Temperature Sensitivity of the Transcriptase of Mutants tsB1 and tsF1 of Vesicular Stomatitis Virus New Jersey Is a Consequence of Mutation Affecting Polypeptide L

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SUMMARY

Two conditional transcriptase-negative mutants of vesicular stomatitis virus (VSV) serotype New Jersey, tsB1 and tsF1, their revertants tsB1/R1 and tsF1/R1 and the wild-type virus were dissociated into pellet, NS and L fractions and, after reconstitution of these in various combinations, the transcriptase activities were assayed in vitro at the permissive (31 °C) and restrictive (39 °C) temperatures. The pellet fractions contained the virion RNA-polypeptide N complexes, while the NS and L fractions were essentially pure preparations of these polypeptides. The synthesis of RNA by the reconstituted pellet and L fractions was inhibited at 39 °C only when the L fractions of tsB1 or tsF1 were used. Addition of the NS fractions to the reconstituted pellet and L fractions did not alter the rates of RNA synthesis. These results demonstrate that polypeptide L is the temperature-sensitive polypeptide of both mutants tsB1 and tsF1 and support previous observations that polypeptide L is the transcriptase itself. The fact that a second mutant of complementation group F, tsF2, is transcriptase-positive but replicase-negative suggests that polypeptide L is involved both in transcription and replication. Intracistronic complementations may account for the observation that the temperature-sensitive mutations affect polypeptide L in complementation groups B and F.

INTRODUCTION

Temperature-sensitive mutants of vesicular stomatitis virus (VSV) serotype New Jersey have been classified into six non-overlapping complementation groups and characterized as replicase-positive or -negative mutants by their ability to synthesize virion RNA in infected cells at the restrictive (39 °C) temperature, and as transcriptase-positive or -negative mutants by their ability to synthesize mRNA species in vitro at 39 °C (Pringle et al., 1971; Pringle, 1977; Szilágyi & Pringle, 1979). Transcriptase-negative mutants have been identified in complementation groups B, E and F (mutants tsB1, tsE1 and tsF1), suggesting the involvement of three viral polypeptides in the transcription process (Szilágyi & Pringle, 1979).

The temperature sensitivity of the transcriptase of tsE1 is the consequence of a ts mutation affecting polypeptide NS, since this polypeptide has an electrophoretic mobility which differs from that of the wild-type virus and the other group E mutants, whereas a spontaneous revertant of this mutant, tsE1/R1, possesses an NS polypeptide with an electrophoretic mobility identical to that of the wild-type virus (Evans et al., 1979; Lesnaw et al., 1979). Dissociation and reconstitution experiments using tsE1, tsE1/R1 and wild-type virus showed that purified polypeptide L synthesizes RNA in vitro on the virion RNA–polypeptide N template both at 31 °C and 39 °C, and that this RNA synthesis is inhibited at 39 °C by the addition of polypeptide NS of tsE1 but not by the NS polypeptides of tsE1/R1 or the wild-type virus (Ongrádi & Szilágyi,

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The purified virions contained the five viral polypeptides, L, G, NS, N and M, while the TNP complexes contained polypeptides L, NS and N. The pellet fractions contained polypeptide N in vitro. A representative mutant of complementation group B, mutant tsB1, is transcriptase-negative since it cannot synthesize RNA in vitro at 39 °C; its spontaneous revertant, tsB1/R1, synthesizes RNA as well as wild-type virus (Szilágyi & Pringle, 1979). Similarly, mutant tsF1 is transcriptase-negative since it synthesizes very little RNA in vitro at 39 °C but the amounts of RNA synthesized at this temperature by its revertant strain, tsF1/R1, are comparable to those of the wild-type virus (Szilágyi & Pringle, 1979). However, while tsB1 possesses a heat-stable transcriptase, that of tsF1 is heat-labile (Szilágyi & Pringle, 1979).

Polyacrylamide gel electrophoresis and Cleveland's partial digestion method failed to identify the temperature-sensitive polypeptide of the mutants tsB1 and tsF1 (unpublished observations). Hybrid infectivity assays specific for primary transcription suggested that mutants tsB1 and tsF1 possess temperature-sensitive L polypeptides (Belle Isle & Emerson, 1982).

Our objective was to establish the temperature-sensitive polypeptides of the mutants tsB1 and tsF1 by dissociation and reconstitution experiments and to study their roles in the transcription process in vitro.

**METHODS**

_Growth and purification of virus._ Wild-type VSV New Jersey, the temperature-sensitive mutants tsB1 and tsF1, and their spontaneous revertant strains tsB1/R1 and tsF1/R1 (Pringle et al., 1971; Szilágyi & Pringle, 1979) were grown and labelled with L-[35S]methionine as previously described (Evans et al., 1979).

_Dissociation of the virion into sub-viral fractions._ A suspension of purified virions was treated with a mixture of a neutral detergent (Triton N-101) and 0.5 M-CsCl which dissolved the envelope of the virion and liberated the viral core, called the transcribing nucleoprotein (TNP) complex. These TNP complexes were isolated by pelleting through a glycerol gradient and, after resuspension, were treated with a mixture of 0.1% digitonin and 0.9 M-LiCl and centrifuged through another glycerol gradient. The resuspended pellet, called the pellet fraction, contained the virion RNA-polypeptide N complex, while the supernatant, the so-called supernatant fraction, contained the solubilized L and NS polypeptides. These polypeptides were then separated by passing the supernatant fraction through a small column of phosphocellulose, which allowed polypeptide NS to flow through but retained polypeptide L. The flow-through material, called the NS fraction, was collected, the column was then repeatedly washed to remove all traces of polypeptide NS, and finally polypeptide L was liberated from the column by elution with 2 M-NaCl. This eluate was called the L fraction. Finally the volumes of the sub-viral fractions were adjusted to make the concentrations of the polypeptides comparable to those in the suspension of the TNP complex.

Detailed description of this dissociation is given in our previous communication (Ongrádi et al., 1985).

_Reconstitution of the sub-viral fractions and assay of the transcriptase in vitro._ For reconstitution, 15 μl pellet fractions, 50 μl NS fractions and 50 μl L fractions were used in various combinations, and each mixture was adjusted to 115 μl by the addition of buffer. To assay the activity of the transcriptase in these reconstituted fractions, we added 80 μl of the reaction mixture (containing the four ribonucleoside triphosphates including [3H]UTP, Tris-HCl pH 8.0, NaCl, dithiothreitol, S-adenosylmethionine, actinomycin D and rat liver ribonuclease inhibitor). These incubation mixtures (195 μl) were then warmed to 31 °C or 39 °C and 1 min later RNA synthesis was started by the addition of 5 μl MgCl2. Samples (20 μl) taken at 30 min intervals were washed and the amounts of [3H]UMP incorporated into RNA were determined by scintillation counting.

A detailed account of this method is given in a previous publication (Ongrádi et al., 1985).

_Polyacrylamide gel electrophoresis of polypeptides._ The [35S]methionine-labelled polypeptides of the virions and sub-viral fractions were separated by electrophoresis on discontinuous SDS-polyacrylamide slab gels, and the individual polypeptides were visualized by autoradiography using Agfa-Gevaert Scopix CR3 NIF X-ray films as previously described (Ongrádi et al., 1985).

**RESULTS**

_Polypeptide composition of the virions and their sub-viral fractions._

The polypeptide compositions of the virions and the sub-viral fractions of mutant tsB1 are shown in Fig. 1 (a), and those of the mutant tsF1 in Fig. 1 (b). Similar results were obtained with wild-type VSV New Jersey and revertants tsB1/R1 and tsF1/R1 (data not shown).

The purified virions contained the five viral polypeptides, L, G, NS, N and M, while the TNP complexes contained polypeptides L, NS and N. The pellet fractions contained polypeptide N in vitro.
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Fig. 1. Polypeptide composition of virions and the sub-viral fractions of (a) mutant tsB1 and (b) mutant tsF1. Polypeptides were separated by polyacrylamide gel electrophoresis, and visualized by autoradiography. The wells contained purified virions (20 μg protein/well), TNP complex (16 μg), pellet fraction (14 μg), supernatant fraction (5 μg), NS fraction (1.5 μg) or L fraction (3.5 μg).

Polypeptide composition of virions and the sub-viral fractions of (a) mutant tsB1 and (b) mutant tsF1. Polypeptides were separated by polyacrylamide gel electrophoresis, and visualized by autoradiography. The wells contained purified virions (20 μg protein/well), TNP complex (16 μg), pellet fraction (14 μg), supernatant fraction (5 μg), NS fraction (1.5 μg) or L fraction (3.5 μg).

RNA synthesis by reconstituted sub-viral fractions of tsB1, tsB1/R1 and wild-type VSV

The pellet, NS and L fractions of the mutant (tsB1), revertant (tsB1/R1) and wild-type (VSV New Jersey) viruses did not on their own synthesize detectable amounts of RNA in vitro at 31 °C. The rates of RNA synthesis after reconstitution of the pellet and L fractions of the three viruses are shown in Fig. 2. The rates of RNA synthesis at 31 °C by the reconstituted wild-type pellet and the L fractions of wild-type and revertant viruses were similar, but less RNA was synthesized when the L fraction of tsB1 was used for reconstitution. The rates of RNA synthesis at 39 °C were again similar when the pellet fractions of wild-type or mutant viruses were reconstituted with the L fractions of the wild-type or revertant viruses. However, when the L fraction of tsB1 was used for reconstitution, transcriptase activity was strongly inhibited.

The rates of RNA synthesis at 39 °C observed after reconstitution of wild-type pellet with the L and NS fractions of the three viruses are shown in Fig. 3. Similar rates of RNA synthesis were observed when the L fractions of the wild-type or revertant viruses were reconstituted with the wild-type pellet fraction in the presence of any of the three NS fractions. However, when the L fraction of tsB1 was used, RNA synthesis was greatly reduced in all combinations.
RNA synthesis by reconstituted sub-viral fractions of tsF1, tsF1/R1 and wild-type VSV

The NS and L fractions of the mutant (tsF1), revertant (tsF1/R1) and wild-type (VSV New Jersey) viruses on their own did not synthesize any RNA at 31 °C, and only residual RNA synthesis was observed when the pellet fraction of the mutant was tested.

The rates of RNA synthesis in vitro after reconstitution of the pellet and L fractions are shown in Fig. 4. After reconstitution of any of the L fractions of the three viruses with the pellet fractions of the wild-type or mutant viruses, RNA synthesis was observed in every combination at 31 °C. However, while RNA synthesis at 39 °C was observed in the presence of the L fractions
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Fig. 4. RNA synthesis in vitro by reconstituted pellet and L fractions of tsF1, tsF1/R1 and wild-type VSV. The pellet fractions of wild-type VSV (a, c) and tsF1 (b, d) were reconstituted with the L fractions of tsF1 (▲), tsF1/R1 (■) or wild-type VSV (●), and the transcriptase activities were assayed in vitro at 31 °C (a, b) and 39 °C (c, d).

Fig. 5. RNA synthesis in vitro after reconstitution of pellet fraction of tsF1 with L and NS fractions of tsF1, tsF1/R1 and wild-type VSV. The pellet fraction of tsF1 VSV was reconstituted with the L fractions of tsF1 (▲), tsF1/R1 (■) or wild-type VSV (●), in the presence of the NS fractions of (a) wild-type VSV, (b) tsF1 or (c) tsF1/R1 and the transcriptase activity was assayed at 39 °C.
of wild-type and revertant viruses, it was greatly reduced when the L fraction of the mutant tsF1 was reconstituted with either of the pellet fractions.

The rates of RNA synthesis in vitro at 39 °C by the reconstituted pellet fraction of the mutant virus with the L and NS fractions of the three viruses are shown in Fig. 5. Synthesis of RNA was observed at 39 °C when the L fractions of the wild-type and revertant viruses were used regardless of which NS fraction was present. However, when the L fraction of the mutant tsF1 was used for reconstitution, RNA synthesis was much reduced in the presence of any one of the three NS fractions.

**DISCUSSION**

Assay of the transcriptase activity at 31 °C and 39 °C after reconstitution in various combinations of the pellet and L fractions of mutants tsB1 and tsF1, their revertants tsB1/R1 and tsF1/R1 and wild-type virus showed that RNA synthesis was inhibited at 39 °C only when the L fractions of the two mutants were used. Since the L fractions were essentially pure preparations of polypeptide L, the results demonstrate that the temperature-sensitive polypeptide of each mutant was polypeptide L and that the temperature-sensitivity of their transcriptases was the consequence of this mutation. Addition of polypeptide NS had no effect on transcription in vitro by the reconstituted pellet and L fractions, thus providing further evidence that polypeptide L is the mutated polypeptide of tsB1 and tsF1.

These results are in agreement with previously reported dissociation and reconstitution experiments using mutant tsE1 where it was shown that polypeptide L is sufficient for in vitro transcription on the virion RNA–polypeptide N template since the mutated polypeptide NS of tsE1 inhibited transcription at the restrictive temperature in vitro (Ongrádi & Szilágyi, 1981; Ongrádi et al., 1985).

Our results also agree with those of Belle Isle & Emerson (1982) who used hybrid infectivity assays to show that both tsB1 and tsF1 possess temperature-sensitive L polypeptides.

The two mutants in complementation group F exhibit differences, since tsF1 is transcriptase-negative whereas tsF2 is transcriptase-positive but replicase-negative (Szilágyi & Pringle, 1979). Since it is reasonable to suppose that mutations within a complementation group affect the same polypeptide, the results strongly suggest that polypeptide L is involved in both transcription and replication.

Gadkari & Pringle (1980), studying ts mutants of Chandipura virus, observed intracistronic complementation within two complementation groups and concluded that the two groups of mutants may either represent different functional domains of the same molecule or the gene product may be a dimer or a multimer and complementation occurred by a protein–protein interaction. Since mutants of VSV New Jersey with a temperature-sensitive defect in polypeptide L appear in complementation groups B and F, we conclude that, similarly to Chandipura virus, mutations in these two groups affect either different functional domains of polypeptide L or different polypeptide L monomers in a multimeric transcriptase complex. The possibility of intracistronic complementation is further supported by the observation that polypeptide L is a multifunctional polypeptide, having a role both in transcription and replication, and that tsB1 and tsF1 may complement each other to a limited extent (Pringle et al., 1971).

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