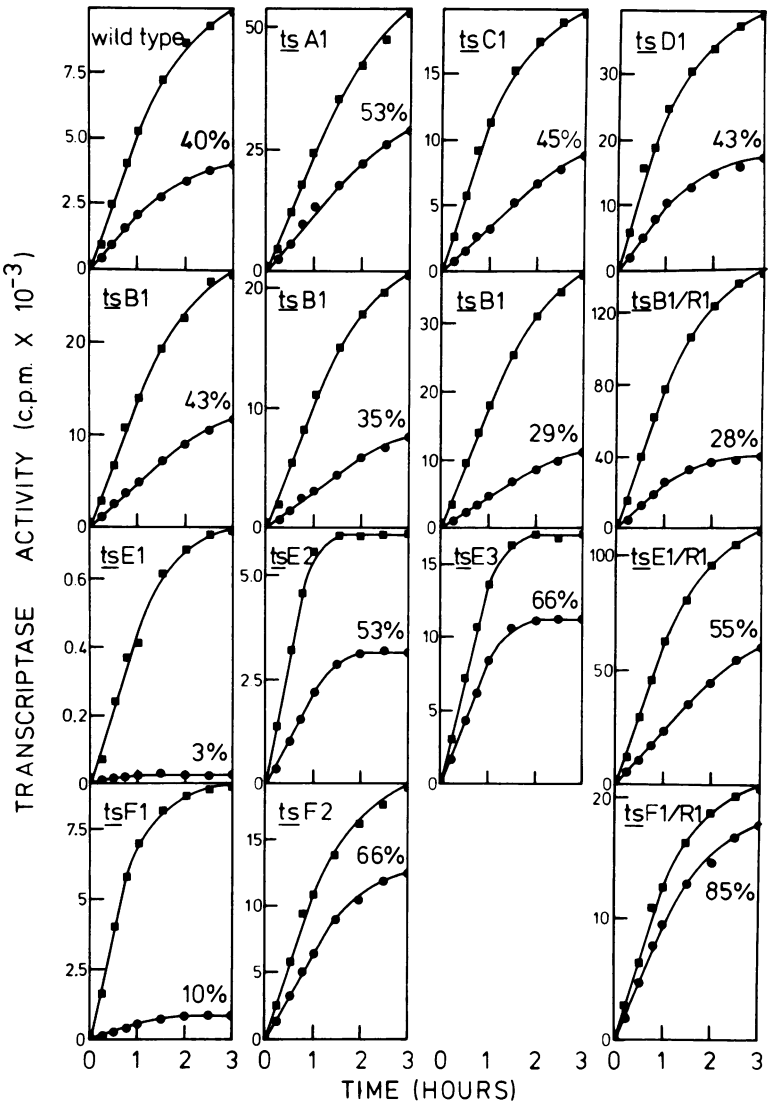

FIG. 2. *Heat stability of the virion-associated RNA transcriptase activity of the ts mutants during incubation at 39°C for 30 min. Identical reaction mixtures containing all ingredients except $MgCl_2$ were incubated at 39°C (●) and 0°C (■) for 30 min, and their residual transcriptase activity was assayed at 31°C after the addition of $MgCl_2$ as described in the text. The mutants were the same as described in Fig. 1 except that two further independently grown and purified preparations of the mutant ts B1 were added.*

stage of RNA synthesis in either of these groups, it is likely that the polypeptides M and G of the virus coat are affected by these mutations. Wunner and Pringle found, paradoxically, that, although in mutant *ts* D1 the electrophoretic mobility of both polypeptides G and N was altered, the *ts* phenotype of this mutant appeared to be due to mutation in the N polypeptide because reversion of temperature sensitivity was accompanied by reversion of the N polypeptide to normal mobility (25). However, the gene assignment of this mutant is under reinvestigation by extensive biochemical and genetic analysis.

In vitro RNA synthesis at 39°C by *ts* A1 was even more pronounced than that by wild-type virus. This makes it very unlikely that transcription in group A is affected by the mutation, and suggests that some function in the RNA replication is defective. This is in good agreement with the results obtained with infected cells, where replication was restricted while primary transcription was unaffected (10, 16).

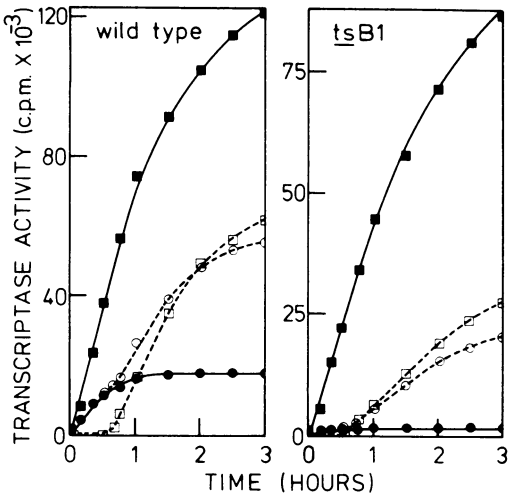

FIG. 3. *Reversibility of the activity of the RNA transcriptase of the mutant ts B1. The transcriptase activity of purified preparations of wild-type VSV New Jersey and the mutant ts B1 was assayed either at 31°C (■) or 39°C (●). A further reaction mixture was incubated at 39°C for 30 min and then transferred to 31°C (○). As control, a reaction mixture which contained all the ingredients except $MgCl_2$ was incubated at 39°C for 30 min, and then $MgCl_2$ was added and the residual transcriptase activity was assayed at 31°C (□). The conditions of assay are described in the text.*

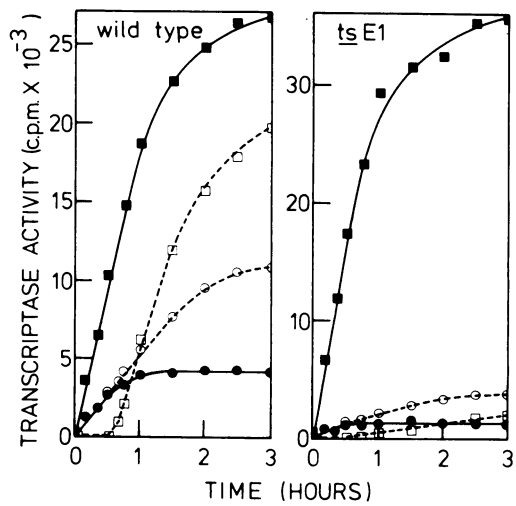

FIG. 4. *Reversibility of the RNA transcriptase activity of the mutant ts E1. Wild-type VSV New Jersey and the mutant ts E1 were used. Transcriptase activity was assayed at 31°C (■), at 39°C (●), and at 31°C after 30 min of incubation at 39°C (○). Another reaction mixture containing all the ingredients except $MgCl_2$ was incubated at 39°C for 30 min, and then $MgCl_2$ was added and the residual transcriptase activity was assayed at 31°C (□). Conditions of enzyme assay are described in the text.*

TABLE 2. *Stability of the infectivity of purified group E mutant preparations during incubation in vitro at 39°C*[a]

Mutants	PFU/ml		% Survival[b]
	After 0°C for 5 h	After 39°C for 5 h	
ts E1 (2nd prepn)	6×10^{10}	6×10^{9}	10
ts E1 (3rd prepn)	6.3×10^{10}	6.3×10^{9}	10
ts E2	1.2×10^{11}	1.7×10^{10}	14
ts E3	1.4×10^{10}	1.2×10^{9}	9
ts E1/R1	2.7×10^{9}	4.1×10^{7}	1.5

[a] The purified virus suspensions were incubated for 5 h at 39°C, and the residual infectivity was titrated at 31°C as described in the text. Two independently grown and purified preparations of the mutant *ts* E1 were used. The preparations of the mutants *ts* E2 and *ts* E3 and the revertant *ts* E1/R1 were the same as described in Fig. 1.

[b] Percent survival is expressed as (PFU after 39°C for 5 h/PFU after 0°C for 5 h) × 100.

Mutants of the other three RNA-negative complementation groups (B, E, and F) are the most likely to be temperature sensitive for transcription, and the in vitro experiments indicated that the representative mutants of these groups, mutant *ts* B1, *ts* E1, and *ts* F1, did possess temperature-sensitive transcriptase activity. Therefore, the investigation was extended to include all three mutants of group E and both of group F.

There was very little RNA synthesis at 39°C in the case of mutant *ts* B1, whereas RNA synthesis was not reduced in the case of the revertant clone *ts* B1/R1, strongly indicating that the mutated gene product is involved in the transcription process. This is in good agreement with the results obtained with *ts* B1-infected cells (10, 16). Temperature-shift experiments with *ts* B1 showed that inhibition is a reversible process presumably brought about by a configurational change of the mutated polypeptide. Thus, the mode of inhibition in this mutant is very similar to that of the temperature-dependent host range mutant *td* CE3 (21, 22).

Mutants of complementation group E showed differences in the amounts of RNA they synthesized in vitro at the restrictive temperature. The involvement of the mutated polypeptide of *ts* E1 in the transcription process is suggested by the fact that this mutant synthesized very little RNA at 39°C, whereas its revertant clone *ts* E1/R1 synthesized normal amounts of RNA at 39°C. On the other hand, mutants *ts* E2 and *ts* E3 both synthesized RNA at 39°C. Since repli-

cation of viral RNA is observed at the restricted temperature in cells infected with *ts* E2 (16), the mutated polypeptide in group E is almost certainly multifunctional. Thus, experiments involving *ts* E1 indicate that the polypeptide takes part in transcription, whereas those involving *ts* E3 suggest that the polypeptide is also involved in replication, and those involving *ts* E2 indicate that the polypeptide is defective either in some later stage of replication or in some stage of virus development following replication. Alternatively, it is possible that the transcription products synthesized at 39°C by *ts* E2 and *ts* E3 are not functional.

The mode of inhibition of the transcriptase activity of *ts* E1 is irreversible thermal inactivation of the mutated polypeptide. However, when undisrupted virus particles are subjected to heat, both the infectivity and transcriptase activity of this mutant and of wild-type virus are very similar. This may be the result of an interaction between the mutated polypeptide and either the M or, less likely, the G polypeptide, resulting in the suppression of the heat lability of the mutated polypeptide. Like interactions, possibly involving some host polypeptide, may account for the primary transcription that takes place in cells infected with *ts* E1 (10).

The two group F mutants are also dissimilar in the amount of RNA they synthesize in vitro at 39°C. One of them, *ts* F1, synthesizes very little RNA at 39°C, and the other, *ts* F2, synthesizes normal amounts of RNA at this temperature. Since the revertant clone, *ts* F1/R1, synthesizes RNA at 39°C, we conclude that the mutated polypeptide of *ts* F1 is also involved in the transcription process. This conclusion accords well with the finding that primary transcription in infected cells was inhibited at the restrictive temperature (10). The heat lability of the mutated polypeptide appears to be responsible for the inhibition of transcriptase activity. The fact that *ts* F1 and *ts* F2 synthesized different amounts of RNA in vitro at 39°C suggests that the polypeptide is multifunctional, being involved in both transcription and replication. However, it is also possible that the RNA species synthesized at 39°C by *ts* F2 are not functional.

Thus in vitro experiments indicate that mutation affected transcription in group B, replication in group A, and both transcription and replication in groups E and F. The mutated polypeptide in group E may also be involved in some late stage of virus development. It is hardly surprising that some of the polypeptides should be multifunctional, since there are only five of them, although some host polypeptide may also be involved in some stages of viral RNA synthesis.

Because there are six complementation groups and only five viral polypeptides, it is difficult to make gene assignments. It is possible that VSV codes for a sixth, so far unrecognized polypeptide, although the possibility that one of the six groups represents an extreme case of intracistronic complementation has to be considered also. Since purified cores isolated from mutants *ts* B1, *ts* E1, and *ts* F1 exhibit the same temperature sensitivity as their virions, it is possible that the core polypeptides L, N, and NS are the sites of the mutational lesion in groups B, E, and F.

We propose that the mutated polypeptide in group B is polypeptide L, since the mode of inhibition in mutant *ts* B1 is similar to that in *td* CE3, where polypeptide L was shown to be the mutated polypeptide (21, 22). This possibility is strengthened by a recent report of dissociation and reconstitution experiments in which it was found that the mutated polypeptide of *ts* B1 was either L or possibly NS (9). If in group B the mutated polypeptide is L, then polypeptides N and NS are the likely ones in groups E and F.

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